To the Graduate Council:

I am submitting herewith a thesis written by Prakash Koderi Narayana entitled "Identification of Microbial Populations in Activated Sludge by 16S rDNA Analysis and Fluorescent in situ Hybridization." I have examined the final copy of the thesis for this form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Life Sciences.

Gary S. Sayler

We have read this thesis
and recommend its acceptance:

Robert N Moore

Accepted for the Council:

Associate Vice Chancellor and
Dean of The Graduate School

Identification of Microbial Populations in Activated Sludge by 16S rDNA Analysis and Fluorescent in situ Hybridization

A Thesis presented

for the

Master of Science Degree

The University of Tennessee, Knoxville

Prakash Koderi Narayana-Karanth

December 1997

DEDICATION

This thesis is dedicated to my beloved parents

Sri. K. S. Narayana Karanth

(ಶ್ರೀ. ಕೆ. ಸ. ನಾರಾಯಣ ಕಾರಂತ)

and

Smt. K. N. Kalyanamma

(ಶ್ರೀಮತಿ. ಕೆ. ನಾ. ಕಲ್ಯಾಣಮ್ಮ)

who have given me invaluable love and educational support

and to my late brother

K. N. Mohan Karanth

(ಕೆ. ನಾ. ಮೋಹನ ಕಾರಂತ)

(08/08/63-08/15/92)

ACKNOWLEDGEMENTS

I would like to thank Dr. Gary S. Sayler for his guidance and for giving me an opportunity to work in an environment conducive to acquire exhaustive knowledge of basic as well as advanced molecular biology techniques.

I also thank my committee members, Dr. Jeffery M. Becker for enabling to me think critically in science and Dr. Robert N. Moore for his suggestions and cooperation.

I extend my gratitude to Dr. Donald K. Dougall, the former Director of Biotechnology Graduate Program, Mohan, Raju and Sunil for their role in my admission to the University of Tennessee. I acknowledge the financial assistance in the form of Graduate Teaching Assistantship provided by the Department of Microbiology.

I am grateful to Dr. Arthur Meyers, Scientist, Tennessee Eastman Chemical Company, for sponsoring this exciting project which enabled me to acquire knowledge in the areas of molecular biology and computer science.

I am indebted to Curtis Lajoie and Alice Layton for their invaluable guidance and ceaseless support throughout my research. The merit of this thesis work is due to their critical suggestions in addition to Ann L. Lacava's useful advice.

I also thank all the members of my family for their everlasting love and emotional support throughout my studies which enabled me to reach the current stage.

I would like to thank Carissa M. Turner and Susan M. Davis for their love, caring and encouragement when it was badly needed. I thank Donya Brown of HIS Mission for her concern to the problems of International students in Knoxville. Finally, I thank my colleagues in CEB and all my friends for their role in my memorable stay in Knoxville.

ABSTRACT

Total DNA was extracted from three different samples of the activated sludge taken from the wastewater system at Eastman Chemical Company, Kingsport, TN. 16S rDNA methodologies were used to characterize the microbial populations of the activated sludge and the 16S rDNA (1500 bp) was amplified by polymerase chain reaction (PCR), and subsequently cloned into the TA cloning vector. The clones were sequenced and the sequences were analyzed by comparing them with the published sequences in the Ribosomal Database Project (RDP) maintained by the Department of Microbiology, University of Illinois, Urbana-Champaigne, IL. Phylogenetic trees based on distance matrix methods were constructed from the aligned sequences. Some of the sequences were similar to the reference sequences from known organisms, but none of the sequences were identical. Comparative sequence analysis of clones from different months indicated a shift in the microbial populations of the activated sludge. Bacteria representing the α, β, γ, and δ subclasses of the Proteobacteria, as well as members of the Cytophagales/Flavobacter/ Flexibacter group, and Gram-positive organisms with low (G+C) content were found in all three samples analyzed (January, April and November 1995). Organisms belonging to the α subclass of the Proteobacteria were dominant in April and November and organisms belonging to the β subclass of the Proteobacteria were dominant in January. Microbial diversity in activated sludge was also demonstrated by restriction fragment length polymorphism (RFLP) analysis.

Sequences similar to *Hyphomicrobium* spp., belonging to the α subclass of the Proteobacteria, were present as 10 of the 64 sequences analyzed. Based on comparative sequence analysis, a fluorescein-labeled oligonucleotide probe complementary to the conserved region of the rRNA of *Hyphomicrobium* spp. was designed. The probe was designed with 1 degeneracy to encompass *Hyphomicrobium* spp. in the RDP and the observed library sequences. Sequencing of a *Hyphomicrobium* spp. isolate (*Hyphomicrobium* M3) from the activated sludge indicated that the library sequences and the isolate were identical in the probe target region, whereas the RDP sequences differed by 1 nucleotide. Whole-cell hybridizations of target organism and reference bacteria with one, two, four and eight mismatches in the target region were used to determine the optimal hybridization conditions for the probe. The efficacy of the probe was demonstrated by in situ hybridization of activated sludge samples. In general, the effectiveness of the use of 16S rDNA libraries for identification of important microorganisms in activated sludge was demonstrated. This was confirmed by fluorescent in situ hybridization with a probe designed based on the library sequences. Independent confirmation was provided by visible, transmission and scanning electron micrographs of the sludge. Transmission and scanning electron micrographs of the sludge indicated the presence of budding bacteria resembling *Hyphomicrobium* spp. in the Bergey's Manual of Determinative Bacteriology (1994).

TABLE OF CONTENTS

1. **INTRODUCTION** 1

2. **LITERATURE REVIEW**
   - Activated Sludge Process 8
   - Role of Microorganisms in Sludge 9
   - Characterization of Activated Sludge with Respect to Settling Problems .. 10
   - Advent of Molecular Techniques 11
   - Ribosomal RNA as a Valuable Tool for Phylogenetic Studies 12
   - Extraction of Total DNA from a Complex Environment 13
   - Selective Amplification of 16S rRNA Gene Fragments from Total DNA by Polymerase Chain Reaction 13
   - Cloning of 16S rRNA Genes 14
   - Comparative Analyses of the Sequence Obtained 14
   - Biases in 16S rRNA Sequence Retrieval 15
   - In Situ Hybridization Techniques for the Identification of Natural Bacteria 16

3. **MATERIALS AND METHODS**
   - Samples 18
   - DNA Extraction and Purification 18
   - Qiagen Protocol for the Purification of Total DNA 20
   - PCR amplification of 16S rDNA 20
   - Agarose Gel Electrophoresis of PCR Products 22
   - Agar*ACE* Protocol for the Purification of PCR Amplified 16S rDNA ... 23
   - Spin Column Protocol for the Purification of PCR Amplified 16S rDNA . 23
   - Electroelution Protocol for the Purification of PCR Amplified 16S rDNA . 24
   - Cloning of 16S rRNA Genes 24
   - PCR Amplification of 16S rDNA from Clones 25
   - Restriction Digestion of the PCR Amplified Products 26
   - Midiprep Plasmid Preparation from Strains with Inserts 26
   - 16S rDNA Sequencing 27
   - 16S rDNA Sequence Analysis 27
   - Fluorescent in-situ Hybridization
     - Organisms 28
     - Cultivation of Cells 29
     - Fixation of Culture Cells 29
     - Fixation of Sludge Samples 30
     - Hybridization 31
     - Washing 32
   - Specimen Preparation for Microscopy Techniques
     - Light Microscopy 33

Negative Staining 33
Transmission Electron Microscopy
Fixation and Embedding 33
Preparation of Plates 34
Scanning Electron Microscopy 34

4. **RESULTS**
DNA Extraction and Purification 36
PCR Amplification of 16S rDNA 36
Cloning of 16S rDNA Genes 40
RFLP Analysis 42
Comparative Sequence Analysis to Determine Phylogeny 44
Hyphomicrobium Probe Design 50
Sequence Analysis of 16S rDNA from *Hyphomicrobium* Species 50
Microscopic Examination of Activated Sludge 52
Fluorescent in-situ Hybridization 55

5. **DISCUSSION** 66

6. **CONCLUSION** 77

7. **BIBLIOGRAPHY** 78

8. **APPENDICES**
Appendix A. Partial 16S rDNA sequences of the clones of January, April and November sludge samples 90
Appendix B. Similarity_Rank result for the clones of January, April and November sludge samples 109
Appendix C. Phylogenetic trees showing relationships of the nucleotide sequences of the 16SrDNA Clones obtained from January Sludge Samples 147
Appendix D. Phylogenetic trees showing relationships of the nucleotide sequences of the 16SrDNA clones obtained from April activated sludge samples 148
Appendix E. Phylogenetic trees showing relationships of the nucleotide sequences of the 16SrDNA clones obtained from November activated sludge samples 149
Appendix F. Multiple sequence alignment of the partial 16S rDNA sequences obtained from January, April and November clones 150
Appendix G. Pairwise evolutionary distances, obtained from the DISTANCES tool of GCG package, between nucleotide sequences of all the clones analyzed and the selected reference bacteria 195

Appendix H. List of ranked best complementarities between the *Hyphomicrobium* probe sequence and the sequences in the RDP alignment as determined by Check Probe tool of RDP 202

Appendix I. Complete 16S rDNA sequence for 3M3 clone of *Hyphomicrobium* 211

Appendix J. Complete 16S rDNA sequence for 6M3 clone of *Hyphomicrobium* 212

Appendix K. Similarity_Rank result for 3M3 and 6M3 clones of *Hyphomicrobium* on submission to RDP database 213

Appendix L. Align Sequence result for 3M3 and 6M3 clones of *Hyphomicrobium* on submission to RDP database 215

9. **VITA** 218

LIST OF FIGURES

Figure	Contents	Page
1	Methodology to identify uncultivable microorganisms by 16S rRNA analysis from sludge samples	4
2	Basic concept of activated sludge treatment	8
3	Ethidium-bromide stained gel of PCR amplified 16S rDNA of *Hyphomicrobium* strain M3	37
4	Ethidium-bromide stained gel of PCR amplified 16S rDNA from April sludge sample	39
5	Ethidium-bromide stained gel of PCR amplified 16S rDNA from clones of PCR products	41
6	Restriction patterns of 16S rDNA clones obtained from April sludge sample	43
7	Similarity ranking (SSU-prokaryotic rRNA database) of one of the cloned 16S rDNA sequences (apr19) from the April sludge sample..	45
8	Phylogenetic tree showing relationships of the nucleotide sequences of the 16S rDNA clones obtained from the activated sludge sample..	46
9	Regions used to edit all the three *Hyphomicrobium* M3 sequences ..	51
10	Light micrograph and Transmission electron micrographs of unidentified microorganisms associated with activated sludge	56
11	Scanning electron micrographs of unidentified budding bacteria in activated sludge, resembling *Hyphomicrobium* species, attached to filaments	57
12	Micrographs of fluorescent in situ hybridization	58
13	Micrographs of in situ hybridization of mixture of *Hyphomicrobium* M3 and reference strains with fluorescently labeled *Hyphomicrobium* probe showing mismatch discrimination	62

14 Micrographs of in situ hybridization of mixture of *Hyphomicrobium* M3 and *Sphingomonas capsulata* with fluorescently labeled *Hyphomicrobium* probe 64

LIST OF TABLES

Table	Contents	Page
1	16S rRNA sequencing primers and their specificity	21
2	Concentrations of total DNA extracted from January, April and November sludge samples	37
3.	DNA concentration of November sludge sample extracted by regular and freeze-dried method, and purified by Qiagen protocol as measured by Spectrophotometer	40
4	Cloning results showing the percentage of white colonies bearing 1500 bp 16S rDNA plasmid insert derived from PCR amplified 16S rDNA extracted from different sludge samples	41
5	Evaluation of different methods of purification of PCR products to optimize cloning	42
6	Representative organisms present in the sludge samples analyzed by 16S rDNA analysis	48
7	The distribution of organisms in January, April and November sludge samples showing the shift in bacterial populations	49
8	Comparison of Align Sequence and Similarity_Rank results for 3M3 and 6M3 clones of *Hyphomicrobium*	53
9	Comparison of pairwise evolutionary distances between 16S rDNA sequences of *Hyphomicrobium* M3(Eastman isolate) and reference strains of *Hyphomicrobium* vulgare and *Hyphomicrobium*-like organism with its closest relatives in the library as determined by phylogram	54
10	Target sequence of various organisms used to check the specificity of the *Hyphomicrobium vulgare* probe	61
11	Hybridization of genomic DNA of control strains with the *Hyphomicrobium vulgare* probe	65

LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetate
EMBL	European Molecular Biology Laboratory
FISH	fluorescent in-situ hybridization
GCG	Genetics Computer Group
KOH	potassium hydroxide
LB	Luria Bertani
NaOH	sodium hydroxide
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rDNA	ribosomal deoxyribonucleic acid (DNA coding for rRNA)
rRNA	ribosomal RNA
SDS	sodium dodecyl sulfate
SEM	scanning electron microscopy
SSU	small subunit
TBE	Tris Borate EDTA
TE	TRIS ethylenediaminetetraacetate

TEM	transmission electron microscopy
USDA	United States Department of Agriculture

CHAPTER I

INTRODUCTION

The activated sludge process is an important and widely used biological means to remove pollutants from manufacturing process wastewater. However, sludge separation problems caused by bulking often increase operating costs and may have potential adverse effects on the environment due to poor effluent quality. The role of filamentous organisms such as *Sphaerotilus* spp, Type 021N, *Thiothrix*, *Microthrix parvicella*, *Haliscomenobacter hydrosis*, *Nostocoida limicola*, *Herpetosiphon* sp. and nocardioform actinomycetes in the deterioration of activated sludge settling properties has been reported (Wanner et al., 1989, Wagner et al., 1994, Bradford et al., 1996, Schuppler et al., 1995). However, the degree of bulking problems differ in characteristics according to the type of filamentous microorganisms present. Also, non-filamentous microorganisms like *Zoogloea* spp. can cause viscous bulking problems in activated sludge (Shin et al., 1993). To rectify bulking problems it is important to identify the causative organisms and provide control measures to prevent their recurrent outgrowth. Scientists from Eastman, in collaboration with researchers at the Center for Environmental Biotechnology, University of Tennessee, Knoxville TN are investigating the molecular based microbiological approaches for identifying and monitoring causative organisms in activated sludge process upsets.

Our understanding of the microbial communities in the environment is very limited owing to the fact that many of the microorganisms found in the environment are poorly culturable. This may be due, in part, to difficulties in laboratory simulation of various metabolic associations among community members in the natural ecosystem. Traditional methods of characterizing microorganisms based on their phenotypic characteristics requires prior cultivation. It has been estimated that this method could select species representing only 0.001- 0.1% (sea water), 0.25% (fresh water), 0.25% (sediments), 0.3% (soil), 0.1-1% (mesotrophic lake), 0.1-3% (unpolluted estuarine water), 1-15% (activated sludge) of the total bacterial community (Amann et al., 1995). Only a small percentage of microorganisms identified morphologically using staining technology can form colonies on common nutrient agar media (Aa et al., 1996; DeLong et al., 1989). Immunofluorescence technique used to identify microorganisms requires prior isolation of pure cultures to raise specific antibodies (Campbell et al., 1983). Recent molecular methods in phylogenetic characterization of microbial communities in complex environment based on the analysis of rRNA sequences (Woese et al., 1987) has produced promising results in the identification of uncultivable organisms and has revolutionized the world of microbial systematics.

Ribosomal RNAs are ubiquitous constituents of ribosomes which are present in high numbers within the cell. The abundance of rRNA molecules (5S, 16S, 23S) in ribosomes (more than 10^3 molecules each in growing bacterial cells) (Amann et al., 1996), and the presence of phylogenetically conserved sequences interspersed with regions of great sequence variation, enables them to be useful tools for the construction of

oligonucleotide probes or amplification primers for the identification of individual cells of various phylogenetic levels ranging from species-specific to universal. 16S rRNA is preferred over 5S rRNA, as the information retrieval from 5S rRNA (~120 nucleotides) is relatively small compared to 16S rRNA which has ~1600 nucleotides (Olsen et al., 1986). Also, it has been found that the 16S rRNA molecule contains a sufficient range of conserved and variable regions to allow the development of a complete taxonomic system.

Extraction of DNA from environmental samples allows for the retrieval of rRNA sequence information by using the polymerase chain reaction (PCR) to amplify the sequence of 16S rDNA (DNA coding for 16S rRNA) with primers complementary to the phylogenetically conserved regions of the target molecule. After confirming the presence of 16S rDNA through agarose gel electrophoresis, the PCR products are cloned and sequenced. Alignment of cloned sequences with a set of published sequences of several reference bacteria in the existing sequence databases allows computation of evolutionary distance for all pairs of organisms (or sequences), and a phylogenetic tree is inferred by considering the relationship between these distance values (Stackebrandt et al., 1991).

With the assumption that the organisms share properties common to its phylogenetic group, the biochemical nature of microorganisms is known by its phylogenetic characterization (DeLong et al., 1989). Since retrieval of rDNA sequences involves various steps (Figure 1), there is a possibility to introduce bias in the analysis. Therefore, it is important to obtain a representative cross section of the microbial community.

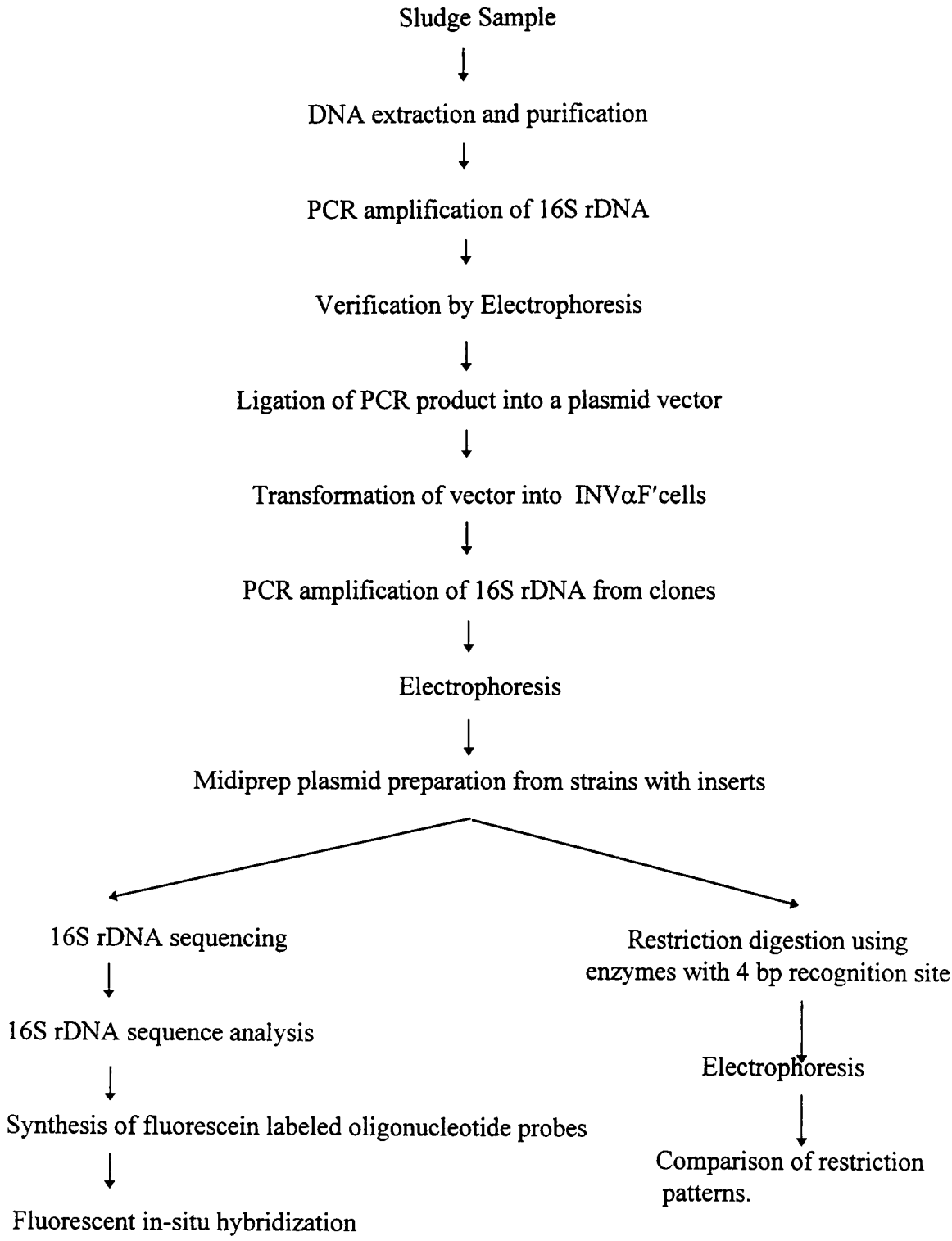


Figure 1. Methodology to identify microorganisms by 16S rRNA analysis of sludge samples.

It is essential to authenticate the presence of retrieved sequences within the cells present in the environmental sample. This can be achieved by hybridization with nucleic acid probes (Sayler et al., 1990; Stackebrandt et al., 1991). By comparative sequence analysis, potential probe target sites are determined and hybridization probes complementary to the target site are synthesized. The synthesized probe is then compared with all the available rRNA sequences in the RDP database (Maidak et al., 1997) and evaluated against known cultured strains with complementary sequences. Targeting the diagnostic probes to the signature sequences in the variable region of 16S rDNA would eventually lead to characterization of unculturable organisms.

In filter hybridization, DNA from unknown strains or environmental samples are extracted and hybridized to filters, and probed with radioactively labeled rRNA oligonucleotide probes and visualized by autoradiography (Giovannoni et al., 1988, Sayler et. al 1990). This method is time consuming and formation of silver grains away from the target cell prevents the localization of small cells in clusters. Fluorescent microscopy enables one to identify whole cells directly on microscopic slides (Stackebrandt et al.,1991), thereby avoiding the extraction of DNA from unknown organisms. Fluorescent probes have found successful applications in aquatic ecosystems, activated sludge and biofilms (Amann R.I., 1995b).

Microbial diversity can also be determined based on the restriction digestion of individual 16S rDNA sequences amplified from a community by PCR (Cardarelli-Leiti et al., 1996; Martinez-Murcia et al., 1995; Moyer et al., 1994; Yoon et al., 1997). The analyses of specific patterns of restriction fragments on agarose gel for each individual

organism reveals the diversity of organisms in a complex environment. In addition to providing a rough estimation of biodiversity, this method can detect major shifts in species composition in natural ecosystems (Wagner et al., 1993) more rapidly and inexpensively than 16S rRNA sequencing.

The effectiveness of molecular methods has been well established for the analysis of microbial communities in activated sludge. Organisms associated with foaming and bulking problems in activated sludge have been characterized by 16S rRNA analysis (Schuppler et al., 1995; Wagner et al., 1994) and by in situ identification with fluorescently labeled oligonucleotide probes (Wagner et al., 1994; Wagner et al., 1996; Reyes et al., 1997). However, probes specific for microorganisms potentially involved in bulking problems at the Eastman Chemical Company activated sludge plant have not been developed, and the use of molecular diagnostics for activated sludge process problems is still in its infancy. Therefore, our objective of creating a set of oligonucleotide probes for rapid identification and enumeration of organisms directly in the sludge sample should play a key role in understanding the microbial basis of activated sludge process upsets.

The purpose of the research described in this thesis was to:

1. Characterize the microbial populations of the activated sludge system at Eastman Chemical Company.
2. Analyze sequences of randomly chosen 16S rDNA clones to determine the evolutionary relationships between organisms in the activated sludge and create

oligonucleotide probes specific for potential causative organisms in sludge quality problems.

3. Determine shifts in the microbial populations by comparing the restriction patterns of the 16S rRNA molecules and the 16SrRNA sequences amplified from samples of sludge collected during different months.
4. Identify organisms directly in sludge samples by fluorescent in-situ hybridization to confirm the results obtained from the analysis of 16S rRNA sequences.

CHAPTER II

LITERATURE REVIEW

Activated Sludge Process:

Domestic and industrial wastewaters contain compounds that may produce an undesirable oxygen demand or are toxic to the aquatic life in receiving waters (where the wastewater is discharged). Activated sludge treatment of wastewater is an important biotechnological process (Figure 2) in which the pollutants of the influent are removed in the aeration basin by activated biomass via degradation or adsorption, and the biological solids are separated from treated wastewater in a clarifier by flocculation and gravity settling. The treated wastewater (effluent) is then discharged into the receiving waters.

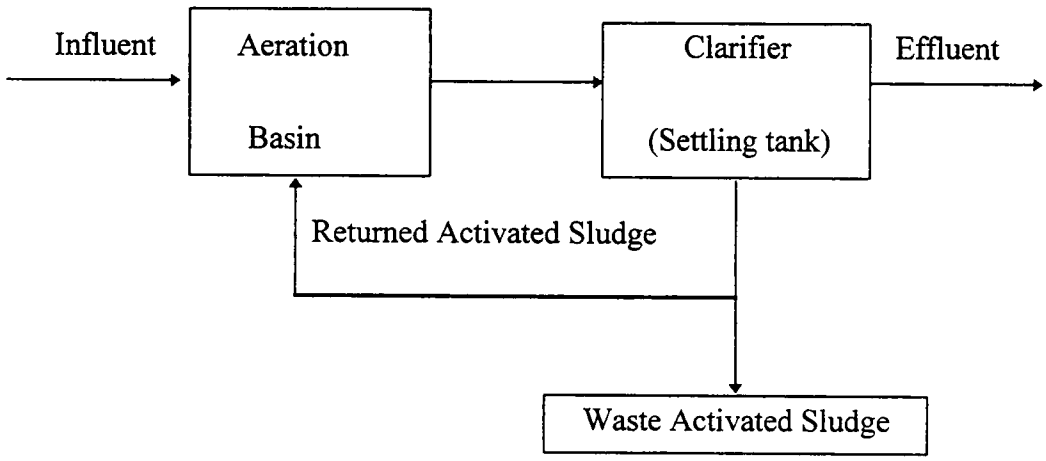


Figure 2: Basic concept of activated sludge treatment.

Role of Microorganisms in Sludge:

Microorganisms grow and reproduce by using organic chemicals present in the wastewater influent as growth substrates. The growing microorganisms are mixed with air in the aeration basin to provide oxygen needed to biodegrade the organic material in the influent. The mixed liquor (mixture of activated sludge and wastewater in the aeration basin) grows as flocs that can be removed by gravity sedimentation in the clarifier. High concentration of microorganisms in the aeration basin are maintained by recycling the settled biological flocs, thereby allowing effective treatment at high hydraulic loading.

The basis for floc formation is the ability of the microbes to adhere and bioflocculate, due to production of extracellular polymer consisting of various types of polysaccharides. Lack of floc formation is referred to as dispersed growth (microorganisms distributed throughout without forming flocs). Other sludge separation problems are viscous bulking (flocs having jelly like consistency), pinpoint floc (small, weak and slow settling flocs), filamentous bulking (filamentous organisms extending from flocs into the bulk solution), and sludge rising (floating flocs due to denitrification) in the clarifier (Jenkins et al., 1986). Therefore, the efficient treatment of waste water depends on the proper functioning of both the aeration basin and clarifier. Process control problems lead to separation problems, resulting in negative effects on the cost of treatment and quality of the treated water. Although coagulants such as aluminum sulfate, ferric chloride and polymer additives are used to ameliorate settling problems, process control changes based on an understanding of the activated sludge microbial populations can provide more cost-effective and long term solutions.

Characterization of Activated Sludge with Respect to Settling Problems:

In order to identify microorganisms, one has to classify them into groups called taxa (Woese 1987). The conventional method of identification and grouping of microorganisms (Jannasch et al., Kampfer et al., 1996) is based on their isolation from mixed cultures and comparison with standard references such as *Bergey's Manual of Determinative Bacteriology* using morphological, biochemical and physiological tests (Wanner et al., 1989) e.g., morphology, staining properties, pigmentation, nutritional requirements (Pace et al, 1996, Stackebrandt et al.,1988). Commercial kits for bacterial counting or bacterial viability are also available (Kampfer et al., 1996). It is difficult to characterize and identify microorganisms by these methods from a complex environment for the following reasons: 1) the cultivation of microorganisms is time-consuming, and it is difficult to simulate various metabolic associations, 2) the detection and phylogenetic characterization of organisms based on morphological characteristics is often difficult without further biochemical and physiological tests which requires cultivation of the organisms, 3) it is difficult to compare the isolates without the availability of pure cultures, 4) the viable plate count or most probable number techniques select certain organisms, 5) there is always a discrepancy between direct microscopic counts and viable cell counts as direct microscopic methods reveal microorganisms regardless of their growth requirements and without any indication of viability(Amann et al., 1995), plus particulate matter could simulate the appearance of microorganisms, 6) commercial kits with nutrient-rich media, high incubation temperatures and short incubation time usually fail to yield positive identifications for environmental organisms (Kampfer et al., 1996)

Advent of Molecular Techniques:

Novel molecular methods such as 16S rRNA analysis (Britschgi et al., 1991, Schmidt et al., 1991), restriction fragment length polymorphism (RFLP) (Moyer et al., 1994), polymerase chain reaction (PCR) (Voytek et al., 1995), quantitative PCR (Lee et al., 1996), fluorescent in situ hybridization (FISH) (DeLong et al, 1989) , and *in vitro* transcription of 16S rRNA (Polz et al.,1997) were developed to classify and estimate abundance of uncultured organisms based on genomic relatedness wherein information obtained from nucleic acids were used for the classification, identification and typing of organisms (Schlefer et al., 1983).

Organisms are phylogenetically classified, based on the rRNA data into three major domains: Archae (formerly archaebacteria), Bacteria (eubacteria), and Eucarya (eucaryotes) (Woese et al., 1977, Woese et al., 1980, Woese, 1987). The rRNA approach used to study natural marine bacterioplankton (Britschgi et al.,1991) and picoplankton (Schmidt et al.,1991) gained popularity in recent years for cultivation independent study of microbial communities in complex environments. 16S rRNA genes retrieved from total DNA extracted from complex environments were amplified by PCR, and the amplified sequence were cloned and sequenced. The information obtained from sequence analyses were used to define phylogenetic groups based on sequence similarities (signatures), and the isolates were placed in bacterial taxa by signature analysis (Woese, 1987). The properties of uncultivable microorganisms were inferred from sequence similarity with known phylogenetic groups. Organisms were identified in situ using oligonucleotide probes designed from these sequence comparisons.

Ribosomal RNA as a Valuable Tool for Phylogenetic Studies:

Ribosomal RNA is a valuable tool for phylogenetic studies (Olsen et al., 1986; Ward et al., 1992 Woese, 1987) because 1) ribosomal RNAs (5S, 16S and 23S rRNA) are ubiquitous constituents of ribosomes and ribosomes (the protein factories of the cell) have almost equal number (10^3-10^5) of ribosomal RNA in all living cells; 2) there is a direct correlation between the number of ribosomes and the growth rate of a cell; and, 3) the presence of phylogenetically conserved sequences between regions of larger sequence variation enable the construction of hybridization probes or amplification primers of any desired specificity ranging from species-specific to universal. Probes are designed by comparing the 16S rDNA sequences of the desired target strain with rRNA sequences of known organisms in existing databases.

Although 5S rRNA was used initially, the limited information available from ~20 nucleotides and the necessity for electrophoretic separation of different 5S rRNA molecules has limited its use for phylogenetic placements of microorganisms from mixed samples. It is hard to detect minor constituents of communities with the analysis of less abundant rRNAs. (Schmidt et al., 1991). The presence of ~1500 and ~3000 nucleotides in 16S and 23S rRNA, respectively, has been found to contain sufficient information for the development of a practical classification method (Giovonnani et al., 1988); however, of the two, 16S rRNA has been more widely used.

Extraction of Total DNA from a Complex Environment:

Extraction of total DNA from a complex environment involves lysis of cells to release DNA or RNA from the cells, and separation of DNA or RNA from other cellular constituents. This must be followed by a purification step if the DNA is to be used for various molecular studies (Sayler et al., 1985) . There are limitations in removing cells from a complex environment. Cells could avoid detachment by firmly attaching to the sediment (Gray et al., 1996) and could create bias in subsequent phylogenetic analysis as they pellet out with sediment during low-speed centrifugation. Microorganisms are detached and washed from soil using procedures involving blending and sonication of soil in pyrophosphate solution (Balkwill et al., 1977). The efficiency of sodium pyrophosphate in solubilizing soil aggregates has been demonstarted (Elsas et al., 1997).

Cells are disrupted chemically by sodium dodecyl sulfate (SDS) plus heat, lysozyme, osmotic shock, NaOH, proteinase K or peptidase or physically by bead mill homogenization, French press, boiling, microwave treatment, freeze-thaw cycles, or sonication (Gray et al., 1996; More et al., 1994). Physical, combined with chemical disruption, has been shown to be an efficient method for extracting total DNA from a complex environment.

Selective Amplification of 16S rRNA Gene Fragments from Total DNA by Polymerase Chain Reaction:

The ability to amplify a single DNA molecule from a complex mixture of genomic sequences using two primers has enabled researchers to use PCR extensively in

environmental studies to recover rRNA genes for analysis of populations from naturally occurring communities. It involves three major steps: denaturation, annealing, and extension. *Taq* polymerase [thermostable DNA polymerase isolated from the thermophilic bacterial species *Thermus aquaticus* (*Taq*)] is the enzyme used to amplify DNA as it can withstand repeated exposure to the high temperatures (94°C) required for the separation of double stranded DNA (Mullis et al., 1986).

Cloning of 16S rRNA Genes:

The TA cloning kit (*Invitrogen*, CA) provides a quick cloning strategy for the insertion of PCR product into a plasmid vector. It utilizes a nontemplate-dependent activity of *Taq* polymerase which adds a single deoxyadenosine (A) to the 3′ ends of PCR products. The linearized vector provided with the kit has single 3′deoxythymidine (T) residues which allows PCR product to ligate efficiently with the help of the enzyme DNA ligase (*Invitrogen* Instruction Manual).

Comparative Analyses of 16S rDNA Sequences :

The database on small ribosomal subunit RNA (SSU rRNA) contains ~ 6000 published nucleotide sequences that are stored in the form of an alignment (Maidak et al., 1997; Van de Peer et al., 1997). New sequences present in the database are classified based on evolutionary trees that are constructed by aligning the new sequences with closely related sequences retrieved from the European Molecular Biology Laboratory

(EMBL) and/or GenBank nucleotide sequence libraries. The new species are assigned to one of the taxa according to the phylogenetic position observed.

Biases in 16S rDNA Sequence Retrieval:

Since retrieval of 16S rDNA involves various steps, care should be taken in making inferences based on the results obtained from sequence analysis.

The introduction of bias may start from the sample collection itself. The potential biases that could be introduced during analysis are as follows; 1) not all microorganisms present in the sample lyse equally well during the initial extraction (Ogram et al., 1987), 2) PCR amplification may result in the selection of certain rRNA genes (Reysenbach et al.,1992), 3) contamination during PCR may result in clones that are not actually present in the original sample, 4) PCR amplification of mixed cultures may form chimeric products (Amann et al., 1995; Liesack et al.,1991; Schuppler et. al., 1995) by premature termination of elongation during PCR or by amplification of low molecular weight DNA caused by shearing action during extraction procedure or by annealing of different rDNAs in highly complementary regions, 5) less common organisms (abundance below 1 in 1000) may not be represented in the resulting 16S rDNA library, 6) the nucleotide sequence of more than a single 16S rRNA gene from one eubacterial genome may differ from each other (Nubel et al., 1996), 7) reannealing of templates may prevent the formation of template-primer hybrids during PCR(Suzuki et al., 1996), and 8) SSU rDNA sequences may not be available in the sequence database for many cultivable strains (Suzuki et al., 1997).

In situ Hybridization Techniques for the Identification of Natural Bacteria:

All these above mentioned problems make it difficult to quantify microbial communities from 16S rRNA analysis as they could select organisms that are not actually present in the sample (contaminants) or provide a skewed indication of population distribution. Therefore, it is necessary to confirm the presence of the retrieved sequences in the original sample from which the DNA was extracted. There are several commonly used approaches for probing natural communities. Applications of DNA hybridization techniques in environmental analyses has been extensively reviewed by Sayler (1990). Information on community diversity was obtained by hybridization with nucleic acid probes (DeLong et al., 1989; Giovannoni et al., 1988; Amann et al., 1995b). Cells were made permeable to probes for hybridization with target rRNAs. Group-specific, radioactively labeled oligonucleotide probes were used in combination with autoradiography to identify single microbial cells (Giovannoni et al., 1988). Nucleic acids extracted from samples were immobilized on membranes and hybridized with radioactive probes. Quantification of certain populations was done by comparing the signal conferred by probes binding to the target rRNA to that conferred by a universal probe. This method was time-consuming and compromised resolution due to long exposure of probes to photographic emulsions which scattered the radioactive disintegration. Fluorescently labeled probes were developed to identify single cells by fluorescence microscopy (DeLong et al., 1989). Genus- and species-specific probes have been developed for filamentous bacteria (Wagner et al., 1994) in activated sludge. Flow cytometry in combination with fluorescent probes has been used to analyze mixed microbial

populations (Amann et al., 1990b). With the advancement in quantitative fluorescence in situ hybridization (Amann et al., 1995, Langendijk et al., 1995) there is a potential for this technique to provide rapid and reliable identification and quantification of microorganisms in complex microbial communities.

CHAPTER III

MATERIALS AND METHODS

Samples :

Activated sludge samples were collected from the Eastman Chemical Company wastewater treatment facility in Kingsport, TN for the months of January, April, and November, 1995.

DNA Extraction and Purification:

DNA from the sludge was extracted by a modified Ogram DNA extraction procedure (Ogram et al., 1987). Sludge samples (150 ml) were centrifuged at 6000×*g* in JA-14 fixed angle rotor in a J-21 series Beckman Centrifuge for 10 minutes at 10°C. Cell pellets obtained were treated with 50 ml of 0.12M sodium pyrophosphate buffer ($Na_4P_2O_7.10\ H_2O$), pH 8.0 and 2.5 ml of 5%(w/v) sodium dodecyl sulfate ($C_{12}H_{25}OSO_3Na$), then incubated in a 70°C for water bath one hour with inversions by hand every ten minutes. The bacteria were then mechanically disrupted by using 5g of 0.1 mm glass beads (approximately a 1:1 ratio of sample and glass beads) in a bead beater for 5 min with a pattern of 2 minutes ON, 1 minute OFF and 2 minutes ON. The sample was then cooled on ice, and the sample/glass bead mixture was centrifuged at 6000 × *g* for 25 minutes at 10°C and the supernatant recovered. Further recovery of DNA was done by successive extractions of the sediment with 0.12M sodium pyrophosphate, pH 8.0 by

adding 50ml of the buffer and centrifuging as above. Supernatants were pooled. Extracts were precipitated at -20°C with 0.1 volume of 2M sodium acetate ($NaC_2H_3O_2.3H_2O$) and 0.8 volume isopropanol. The precipitates were centrifuged (10,000×*g* at 4°C for 30 minutes) and dried using a Savant Speed Vac Concentrator to remove the residual alcohol. The dried sample was resuspended with TRIS ethylenediaminetetraacetate (TE) [10mM Tris, 1mM EDTA (pH 7.5-8.0)] buffer , dialyzed (to remove impurities such as salts) overnight in dialysis tubing (Mol wt cut off 6000-8000) against 1X (TE) buffer and extracted with phenol followed by chloroform/iso-amyl alcohol (24:1) to denature proteins. Sodium acetate (0.1 volume of 2M solution) was added, and the sample incubated at -20°C to precipitate most of the humic-like materials (Ogram et al., 1987). Following centrifugation, the supernatant was removed and two volumes of absolute ethanol were added to precipitate the purified DNA. The samples were centrifuged (10,000×*g* for 30 minutes at 4°C and dried in a speed vac to remove the ethanol. The dried sample was resuspended in 1ml sterile TE buffer. DNA was further purified using the Elu-Quick DNA purification kit (Schleicher and Schuell, Keene, NH). The purified DNA was treated with RNAse, and subsequently used for PCR amplification.

The optical density, at 260nm, of a pure solution of double-stranded DNA at 50 μg/ml is 1.0 (Becker et al., 1990). Therefore, DNA concentration was estimated by measuring the absorption at 260nm using a Beckman DU-70 spectrophotometer.

Qiagen Protocol for the Purification of Total DNA:

DNA extracted from sludge samples by the freeze-dried method was purified by the Qiagen protocol (Qiagen, Chatsworth, CA). 10 ml of equilibration buffer (QBT) containing NaCl, 750mM; MOPS, 50 mM; ethanol, 15%; triton X-100, 0.15%, pH 7.0 was passed through the Qiagen column. DNA, resuspended in 10 ml TE buffer was then passed through the column. The column was then washed thrice with 15 ml of wash 1 buffer (NaCl, 50 mM; MOPS, 50mM; ethanol, 15%; pH 7.0), once with 15ml of wash 2 buffer (NaCl, 1000mM; MOPS, 50mM; ethanol, 15%; pH 7.0) and once with 15 ml of wash 3 buffer (NaCl, 1600mM; MOPS, 50 mM; ethanol, 15%; pH 7.0). The elutate was saved after each wash step, and the DNA precipitated with 0.7 volumes of isopropanol, and centrifuged for 30 min. The pellets were washed with 70% ethanol, dried and resuspended in 1ml TE buffer for further analysis. DNA concentration was measured in the absorbance range of 200 - 300 before and after purification.

PCR Amplification of 16S rDNA:

The DNA coding for 16S rRNA was amplified from the purified genomic DNA of the activated sludge sample by PCR using primers complementary to the conserved regions in the 5′ and 3′ regions of the 16S rRNA genes of most organisms. The primers 27f: 5′-AGA GTT TGA TCM TGG CTC AG-3′ and 1492r: 5′-TAC GGY TAC CTT GTT ACG ACT T-3′ (Table 1) were synthesized using an Oligo 1000 DNA synthesizer (Beckman, Fullerton, CA), and the synthesized primers were cleaved from the solid support in the reactor column. The protecting groups blocking the oligomers' reactive

Table 1: 16S rRNA sequencing primers and their specificity. Sequence for m13f (-40) and m13r were reproduced from the Invitrogen Manual. All other sequences were reproduced from Nucleic Acid Techniques in Bacterial Systematics, Stackebrandt et al., 1991. Primers 27f, 1492r and 1525r were used for the amplification of 16S rDNA from the total DNA. Primers 530f, 907r, 926f, 1492r, m13f (-40), and m13r were used for 16S rDNA sequencing.

Name	Sequence (5′ -3′)	Comments
27f	AGAGTTTGATC(C/A)TGGCTCAG	PCR and sequencing, most eubacteria
109r1	ACGVGTTAC(G/T)CACCCGT	Broad specificity
109r2	A(G/T)(A/G)CATTACTCACCCGT	Most gamma and some beta proteobacteria
342r	CTGCTGCS(C/T)CCCGTAG	Most eubacteria
357r	CTCCTACGGGAGGCAGCAG	Most eubacteria
519r	G(A/T)ATTACCGCGGC(G/T)GCTG	Most eubacteria, eukaryotes, archaebacteria
530f	GTGCCAGC(C/A)GCCGCGG	Most eubacteria, eukaryotes, archaebacteria
685r1	TCTACG(A/G)ATTTCACC(C/T)CTAC	Alpha and delta proteobacteria, fusobacteria
685r2	TCTACGCATTTCAC(C/T)GCTAC	All beta and gamma proteobacteria
685r3	TCT(A/G)CGCATT(C/T)CACCGCTAC	Most Gram-positive, cyanobacteria, some miscellaneous bacteria
907r	CCGTCAATTC(C/A)TTT(A/G)AGTTT	Most eubacteria, eukaryotes, archaebacteria
926f	AAACT(C/T)AAA(G/T)GAATTGACGG	Most eubacteria, eukaryotes, archaebacteria
1100r	GGGTTGCGCTCGTTG	Most eubacteria
1114f	GCAACGAGCGCAACCC	Most eubacteria
1392r	ACGGGCGGTGTGT(A/G)C	Most eubacteria, eukaryotes, archaebacteria
1406f	TG(C/T)ACACACCTCCCGT	Most eubacteria, eukaryotes, archaebacteria
1492r	TACGG(C/T)TACCTTGTTACGACTT	PCR and sequencing, most eubacteria, archaebacteria
1525r	AAGGAGGTG(A/T)TCCA(A/G)CC	PCR and sequencing, most eubacteria, archaebacteria
m13f(-40)	GTTTTCCCAGTCACGAC	
m13r	CAGGAAACAGCTATGAC	

phosporyl and amino groups were removed using the DNA Ultra Fast cleavage and deprotection kit . Amplification was performed in a 100µl reaction mixture (distilled water, 75µl; primers, 2.5µl each; dNTP's, 2µl each; buffer,10µl; template DNA, 2µl; *Taq* polymerase, 0.5µl) in an automated thermal cycler (Perkin Elmer-Cetus, Norwalk, CT) by denaturing samples at 99°C for 5 minutes and subsequent amplification by 38 cycles of denaturing (94°C/1minute), annealing (45°C/1minute) and elongation (72°C/1minute), plus an additional cycle with a final 10 minutes elongation at 72°C to increase the yield of PCR products and to reduce the faint smear observed on the gel directly below the PCR product (Promega, Madison WI). The amplified samples were stored at 4°C. A negative control with no addition of template DNA was also included.

Agarose Gel Electrophoresis of PCR Products:

PCR amplified products (20µl) and 2.5µl of loading dye [50% glycerol, 1mM EDTA (pH 8.0), 0.25% bromophenol blue and 0.25% xylene cyanol] were loaded in the wells and run on 1% (w/v) agarose gels in 1X TBE [prepared from 10X TBE stock solution (Tris base 108g; Boric acid, 55g; EDTA, disodium salt ($C_{10}H_{14}O_8N_2Na_2.2H_2O$), 0.5M; and distilled water, 1 liter; pH 8.0)] for approximately 2 hours at 80V (Sambrook et al, 1989) to check for the presence of an approximately 1500 bp 16S rDNA fragment by comparing it with DNA molecular weight markers run on the same gel. The gels were then stained with ethidium bromide (0.5µg/ml) for 20 minutes and destained with de-ionized water for 20 to 30 minutes. The destained gels were then visualized on a Foto UV

300 DNA transilluminator (Fotodyne, New Berlin, WI), and the ethidium bromide-DNA complex was photographed with a Polaroid camera. Several approaches were compared for the further purification of the amplified 16S rDNA sequences as described below.

Agar*ACE* Protocol for the Purification of PCR Amplified 16S rDNA:

The PCR amplified 16S rDNA was further purified by Agar*ACE* protocol (Promega, Madison, WI). PCR amplified products were run on 1%(w/v) agarose gels in 1X TBE solution for approximately 2 hours at 80V. A slot was cut in front of the 1500 bp 16S rDNA fragment, and the slot was filled with 1.8% LMP agarose. The gel was run again at 80V until the required band ran into LMP agarose which was cut and melted in an Eppendorf tube at 65°C. One unit of Agar*ACE* (5µl) was added to the melted agarose and was incubated overnight at 65°C. The Agar*ACE* treated PCR products were purified by phenol/chloroform extraction and precipitated for 2 hours in 0.1 volume sodium acetate and 2 volume ethanol. The precipitated products were centrifuged (4°C) at 14,000 rpm for 20 minutes and dried in a speed vac for 20 min. The dried sample was resuspended in 200µl TE buffer and used for further analysis.

Spin Column Protocol for the Purification of PCR Amplified 16S rDNA:

PCR amplified products were run on 1% (w/v) agarose gels in 1X TBE solution for approximately 2 hours at 80V. The 1500 bp 16S rDNA fragment was cut and stored overnight in the refrigerator in a vial provided with a filter at the top. The PCR product

was then centrifuged at 14,000 × *g* for 15 minutes at 4°C. The top filter was removed after collecting the liquid in the bottom of the tube. Potassium acetate (0.1 volume) and ethanol (3 volumes) were added to the estimated volume in the bottom tube and stored in a freezer for at least 30 minutes. The precipitated sample was then centrifuged (4°C) at 14,000 × *g* for 15 minutes, washed with 70% ethanol and dried in speed vac. The dried sample was resuspended in 10µl sterile TE buffer and used for further analysis.

Electroelution Protocol for the Purification of PCR Amplified 16S rDNA:

PCR amplified products (35µl each) were run on 0.8% agarose gel at 80V in two wells side-by-side. The gel around the 1500 bp band was cut and transferred into a dialysis bag containing 1ml TE buffer. The gel was run again until the desired band in the excised gel moved into the TE buffer. TE buffer containing the PCR products was used for further analysis.

Cloning of 16S rRNA Genes:

16S rDNA fragments amplified by PCR were ligated into a plasmid vector by using the Original TA Cloning kit (Invitrogen, San Diego, CA). The reaction mixture (10µl) consisting of distilled water,5µl; ligation buffer, 1µl; vector, 2µl; template DNA, 1µl; and DNA ligase, 0.5µl, was incubated overnight at 12°C, the INVαF′ competent cells were transformed with the plasmids using the one-step TA cloning strategy. Frozen one shot competent cells (50µl) were mixed with 2µl of 0.5M β-mercaptoethanol and 2µl

of ligation reaction mixture. The reaction mixtures were incubated on ice for 30 minutes, and heat-shock treatment was given to the cells for exactly 45 seconds (subsequently changed to 30 seconds by the manufacturer) in a 42°C water bath; the cell suspension was then placed on ice for 2 minutes. Aliquots of cell suspension in 250µl SOC medium (room temperature) were plated onto Luria Bertani (LB) Agar medium (tryptone, 10g/l; yeast extract, 5g/l; NaCl, 10g/l; agar 17%, distilled water 1 liter, pH7.5) containing ampicillin (50µg/ml) and 25µl of 40mg/ml X-Gal, equilibrated at 37°C for 30 minutes, and incubated overnight at 37°C. Colonies were selected for analysis after the agar plates were transferred to 4°C for 2-3 hrs for proper color development (Invitrogen Instructions Manual).

PCR Amplification of 16S rDNA from Clones:

The white colonies picked using sterile toothpicks were streaked onto LB agar containing ampicillin (50µg/ml). The streaked colonies were then screened for plasmids containing full-length (1500bp) 16S rDNA inserts by PCR amplification of 16S rDNA using SP6 and T7 primers to distinguish between TA Cloning inserts and 16S rDNA from *Escherichia coli*. Primers were synthesized and purified as described earlier. The amplified products were run on 1%(w/v) agarose gel as before to verify that the positive clones had 16S rDNA plasmid inserts.

Restriction Digestion of the PCR Amplified Products:

The restriction endonucleases *Cfo*I, *Hae*III, and *Taq*I with 4 base pair recognition sites (Life Technologies, Inc. Gaithersburg, MD) were used to cleave the PCR amplified 16S rDNA gene sequences using React1, React2 and React2 buffers respectively. The 20µl reaction mixtures (PCR product, 17µl; buffer, 2µl; enzyme, 0.5µl) were incubated overnight at 65°C for *Taq*I digestion, and at 37°C for *Cfo*I and *Hae*III digestion. The digested samples were run on 1.5% Nusieve gel, stained with ethidium bromide, and DNA fragments were visualized by UV transillumination, and photographed as described earlier.

Midiprep Plasmid Preparation from Strains with Inserts:

Large amounts of plasmid DNA required for subsequent sequence analysis of 16S rDNA were prepared. The bacteria containing plasmids were grown on larger scale (125 ml), harvested, and lysed by a modified Promega midiprep plasmid preparation method. (Promega, Madison, WI).

Bacteria were grown overnight in 125 ml LB broth in the presence of kanamycin (25 µg/ml) at 37°C in a shaking incubator (Labline Instruments, Inc. Melrose Park, IL). Cell suspension (0.5ml) were preserved in cryotubes with 0.5ml of 30% sterile glycerol at -80°C, and the rest of each sample was centrifuged at 8,000×*g* for 10 minutes at 10°C. The pellets obtained were resuspended in 3ml of cold lysis buffer. Aliquots (6ml) of freshly prepared 0.2 N sodium hydroxide (NaOH), 1% sodium dodecyl sulfate

($C_{12}H_{25}OSO_3Na$) and 3.75 ml of 5M potassium acetate ($KC_2H_3O_2$) were added and the mixture was at 10,000×*g* centrifuged for 30 minutes at 10°C . Equal volumes of isopropanol were added to the supernatant, and the samples were centrifuged at 10,000×*g* for 30 minutes at 10°C. The pellet were washed with 70% ethanol and dried in a speed vac. The dried pellets were RNAse-treated and purified by phenol/chloroform extraction. The purified plasmids were dried, resuspended in 200µl of TE buffer and stored at -80°C.

16S rDNA Sequencing:

30µl of plasmid containing the insert was sent to Retrogen, Inc. (San Diego, CA) for sequencing of approximately 400 bp of the 1.5kb 16S rDNA molecule by the single primer extension method using the 1492r primer (Table 1). Full-length (1500 bp) 16S rRNA sequences amplified from an activated sludge isolate (*Hyphomicrobium* M3) obtained from Dr. Arthur Meyers at Eastman Chemical Company was determined at the Molecular Biology Resource Facility at the University of Tennessee (Knoxville, TN) using the primers m13f, 530f, 907r and m13r (Table 1). All primers were synthesized using an Oligo 1000 DNA synthesizer.

16S rDNA Sequence Analysis:

16S rDNA sequences were analyzed using different software packages. The sequences were ranked against all organisms in the small subunit prokaryotes (SSU-Prok) rRNA database using Niels Larsen's SIMILARITY_RANK software available in the

Ribosomal Database Project (Maidak et.al., 1997, Van de Peer et al., 1997). The Genetics Computer Group's (GCG) sequence analysis software package (version 8.1 on UNIX) was used to cluster sequences by similarity to produce a dendrogram. Multiple sequence alignments from a group of related sequences using progressive, pairwise alignment were created by using the pileup function. Multiple sequence format (msf) files were then analyzed to create oligonucleotide probes specific for the desired target strain.

Fluorescent in-situ Hybridization:

Organisms:

Hyphomicrobium strain M3, previously isolated from activated sludge at the Eastman Chemical Company wastewater treatment facility, was obtained from Dr. Arthur J. Meyers. *Hyphomicrobium MC-750* (ATCC 27500) and *Zoogloea ramigera* (ATCC 25935) were obtained from the American Type Culture Collection (ATCC). *Sphingomonas capsulata* (ATCC 14666) was obtained from Dr. David White's laboratory, Center for Environmental Biotechnology, University of Tennessee, Knoxville. *Bradyrhizobium japonicum* (USDA 110) was obtained from Dr. Gary Stacey's laboratory, Dept. of Microbiology, University of Tennessee, Knoxville. *Sphingomonas* sp. strain *A8AN3*, and *Escherichia coli* were obtained from the culture collection at the Center for Environmental Biotechnology, University of Tennessee, Knoxville.

Cultivation of Cells:

Hyphomicrobium strains were grown on ATCC culture medium 656 [KH_2PO_4, 1.36g/l; Na_2HPO_4, 2.15g/l; $(NH_4)_2SO_4$, 0.5g/l; $MgSO_4.7H_2O$, 0.2g/l; trace solution ($CuCl_2$, 0.15g; $FeSO_4.7H_2O$, 0.1g; $MnSO_4.H_2O$, 0.035g; $Na_2MoO_4.2H_2O$, 0.05g; distilled water, 100ml) 5.0ml/l; filter sterilized methylamine hydrochloride, 3.38g/l; Agar Noble (Difco 0142), 18.0g/l; distilled water, 1 liter; pH 7.1] at 30°C.*Bradyrhizobium japonicum* was grown on RDY medium [yeast extract, 1g/l; K_2HPO_4, 0.12g/l; $MgSO_4$, 0.1g/l; trace element (H_3BO_3, 3g/l; $MnSO_4.4H_2O$, 2.23g/l; $ZnSO_4.7H_2O$, 0.29g/l; $CuSO_4.5H_2O$, 0.125g/l; $CoCl_2$, 0.065g/l; $Na_2MoO4.2H_2O$, 0.12g/l; 1mM $FeCl_3$), 1ml/l; L-glutamate, 1.0g/l; Na-gluconate, 5.0g/l, distilled water, 1 liter; pH 7.0] at 30°C. *Sphingomonas capsulata* and *Sphingomonas* strain *A8AN3* were grown on Nutrient broth (8.0g/l peptone), pH 7.0 at room temperature. *Zoogloea ramigera* strain 25935 was grown on Stokes medium [Peptone, 5g; 100X stock ($MgSO_4.7H_2O$, 20g; NH_4SO_4, 7.5g; Sodium citrate, 10g; $CaCl_2$, 5g; $MnSO_4$, 5g; $FeCl_3.6H_2O$, 1g, $FeSO_4$, 7.5g; distilled water, 1 liter), 2ml; pH 7.2 at 30°C. *Escherichia coli* was grown on LB agar at 37°C.

Fixation of Culture Cells:

Cells were harvested during the exponential growth phase to optimize the rRNA content. Cultured cells (1.7ml) were spun at 14,000×*g* for 5 minutes. Aliquots (750µl) of the supernatant were removed and the bacterial cells were fixed with equal volumes of

4% paraformaldehyde (Sigma, MO) in 0.2M sodium phosphate buffer (pH 7.2) for 3-24 hrs at 4°C (Amann et al., 1990).

The incubated cells were sonicated for 5 minutes in an ice water bath and spun at 6,000×*g* for 5 min. Pellets were resuspended in 900µl 1X PBS (130mM NaCl in 30mM NaPO4 buffer, pH 7.2) buffer and 100µl 0.1% Igepal, then spun at 6,000×*g* for 5 minutes. Cells were resuspended again in 500µl 0.1% Igepal, vortexed, and spun at 6,000×*g* for 5 minutes. Pellets were resuspended in 200µl 2X storage buffer [40mM Tris pH 7.5, 0.2% Igepal CA-630 (Sigma, MO)], vortexed for at least 1 minute, and mixed with equal volumes of 96% ethanol and stored at -20°C.

Fixation of Sludge Samples:

Activated sludge samples (10 ml) were centrifuged at 2,000×*g* for 5 minutes in sterile corex tubes. One ml of the supernatant was pipetted to a 1.7 ml eppendorf tube, and centrifuged at 14,000×*g* for 5 minutes. Supernatant (750µl) was removed, and was replaced by an equal volume (750µl) of 4% paraformaldehyde (Sigma, MO) in 0.2M sodium phosphate buffer (pH 7.2) to fix the cells. The formaldehyde treated cells were mixed in a vortex mixer for 1 minute, and incubated for 3-24 hrs at 4°C.

The incubated cells were centrifuged at 14,000×*g* for 5 minutes, and the supernatant was replaced with 10mM EDTA. The cells were then sonicated for 10 minutes in an ice water bath and spun at 3,000×g for 5 min. Pellets were resuspended in 900µl 1X PBS (130mM NaCl in 30mM $NaPO_4$ buffer, pH 7.2) buffer and 100µl 0.1%

Igepal and spun at 3,000×*g* for 5 minutes. Cells were resuspended again in 500µl 0.1% Igepal, votexed and spun at 3,000×g for 5 minutes. Pellets were resuspended in 200µl 2X storage buffer, vortexed for at least a minute and mixed with equal volumes of 96% ethanol and stored at -20°C.

Hybridization:

Small aliquots (3µl) of each sample of fixed cells was placed on a poly-lysine coated slides, air-dried, dehydrated in an ethanol series(50, 80, and 100%) for 3 minutes each, and again air-dried. Hybridization solution (9µl) consisting of 5M NaCl, 1M Tris pH7.2, 10% SDS and distilled water, pH7.2, with various concentrations (0, 10, 25, 30, 35, 45 and 75%) of formamide [stock solution of formamide was prepared by stirring formamide (40ml) and 5g mixed-bed ion-exchange resin (25-50 mesh, Bio-Rad AG 501-X8) at room temperature for 30 minutes. The resin-treated formamide was filtered twice through Whatman No.1 filter paper] were added onto the fixed cells. The slides were transferred to a hybridization chamber with 1M Whatman paper equilibrated earlier with equilibration buffer (30% formamide, 0.9M NaCl, 0.1% SDS, 100mM Tris pH 7.2]. The slides were incubated at 37°C for 30 minutes (Poulsen et al., 1993). The Whatman paper was used to prevent drying during the hybridization process. The pre-hybridized cells were then exposed to 1µl of 25-50 ng/µl degenerate *Hyphomicrobium* probe [5′ GCT GC(C/G) CAT TGT CAC CGC C 3′] labeled with fluorescein (Genosys, The Woodlands, TX.) and incubated overnight at 37°C. The appropriate formamide concentration needed

to achieve the desired probe specificity was determined using formamide concentrations of 0, 10, 25, 30, 35, 45 and 75% .

Washing:

The hybridized cells were rinsed with distilled water, incubated in a coplin jar with 100 ml of appropriate concentrations of hybridization solution (pre-warmed to 37°C) for 20 minutes, rinsed with distilled water, and incubated in a coplin jar containing 100 ml of washing solution [4ml 5M NaCl, 10ml 0.5M sodium phosphate buffer(stock: 28ml 0.5M NaH_2PO_4 and 72ml 0.5M Na_2HPO_4), 1ml 10% SDS, 1 ml 0.5M EDTA and 84ml distilled water] at 37°C for 15 minutes. The washed slides were dipped in 100 ml of distilled water and air dried.

A drop of anti-fade solution (Vectashield mounting medium for fluorescence, H-1000, Vector Laboratories, Inc., Burlingame, CA) was added to the hybridized cells, and the cells were covered with a glass cover slip. The bacterial cells were visualized using an oil-immersion objective (1000X). Fluorescence was detected with a microscope fitted for fluorescence microscopy, and photographs were taken. Exposure times were 0.01- 0.05s for phase contrast and 20-40s for fluorescence micrographs.

Specimen Preparation for Microscopy Techniques:

Light Microscopy:

A thick section(1μm) was obtained from the embedded sample by cutting it with a glass knife using a Reichert Ultramicrotome. The section was then treated with a drop of 10% acetone on a gelatin coated glass slide. The slide was dried over a slide warmer and stained with 1% Toluidine Blue in 1% Borax and observed under a light microscope.

Negative Staining:

The sludge sample was settled on a glow discharged, carbon coated formvar grid for 1 minute, and was then treated with a drop of stain containing 0.25% phosphotungstic acid (pH 7.0 with KOH) and 2% uranyl acetate, pH 4.5 , dried and observed with a Hitachi H-600 transmission electron microscope.

Transmission Electron Microscopy (TEM)

Fixation and Embedding :

The sludge sample was fixed using 3% glutaraldehyde (in 0.1M phosphate buffer, pH 7.2) for 1 hour at room temperature and post-fixed with 5ml of 2% osmium tetroxide (in 0.1M phosphate buffer, pH 7.2) for 1 hour at room temperature. The fixed sample was

dehydrated sequentially in 50% ethanol (15 min.), 70% ethanol (15 min.), 95% ethanol (15 min.), 100% ethanol (20 min. x 3 changes), propylene oxide (15 min. x 2 changes), and 1:1 propylene oxide/Epon mix (overnight). The sample was embedded in a complete Epon mix in beem capsules and cured in an oven at 60°C for 2 days.

Ultrathin sections obtained from the embedded sample using an ultramicrotome were placed on a carbon coated grid and stained with uranyl acetate (saturated in 50% ethanol) followed by lead citrate (0.3% lead citrate in glass distilled water, pH 12.0) with appropriate washings. The sections were finally observed with a Hitachi H-600 transmission electron microscope.

Preparation of Plates:

Images captured with a Dage video camera (Model 65 with Nicon macro lens) were formatted digitally by a data translation capture card and were read into an NIH image. Images were electronically manipulated to create a plate on the computer, and the final products were printed on a Kodak XLS 8600 high resolution printer.

Scanning Electron Microscopy:

At the 70% dehydration step in the TEM procedure, some of the sample was quickly transferred to 12mm round cover slips that had been coated with 0.1% aqueous polylysine. Further dehydration of sample took place on the cover slips. Samples on cover slips were transferred to a Critical Point Dryer (Ladd Research Industries, Inc.,

#28000), using liquid carbon dioxide as a transition fluid. After critical point drying with palladium-gold, samples were examined in an ETEC Autoscan Scanning Electron Microscope and photographed using Type 55 P/N Polaroid Film.

CHAPTER IV

RESULTS

DNA Extraction and Purification:

DNA extraction from sludge samples was problematic. The samples which were originally brown, turned dark brown after bead beating, possibly due to the release of organic carbon from the combination of SDS and frictional heat (Ogram et al., 1987). DNA was further extracted by phenol/chloroform and concentrated by ethanol precipitation. Although the dark brown color was drastically reduced, the DNA sample still had a light brown color indicating the presence of contaminants, presumably humic materials. Therefore, DNA was further purified by using the Elu-Quick DNA purification kit to obtain an acceptable ratio of absorbance at 260nm and 280nm. RNAs in the sample were removed by RNAase treatment before the DNA was quantified using a UV-visible spectrophotometer (Becker et al., 1990). DNA yields were low for January and November sludge samples (Table 2), indicating either the presence of impurities in the samples or the degradation of DNA over a period of time after sampling.

PCR Amplification of 16S rDNA:

Following the standard PCR protocol, the results of amplification of the template DNA extracted from the sludge were variable. The fuzzy bands of DNA (Figure 3A) viewed by electrophoresis suggested that the samples had impurities. This was later

Table 2: Concentrations of total DNA extracted from January, April and November sludge samples.

Sample	Date of Sampling	Date of DNA Extraction	Concentration of Total DNA (μg/μl)	A_{260}/A_{280}
January	(01/20/95)	10/25/95	0.653	1.266
April	(04/13/95)	06/28/95	7.072	1.910
November	(11/14/95)	06/12/96	0.200	13.33

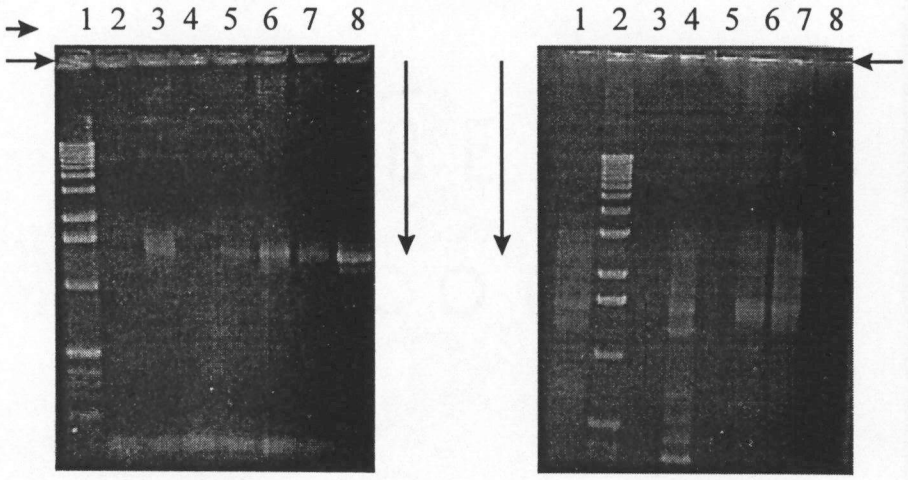


Figure 3: Ethidium-bromide stained gel of PCR amplified 16S rDNA from April sludge sample. (A) Purified DNA, diluted to various concentrations (1:10 in lanes 2, 4 and 6; 1:100 in lanes 3, 5 and 7) were used as a template in the reaction mixture. Different buffers (buffer B in lanes 2 and 3, buffer C in lanes 4 and 5, and buffer D in lanes 6 and 7) were used to optimize the amplification of 16S rDNA. Note the appearance of a band in the control lane (lane 8) indicating possible contamination of the sample. (B) Primer-dimers, false priming and incomplete amplification of 16S rDNA (lanes 1 and 4) were eliminated using different combinations of forward and reverse primers (diluted and undiluted). More clearly defined PCR products (lane 6) were used for cloning.

corroborated by easier amplification of 16S rDNA and cleaner separation on agarose gel when total DNA from pure culture was amplified by PCR (Figure 4). However, the use of the PCR optimizer kit eased the difficulty to some extent by indicating the proper buffer conditions to be used for amplification. The amplified products were run on agarose gel. More clearly defined DNA fragments of approximately 1500 bp was obtained using suitable buffer and were cloned (Figure 3B). The appearance of a band in the control lane indicated possible contamination of the sample since no template DNA was added to the reaction mixture. *AmpliTaq* DNA polymerase LD, (Low DNA) from Perkin Elmer was used to avoid the amplification of contaminant DNA in the control reaction.

This recombinant, thermostable DNA polymerase (a modified form of the *Thermus aquaticus* DNA polymerase gene inserted in *Escherichia coli*) is identical to *Ampli*Taq DNA polymerase but purified to a greater extent to reduce non-specific, false positive DNA products via the amplification of bacterial DNA introduced from the environment. The possibility of problems arising from the quality of DNA from sludge extracts was tested by extracting DNA through freeze-drying, and purifying the extracted DNA using either the Qiagen protocol (Table 3) or hexadecyl trimethyl ammonium bromide (CTAB) treatment (Elsas et al., 1997) (data not shown). These procedures did not yield improved results.

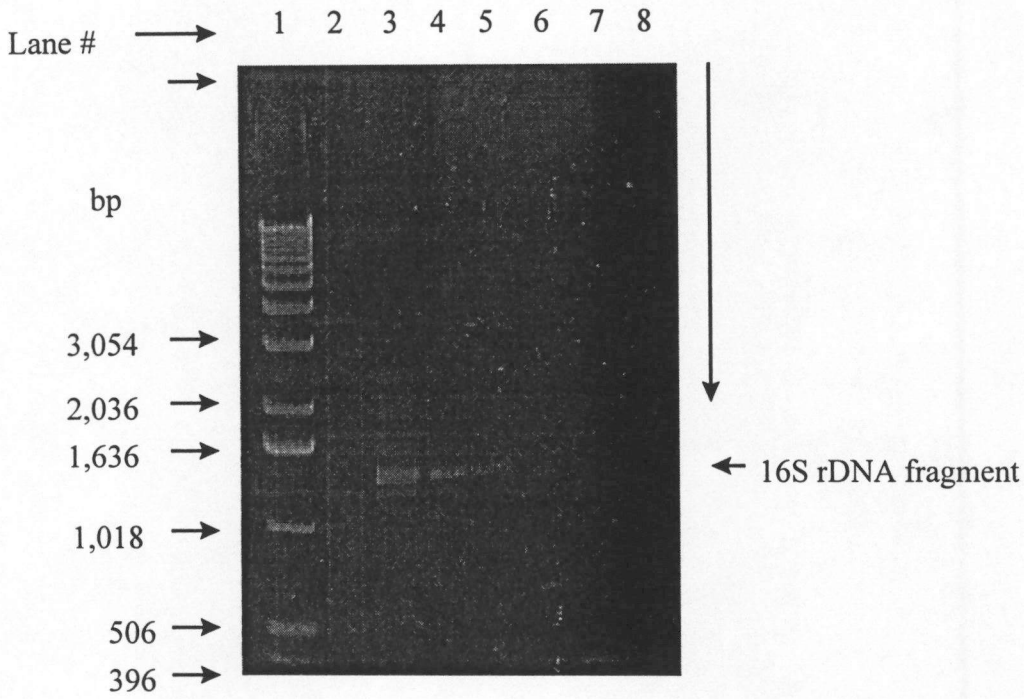


Figure 4: Ethidium-bromide stained gel of PCR amplified 16S rDNA of *Hyphomicrobium* strain M3. Purified DNA, diluted to various concentrations (1:10, lane 3; 1:100, lane 4; 1:1000, lane 5 and 1:10,000 lane 6) were used as a template in the reaction mixture. No bands were found for the reaction mixture containing undiluted DNA as a template (lane 2). More clearly defined PCR products (lane 4) were used for cloning. Negative control reaction was run on lane 7 and lane 8.

Table 3: DNA concentration of November sludge sample extracted by standard (modified Ogram's) method and freeze-dried method and purified by Qiagen protocol as measured using a spectrophotometer.

	Before purification		After purification by Qiagen protocol			
	Standard	Freeze-dried	Standard		Freeze-dried	
			Wash2	Wash3	Wash2	Wash3
A_{260}	0.16	0.2225	0.0137	0.0217	0.0191	0.0905
A_{280}	0.012	0.1121	0.0115	0.0130	0.0114	0.0497
A_{260}/A_{280}	13.33	1.985	1.191	1.67	1.675	1.82
DNA Conc (in μg/μl)	0.2	0.28	0.017	0.027	0.024	0.0113

Cloning of 16S rDNA Genes:

The PCR amplified 16S rDNA were cloned using the TA cloning kit (Invitrogen, San Diego, CA). Although sludge samples from different months were not processed simultaneously, it appears that the cloning efficiency of 16S rDNA was higher for the April sludge sample. The number of colonies containing inserts as determined by agarose gel electrophoresis (Figure 5) was much lower for the January sample (Table 4). Since the recovery of plasmids with inserts was lower than expected, different methods of purification of the PCR product (Electroelution, Spin-column, Agar*ACE* protcol) were evaluated in an attempt to optimize cloning. However, for unknown reasons, PCR product purification did not improve the cloning efficiency above that obtained with unpurified PCR product (Table 5). Also, cloning of 16S rDNA sequences following Agar*ACE* treatment did not improve transformation efficiency.

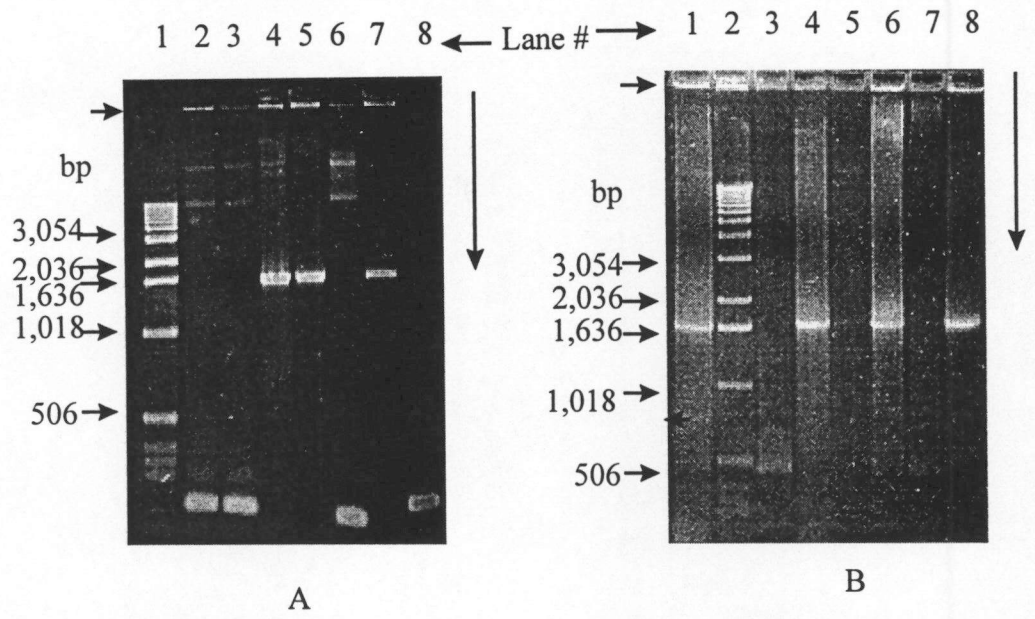


Figure 5: Ethidium bromide-stained gel of PCR amplified 16S rDNA from clones of PCR products. The positive clones containing 1500 bp 16S rDNA inserts (lanes 4, 5 and 7 in A and lanes 1, 4, 6 and 8 in B were selected for plasmid preparation and sequence analysis

Table 4: Cloning results showing the percentage of white colonies bearing 1500 bp 16S rDNA plasmid insert derived from amplified 16S rDNA extracted from different sludge samples.

Sample	Total number of white colonies checked for the presence of insert	Number of colonies having insert	% recovery $\frac{\text{No. of colonies with inserts}}{\text{No. of colonies screened}} \times 100$
January	353	27	8
April	69	32	46
November	78	23	29

Table 5: Evaluation of different methods of purification of PCR products to optimize cloning. Clones contained 16S rDNA inserts obtained from unpurified PCR product and from the amplified DNA purified by electroelution and spin-column. The percentage of white colonies bearing 1500 bp 16S rDNA inserts are shown.

Method	Total number of white colonies checked for the presence of insert	Number of colonies having 1500 bp insert	% recovery
Standard (unpurified)	17	09	53
Electroelution	36	11	31
Spin-column	39	11	28

RFLP Analysis:

Restriction fragment length polymorphism analysis of a PCR amplified 16S rDNA was performed on the clones containing inserts for rapid detection of biodiversity in the sample. The PCR restriction patterns obtained using three restriction endonucleases *Cfo*I, *Hae*III and *Taq*I for April clones are shown in Figure 6. A 100bp DNA ladder was used as a marker to size DNA fragments. Diversity in the bacterial population was observed from the restriction patterns. None of the cloned inserts produced the same restriction patterns. Although, the PCR products were run on 1.5% NuSieve gel for efficient separation of low molecular weight DNA as recommended by the manufacturer, fuzzy bands limited the accurate measurement of molecular weights. Restriction patterns obtained from the January sludge sample showed different banding patterns (not shown) from that obtained from the April sample (Figure 6) indicating a shift in the bacterial populations.

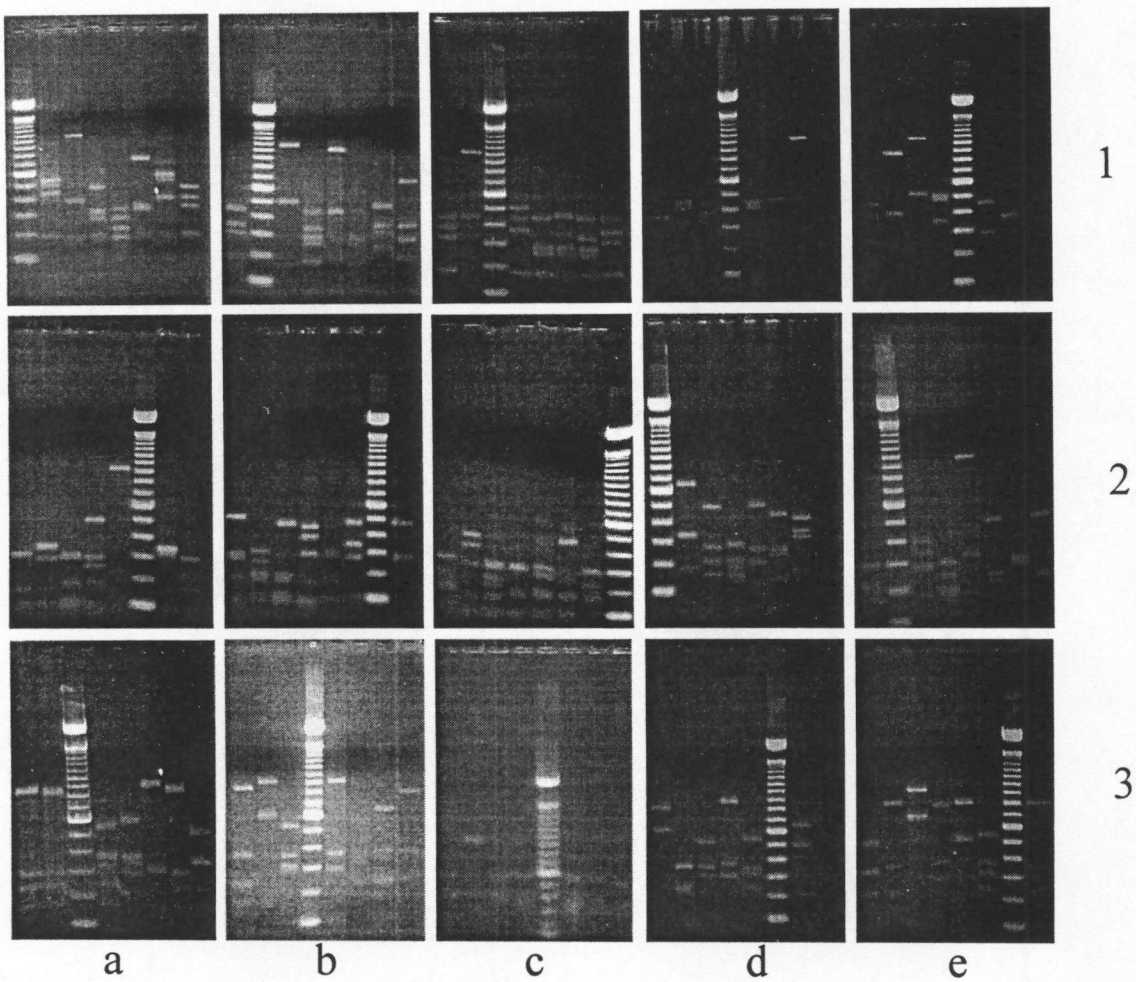


Figure 6: Restriction patterns of 16S rDNA clones obtained from April sludge sample. 1a-1e: 16S rDNAs digested with *Cfo*I. 2a-2e: 16S rDNAs digested with *Hae*III. 3a-3e: 16S rDNAs digested with *Taq*I. The banding patterns obtained from *Cfo*I digestion in lane 5 of 1a were similar to that of lane 3 of 1b, lane 2 of 1c, lane 7 of 1d, and lane 3 of 1e. The banding pattern in lane 4 of 1a was similar to the banding patterns in lane 5 of 1c, and lane 6 of 1c. The banding patterns in lane 1 of 1c were similar to the banding pattern in lane 8 of 1c, lane 3 of 1d, and lane 1 of 1e. Similar analyses were made with restriction patterns obtained using other restriction endonucleases.

Comparative Sequence Analysis to Determine Phylogeny:

The clones were sequenced using the 1492r primer. The sequenced 16S rDNA plasmid inserts from the clones were analyzed by comparing the sequences with the published reference sequences in the RDP database (Appendix B, F and G). The results of similarity rank analysis of the sequence of one of the clones from the April sample (apr19) ranked against all organisms in the small subunit prokaryotes (SSU-Prok) rRNA database of Niels Larsen's SIMILARITY_RANK software of the Ribosomal Database Project is shown in Figure 7.

Phylogenetic trees (Figure 8) based on the distance matrix method (Appendix C, D and E) were constructed from the aligned sequences. Organisms belonging to the lineage of the Bacteria domains consisting of the α, β, γ, and δ subclasses of the Proteobacteria, Cytophagales/Flavobacter/ Flexibacter group, and Gram-positive organisms with low (G+C) content were found in all three activated sludge samples analyzed.(Table 6). Differences in the bacterial populations in each monthly sample were found (Table 7). For example, in April, organisms belonging to α subclass of Proteobacteria (50%) were dominant over the β subclass (23%), γ subclass (3.85%), cytophagales (11.54%), low G+C (3.85%) and unknown organisms (7.7%). However, in January, clones representing the β subclass of the Proteobacteria (44%) were dominant over the α (20%) and γ (20%) subclasses, the cytophagales group (4%), the δ subclass (4%), and the unknown group (8%). Finally in November, members of the α subclass of the Proteobacteria (31.25%) were dominant over the β (18.75%) and γ (3.85%)

RDP data : SSU_Prok
Comments : A minimum of 50 unique oligos required
: A total of 0 sequences were excluded
: 4332 sequences were included in the search
: The screening was based on 7-base oligomers
CPU time : 0.96 seconds

527 apr19 Unknown

S_ab	Olis	Short-ID	Full name
0.723	1374	str.Hyp353	str. US-353.
0.719	1315	Hyp.vulgar	Hyphomicrobium vulgare str. MC-750.
0.638	1325	Rhp.elegan	Rhodoplanes elegans str. AS130.
0.617	1396	Azr.cauli3	Azorhizobium caulinodans.
0.615	1342	Rps.palus7	Rhodopseudomonas palustris.
0.615	1414	Bdr.japon5	Bradyrhizobium japonicum
0.615	1394	Bdr.japon4	Bradyrhizobium japonicum
0.609	1341	Rps.viridi	Rhodopseudomonas viridis.
0.609	1343	Rps.virid3	Rhodopseudomonas viridis.
0.607	1366	Azr.cauli2	Azorhizobium caulinodans.
0.605	1357	Blb.denitr	Blastobacter denitrificans.
0.605	1406	Bdr.sp129	Bradyrhizobium sp. str. 129.
0.605	1364	Bdr.japoni	Bradyrhizobium japonicum
0.603	1359	Bdr.japon2	Bradyrhizobium japonicum
0.598	1373	Rps.palust	Rhodopseudomonas palustris str. GH
0.596	1404	Zoo.ramig5	Zoogloea ramigera.
0.596	1360	Rhb.loti4	Rhizobium loti.
0.596	1359	Rhb.huakui	Rhizobium huakuii.
0.596	1337	Zoo.ramig2	Zoogloea ramigera.
0.596	1370	Bdr.sp9980	Bradyrhizobium sp.

Figure 7: Similarity ranking (SSU_Prok rRNA database) of one of the cloned 16S rDNA sequences (apr19) from the April sludge sample. The cloned 16S rDNA was similar to organisms belonging to the alpha subgroup of Proteobacteria with a maximum of 72.3% similarity with a *Hyphomicrobium-like* organism (str US 353) sequence, followed by a 71.9% similarity with a *Hyphomicrobium vulgare* str. MC-750 sequence.

Figure 8: Phylogenetic tree (see next page for figure) showing relationships of the nucleotide sequences of the 16S rDNA clones obtained from the activated sludge sample. 16S rDNA sequences for organisms from each of the different phylogenetic groups were retrieved from the RDP based on the highest similarity ranking to identify the phylogenetic position of all the clones. The phylogenetic tree obtained was based on distance matrix methods from the growtree function of the GCG. Organisms, used for analysis, with GenBank accession number are given below. Brady (*Bradyrhizobium japonicum*, D11345); Sphingo (*Sphingomonas capsulata*, D16147); Hypho (*Hyphomicrobium vulgare*, X53182); US353 (*Hyphomicrobium*-like organism *str. US353*, U06473); Zoogloea2 (ATCC 25935 strain of *Zoogloea ramigera*, X74914); were selected from the alpha subclass of Proteobacteria. Leptothrix (*Leptothrix discophora*, Z18533); Rubrivivax (*Rubrivivax gelatinosus*, D16214); Variovorax (*Variovorax paradoxus*, D30793); Sphaero (*Sphaerotilus natans*, Z18534); Zoogloea1 (ATCC 19623 strain of *Zoogloea ramigera*, X74915) were selected from the beta subclass of Proteobacteria. Legionella (*Legionella anisa*, X73394) was selected from γ subclass of Proteobacteria. Polyangium (*Polyangium* sp., M94280 was selected from δ subclass of Proteobacteria. Flavobacter (*Flavobacterium spiritivorum*, M58778) Flavobacter); Cytophaga (*Cytophaga heparina*, M11657) were selected from Cytophagales / Flavobacter / Flexibacter group. Bacillus (*Bacillus viscosus*, X77792); Lacto (*Lactobacillus homohiochii*, D31694) were selected from low G+C Gram Positive group. These organisms were selected based on the ranking of the clones with the reference strains.

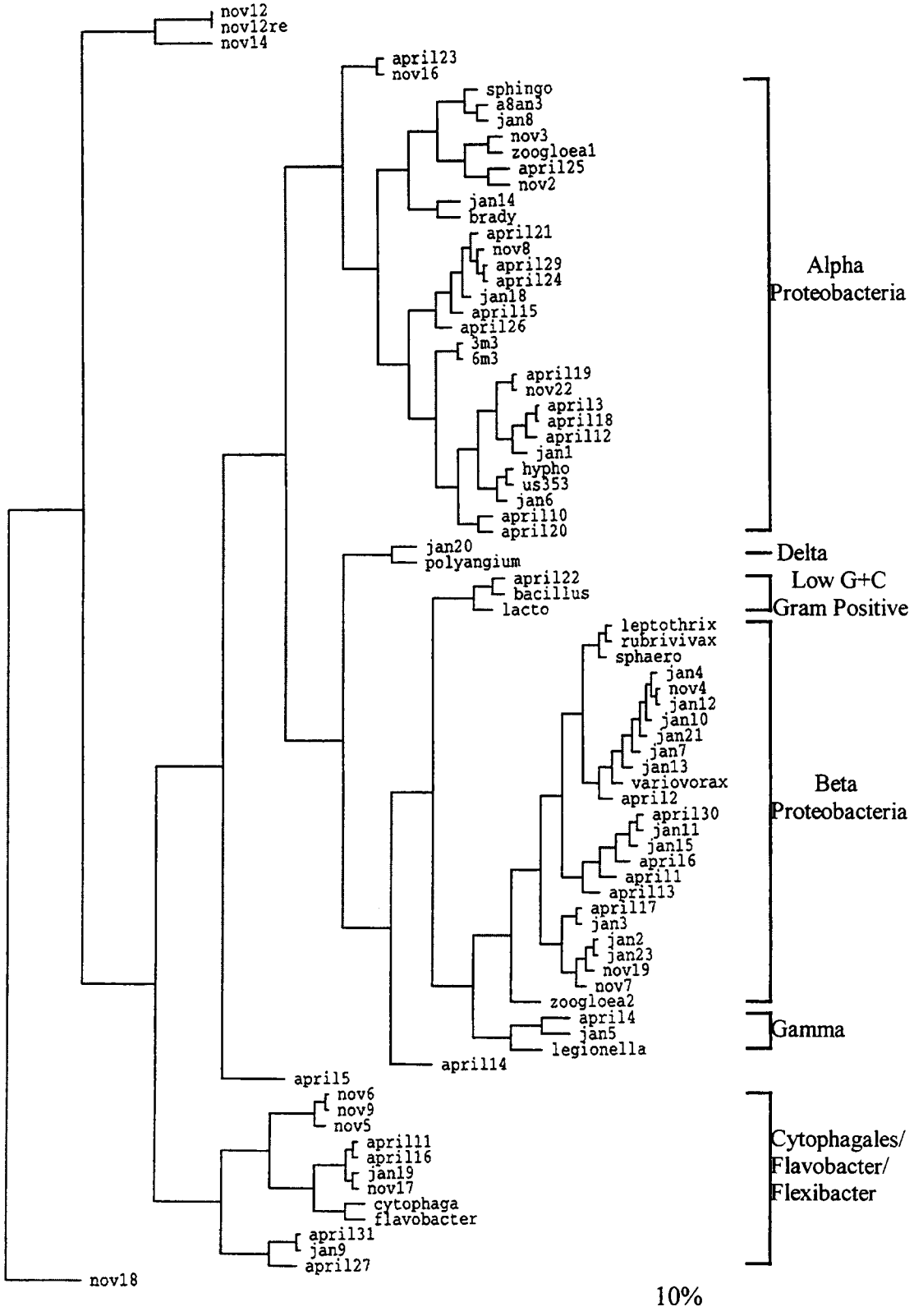

nov12
nov12re
nov14
april23
nov16
sphingo
a8an3
jan8
nov3
zoogloea1
april25
nov2
jan14
brady
april21
nov8
april29
april24
jan18
april15
april26
3m3
6m3
april19
nov22
april3
april18
april12
jan1
hypho
us353
jan6
april10
april20
jan20
polyangium
april22
bacillus
lacto
leptothrix
rubrivivax
sphaero
jan4
nov4
jan12
jan10
jan21
jan7
jan13
variovorax
april2
april30
jan11
jan15
april6
april1
april13
april17
jan3
jan2
jan23
nov19
nov7
zoogloea2
april4
jan5
legionella
april14
april5
nov6
nov9
nov5
april11
april16
jan19
nov17
cytophaga
flavobacter
april31
jan9
april27
nov18
Alpha
Proteobacteria
Delta
Low G+C
Gram Positive
Beta
Proteobacteria
Gamma
Cytophagales/
Flavobacter/
Flexibacter
10%

Table 6: Representative organisms present in the sludge samples analyzed by 16S rDNA analysis. Organisms were grouped into α, β, γ, and δ Proteobacteria, Cytophagales /Flavobacter / Flexibacter and Gram Positive organisms with Low (G+C) content based on their classification in small subunit rRNA database. Clones with their highest ranking to the reference bacteria is given in the parenthesis.

Alpha	**Beta**	**Gamma**	**Cytophagales/Flavobacter/ Flexibacter**
Magnetospirillum magnetotacticum (apr25, 70.8%)	*Variovorax paradoxus* (jan4, 83%)	*Legionella anisa* (apr4, 57.9%)	*Flexibacter ruber* (apr27, 54.1%)
Zoogloea ramigera	*Rubrivivax gelatinosus* (apr1, 82%)	*Haemophilus* sp.	*Flavobacterium spiritivorum* (apr11, 51.8%)
Thiobacillus acidophillus	*Leptothrix discophora* (apr1, 78.6%)	*Aeromonas* sp.	*Sphingobacterium heparina* (apr11, 53.9%)
Sphingomonas capsulata (jan8, 75.2%)	*Rhodocyclus tenuis* (apr13, 75.3%)		*Prevotella veroralis*
Rhodospirillum molischianum (apr25, 68.8%)	*Rhodoferax fermentus* (jan4, 79.8%)	**Delta**	*Flavobacterium ferrugineum* (nov6, 76.6%)
Rhodopsueudomonas viridis (apr29, 65%)	*Zoogloea ramigera* (apr13, 74.6%)	*Pelobacter propionicus* (apr4, 43.6%)	
Methylobacterium sp.	*Pseudomonas testosteroni* (apr6, 79.2%)	*Desulfovibrio desulfuricans* (jan9, 41.4%)	**Low (G+C) Gram Positive**
Hyphomicrobium vulgare (apr3, 74%)	*Brachymonas denitrificans* (jan23, 82.3%)	*Polyangium* sp. (jan20, 53%)	*Lactobacillus homohiochii* (apr27, 52.5%)
Hyphomicrobium-like organism str. US353 (apr3, 72.7%)	*Anoxobacterium dechloaticum* (jan2, 78.5%)	*Chondromyces* sp.	*Bacillus viscosus* (apr22, 73.1%)
Azospirillum sp. (apr25, 68.3%)	*Sphaerotilus natans* (apr1, 68.7%)		*Bacillus polymyxa* (apr22, 70.7%)
Azorhizobium sp.	*Bordetella bronchiseptica* (apr30, 71.6%)		
Agrobacterium tumefaciens (apr26, 59.7%)			**Others**
Bradyrhizobium japonicum (apr20, 65.2%)			str.FIE1 (apr1, 92%)
Rhodobacter sphaeroides (apr23, 71%)			str. PAD4 (nov9, 78.8%)

Table 7: The distribution of organisms in January, April and November sludge samples showing the shift in bacterial populations as determined by 16S rDNA analysis.

	Samples		
Organisms	**January**	**April**	**November**
Alpha subclass of the Proteobacteria	5	13	5
Beta subclass of the Proteobacteria	11	6	3
Gamma subclass of the Proteobacteria	0	1	0
Delta subclass of the Proteobacteria	1	0	0
Cytophaga/Flavobacter/Flexibacter group	1	3	4
low G+C Gram-positive group	0	1	2
Unknown	2	2	2

subclasses, the cytophagales group (25%), the low G+C group (12.5%) and the unknown group (12.5%).

Similarity_Rank results, from RDP, for all the clones is given in the appendix. None of the clones were identical to any of the known 16S rRNA sequences in the database. For example, in April sample, of the 26 clones, only two clones (april3 and april18) were nearly identical to each other while 20 clones were distinctively different from one another. The largest number of clones were found in the α subclass of the Proteobacteria (37.1%) followed by the β subclass of the Proteobacteria (32.26%) and the Cytophagales /Flavobacter /Flexibacter group(11.3%), the γ subclass of the Proteobacteria (1.6%), and the low G+C Gram-positive group (1.6%).

***Hyphomicrobium* spp. Probe Design:**

Multiple-sequence alignments from a group of related sequences using progressive, pairwise alignment were created by using the pileup function. The pairwise evolutionary distances between the aligned sequences, expressed as substitutions per 100 bases, were obtained from the distance function of the GCG package (Appendix G). A multiple-sequence format (msf) file (Appendix F) containing the multiple-sequence alignments of all the cloned sequences in the 16S rDNA sludge library was then analyzed to create oligonucleotide probes. The signature nucleotides [5′ GCT GC(C/G) CAT TGT CAC CGC C 3′] for *Hyphomicrobium vulgare* and *Hyphomicrobium*-like organism 16S rDNA sequences retrieved from RDP were identified (page 185). The degeneracy of the probe was designed to encompass the degeneracy of *Hyphomicrobium* strains as observed in the library and RDP sequences as determined by multiple sequence alignment.

Sequence Analysis of 16S rDNA from *Hyphomicrobium* Species:

To further validate the sequencing results, 16S rDNA was amplified from three identical samples of *Hyphomicrobium* M3 previously isolated by Eastman Scientists. Initially, the 16S rDNA from the positive clones (Figure 5) were partially sequenced using m13f, 926f and m13r primers. The sequence aligned with *Hyphomicrobium vulgare* with a mere 45% similarity.

After careful observation it was found that there were discrepancies in the sequence beyond 350 - 400 nucleotides. However, after editing (Figure 9), the percentage similarity of the *Hyphomicrobium* M3 sequence to *Hyphomicrobium vulgare* and

A

301 350

3926f CACGTTGCTA CAAT**GGCGGT GACAATGCGC** AGCCACCTAG TTAATAGGGA

6926f CACGTTGCTA CAAT**GGCGGT GACAATGCGC** AGCCACCTAG TTAATAGGGA

4926f CACGTTGCTA CAAT**GGCGGT GACAATGCGC A**GCCACCTAG TTAATAGGGA

Hyphomicrobium target site

B

551 600

4m13r ACAACACGTG .TAACAAT**GG CGGTGACAAT .GCGCA**GCCA ACCTAGTAAT

6m13r AACACAAGTG .TAACAAT**GG CGGTGACAAT GGCGCA**GCCA ACCTAGTAAT

3m13r ACAACAAGTG CTAACAAT**GG CGGTGACAAT .GCGCA**GCCA ACCTAGTAAT

Hyphomicrobium target site

C

801 850

4m13r TAAGGTCAGC GACTGGGGTG **AAGTCGTAAC AAGGTAGCCG**TAAAGCCGAA

6m13r TAAGGTCAGC GACTGGGGTG **AAGTCGTAAC AAGGTAACCG**TAAAGCCGAA

3m13r TAAGGTCAGC GACTGGGGTG **AAGTCGTAAC AAGGTAGCCG**TAAAGCCGAA

1492r site

Figure 9: Regions used to edit all the three *Hyphomicrobium* M3 sequences. A. The potential target site for the *Hyphomicrobium* probe (315 - 331) obtained using the 926f primer. B. The potential target site for the *Hyphomicrobium* probe (569 - 586) on the complementary strands of the sequences obtained by using the m13r primers. C. Regions at bp 1492 (821 - 840).

Hyphomicrobium-like organism (US353) sequence in the published database was increased. The sequences were edited by deleting the sequence obtained using the 926f primer after the potential target site of the *Hyphomicrobium* probe, and this sequence was substituted with the complementary strand of the sequences obtained using the m13r primer up to the 1492 consensus region (Figure 9). This resulted in drastic increase (data not shown) in the percentage similarity between the replicate sequences from the same isolate (from 75% to 99%) and also with the published sequences in the RDP database [over 70% to both *Hyphomicrobium*-like organism (strain US 353) and *Hyphomicrobium vulgare*].

Two of the positive clones (3M3 and 6M3) were then sequenced using 530f and 907r sequencing primers to obtain the complete (~1500 bp) sequence of 16S rDNA (Appendix I and Appendix J). Comparison of these clones with reference bacteria in the RDP database indicated their relatedness to *Hyphomicrobium*-like organism strain US353 and *Hyphomicrobium vulgare* (Table 8). The evolutionary distances between the clones of *Hyphomicrobium* M3 and its closest relatives as observed in the form of a cluster in the phylogenetic tree (Figure 8) were calculated using the DISTANCES tool of the GCG package (Table 9).

Microscopic Examination of Activated Sludge.

Microscopic examinations for morphological characteristics were made using light microscopy, transmission electron microscopy (TEM) and scanning electron

Table 8. Comparison of Align Sequence and Similarity_Rank results for 3M3 and 6M3 clones of *Hyphomicrobium*

Sample	No. of nucleotides sequenced	Align Sequence result	Similarity_Rank result	
			strain	S-ab value
3M3	1483	str. US353	str. US353	0.640
			Hyp. Vulgare	0.638
6M3	1449	str. US353	str. US353	0.658
			Hyp. Vulgare	0.652

Table 9. Comparison of pairwise evolutionary distances between 16S rDNA sequences of *Hyphomicrobium* M3 (Eastman isolate) and reference strains of *Hyphomicrobium vulgare* and *Hyphomicrobium*-like organism (US353) with its closest relatives in the library as determined by phylogram. Distances were calculated over 1 to 1583 bp, considering all base positions. The Jukes-Cantor correction method was used for estimating the number of substitutions per 100 bases.

	Number of nucleotide substitutions per 100 bases												
Clones	3M3	6M3	apr19	nov 22	apr3	apr 18	apr 12	jan 1	hypho	US353	jan 6	apr 10	apr 20
3M3	0.00	2.68	12.15	11.30	18.56	5.71	24.66	9.98	17.07	19.27	9.23	17.73	15.20
6M3	2.68	0.00	13.23	13.06	19.93	7.1	26.04	10.73	17.48	19.77	9.77	19.01	16.4
Hypho	17.07	17.48	10.01	10.43	20.68	8.32	26.78	8.51	0.00	4.46	6.10	14.93	9.93
US353	19.27	19.77	9.06	10.43	22.60	9.77	27.75	9.49	4.46	0.00	6.61	14.62	8.62

microscopy (SEM) to further confirm the legitimacy of phylogenetic trees (presence of *Hyphomicrobium* spp.) obtained by 16S rRNA analysis. Organisms with various morphologies were observed. Cell septa which could hardly be seen under light microscopy (Figure 10a) were visible under transmission (Figure 10b-10d) and scanning electron microscopy (Figure 11). Organisms resembling *Hyphomicrobium* [a group of gram-negative (Holm et al., 1996) organisms consisting of cells (0.5 to 1.0 × 1.3μm) which are either rod-shaped with pointed ends, oval- or bean-shaped, and produce mono- or bipolar filamentous outgrowths (prosthecae or hyphae) of varying length and capable of utilizing one carbon compounds such as methane (Lee et al.,1996) or methylamine (Harder et al.,) for growth], *Zoogloea* spp. and filamentous bacteria were observed. The identification of the isolates as budding and prosthecate bacteria (*Hyphomicrobium, Hyphomonas, Pedomicrobium*) was based on the budding morphology and their ability to produce prosthecae (Staley et al., 1988).

Fluorescent In Situ Hybridization:

The ability of the degenerate fluorescent *Hyphomicrobium* probe to hybridize with the target sequence was examined using the cultured strains of *Hyphomicrobium* M3 previously isolated at Eastman and *Hyphomicrobium vulgare* obtained from ATCC (Figure 12). The degenerate probe was also targeted for *Hyphomicrobium* species in the sludge. Binding of the probe was seen at all formamide concentrations (Figure 12). The specificity and sensitivity of the probe was further evaluated by hybridization to mixtures of morphologically distinct strains with known mismatches within the target sequence.

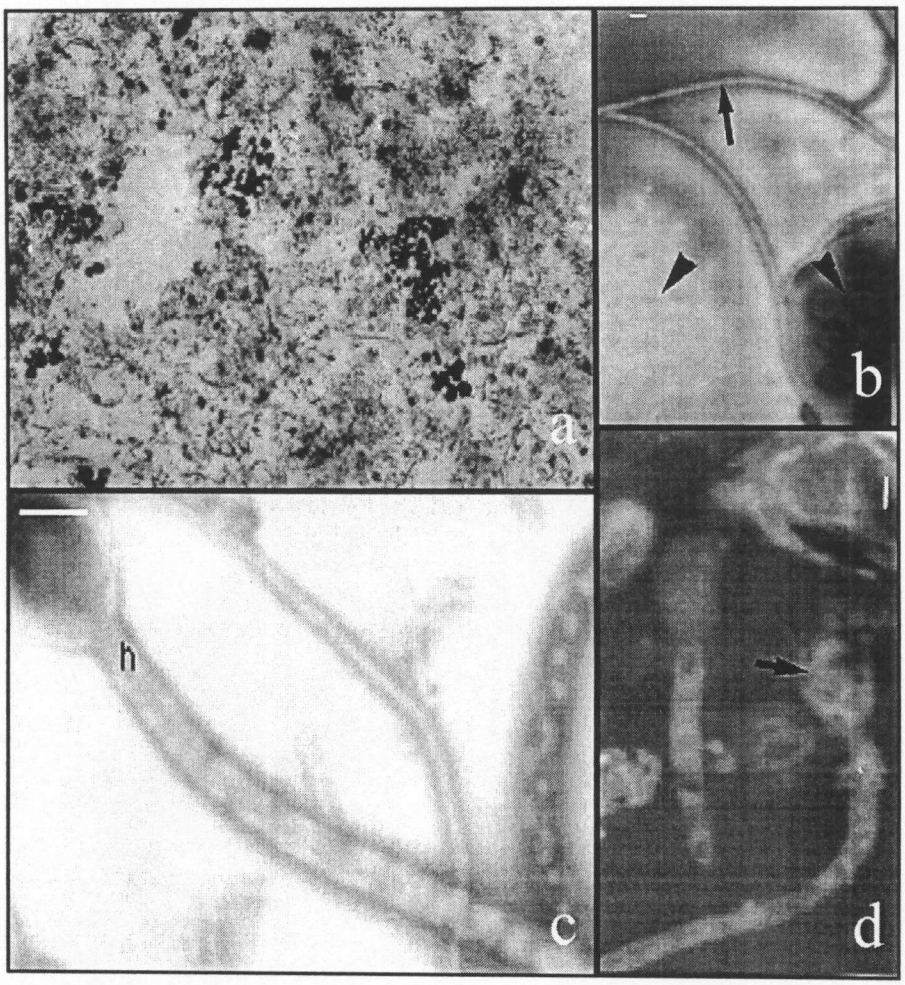


Figure 10: Light micrograph (a, bar=0.1μm), and Transmission electron micrographs from negative stain preparation (b, bar=1μm and c, bar=0.5 μm) and ultrathin sectioning (d, bar=0.1micrometer) of unidentified microorganisms associated with activated sludge. Note the budding bacteria resembling *Hyphomicrobium* species (shown by h in c and arrow in d) and the agglomerating bacteria (arrow head) developed on the filamentous skeleton (arrow) in b. It is difficult to identify such organisms in light micrograph.

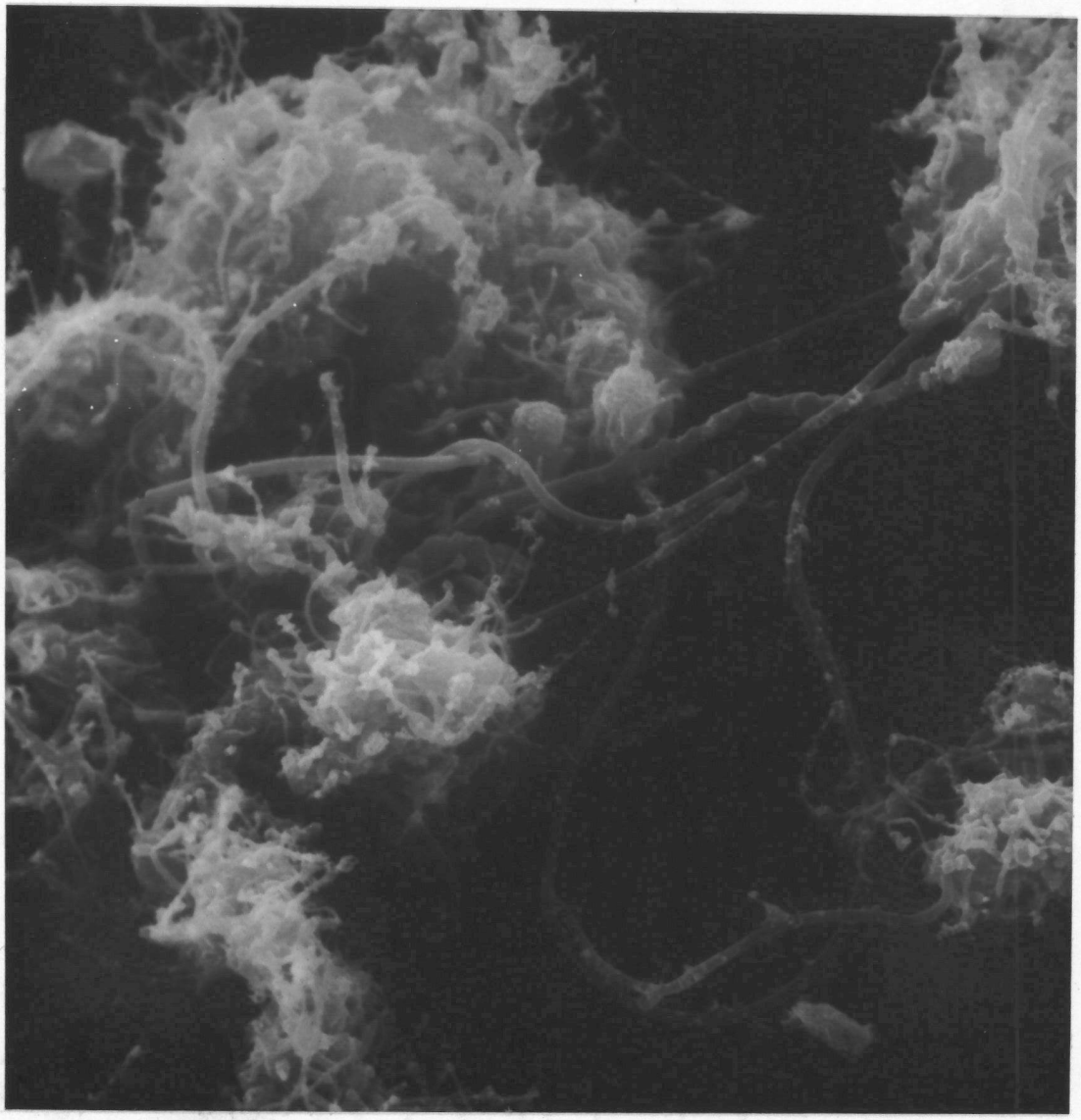


Figure 11: Scanning electron micrograph of unidentified budding bacteria in activated sludge, resembling *Hyphomicrobium* species, attached to filaments.

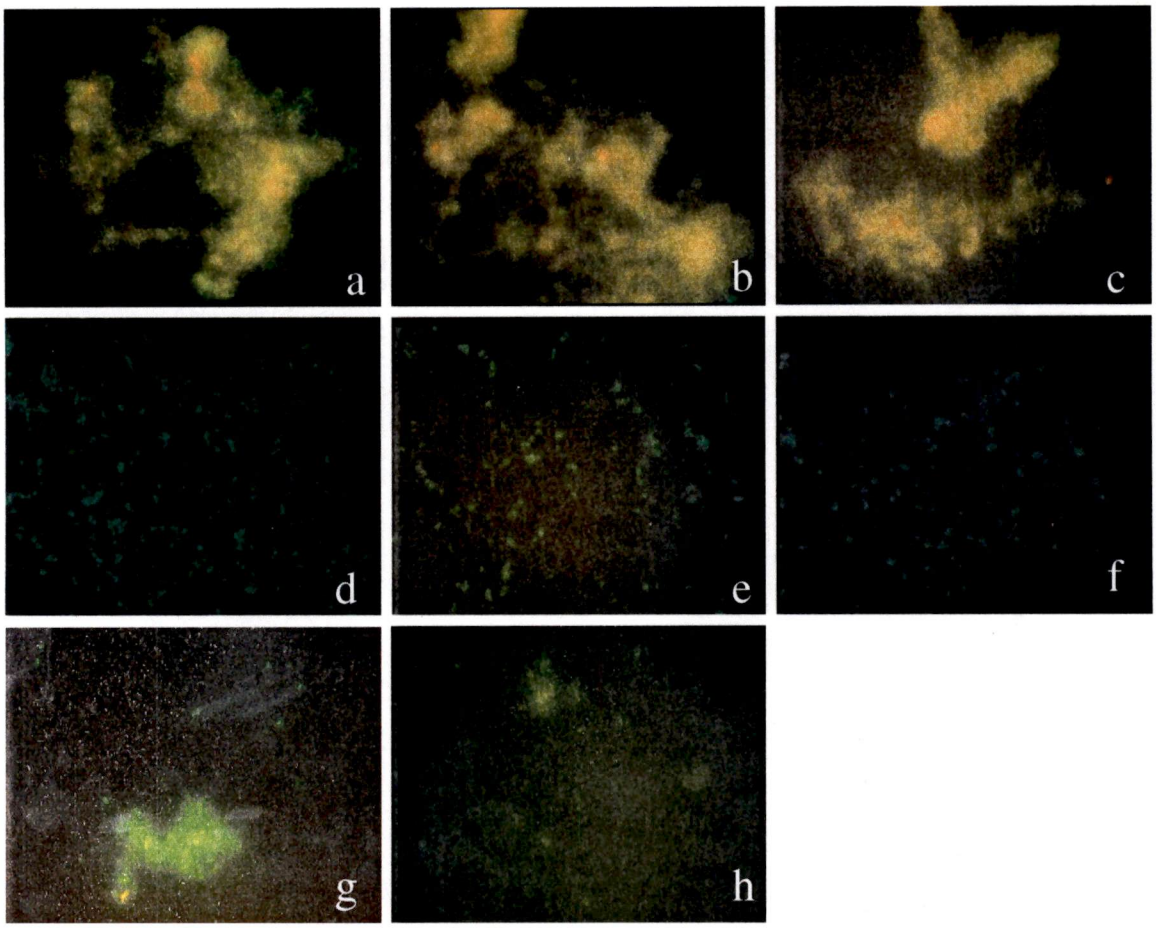
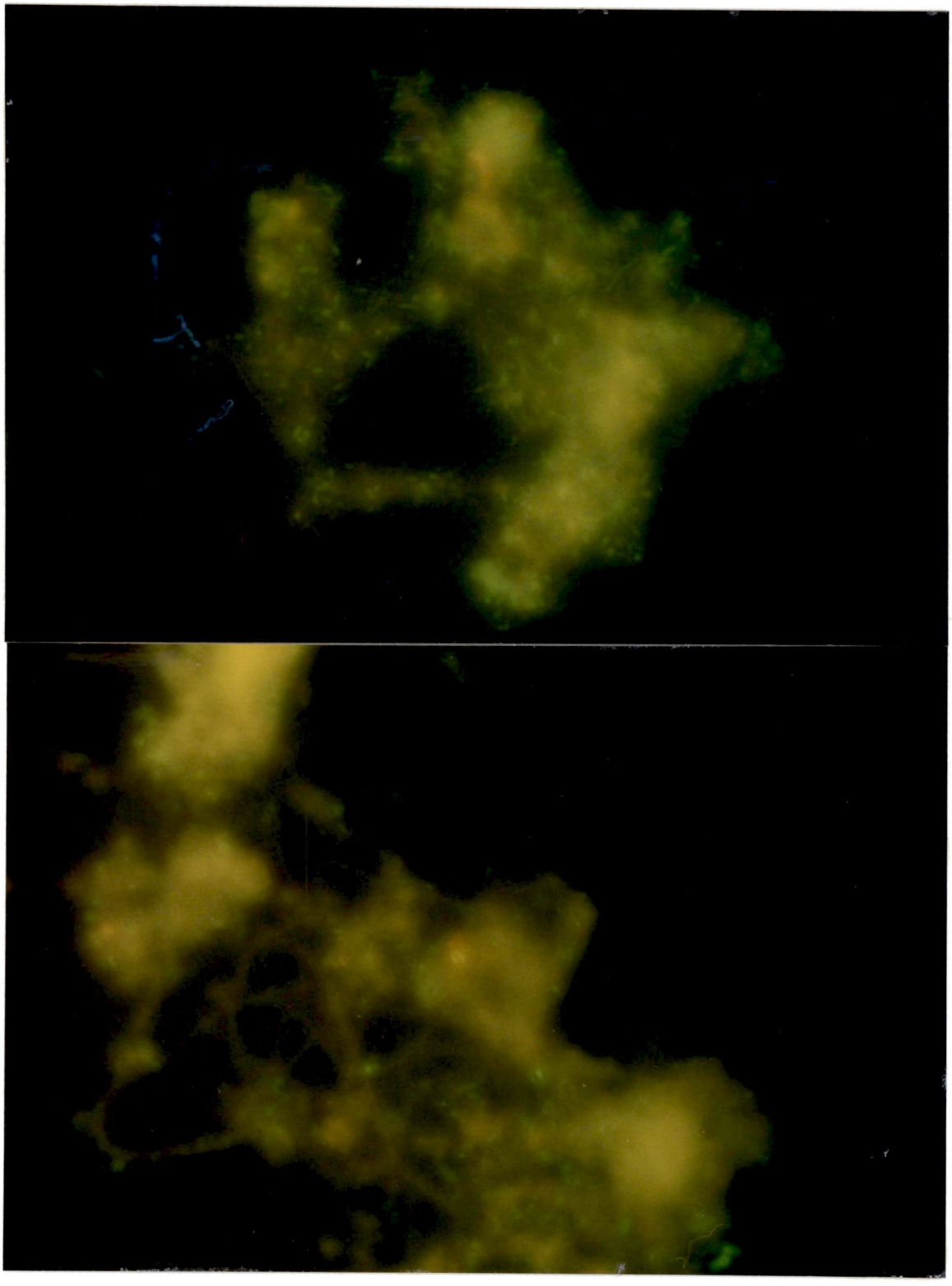


Figure 12: Micrographs of fluorescent in situ hybridization. 12a-12c: Binding of *Hyphomicrobium* probe in activated sludge at 10, 45 and 70% formamide respectively. 12d-12f: Binding of *Hyphomicrobium* probe to pure culture of *Hyphomicrobium* M3 at 10, 45 and 70% formamide respectively. 12g-12h: Binding of *Hyphomicrobium* probe to pure culture of *Hyphomicrobium vulgare* (ATCC 27500) at 45 and 70% formamide respectively. The enlarged view of 12a and 12b are shown on the next page.



Organisms with zero, one, two, four and eight mismatches in the probe target region as determined from the Check Probe tool of the RDP database (Appendix H) were selected to check for probe specificity. The differences in the target site for control organisms used to optimize probe specificity are given in Table 10. The stringency of hybridization (to differentiate between organisms with mismatches in the probe target region) was determined by increasing the formamide concentration in the hybridization buffer, while keeping the temperature constant at 37°C. Effective binding of the probe to *Hyphomicrobium* M3 and *Hyphomicrobium vulgare* was observed at all formamide concentrations (Figure 12). The results of mismatch discrimination experiments (Figure 13 and 14) are summarized in Table 11.

The probe distinguished *Sphingomonas A8AN3* and *Escherichia coli* from *Hyphomicrobium* M3 at 10% formamide concentration. *Zoogleoea ramigera* and *Bradyrhizobium japonicum* were distinguished from *Hyphomicrobium* species at 30% and 70% formamide concentrations, respectively (Figure 13). Unexpectedly, at 10% formamide concentration, the probe bound to *Zoogleoea ramigera* with 8 mismatches but not to *Sphingomonas A8AN3* that has four mismatches. Although the amount of bound probe gradually decreased, as expected, by increasing the formamide concentration, the probe still showed binding to *Hyphomicrobium vulgare* and *Sphingomonas capsulata* at 70% formamide concentration (Figure 14).

Table 10: Target sequence of various organisms used to check the specificity of the *Hyphomicrobium* probe. Mismatched nucleotides are underlined. Nucleotide C underlined in *Hyphomicrobium* strain M3 is degenerate.

Organism	**Sequence in the binding region of degenerate probe 5′ GCTGC(C/G)CATTGTCACCGCC 3′**	**No. of mismatches**
Hyphomicrobium strain M3	GGCGGTGACAATGCGCAGC	0
Hyphomicrobium vulgare (ATCC 27500)	GGCGGTGACAATGGGCAGC	0
Sphingomonas capsulata (ATCC 14666)	GGCGGTGACAGTGGGCAGC	1
Bradyrhizobium japonicum (USDA 110)	GGCGGTGACAATGGGATGC	2
Sphingomonas A8AN3	GGCGACTACAGTGGGCAGC	4
Zoogloea ramigera (ATCC 25935)	GGTACATACAGAGGGATGC	8

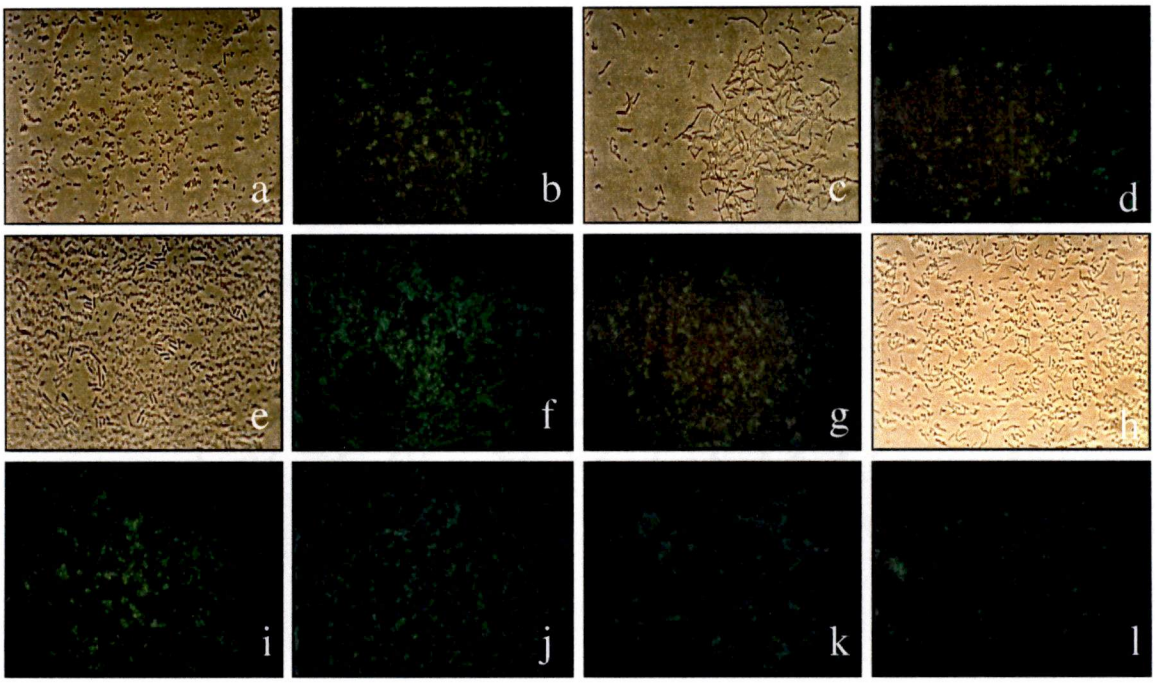
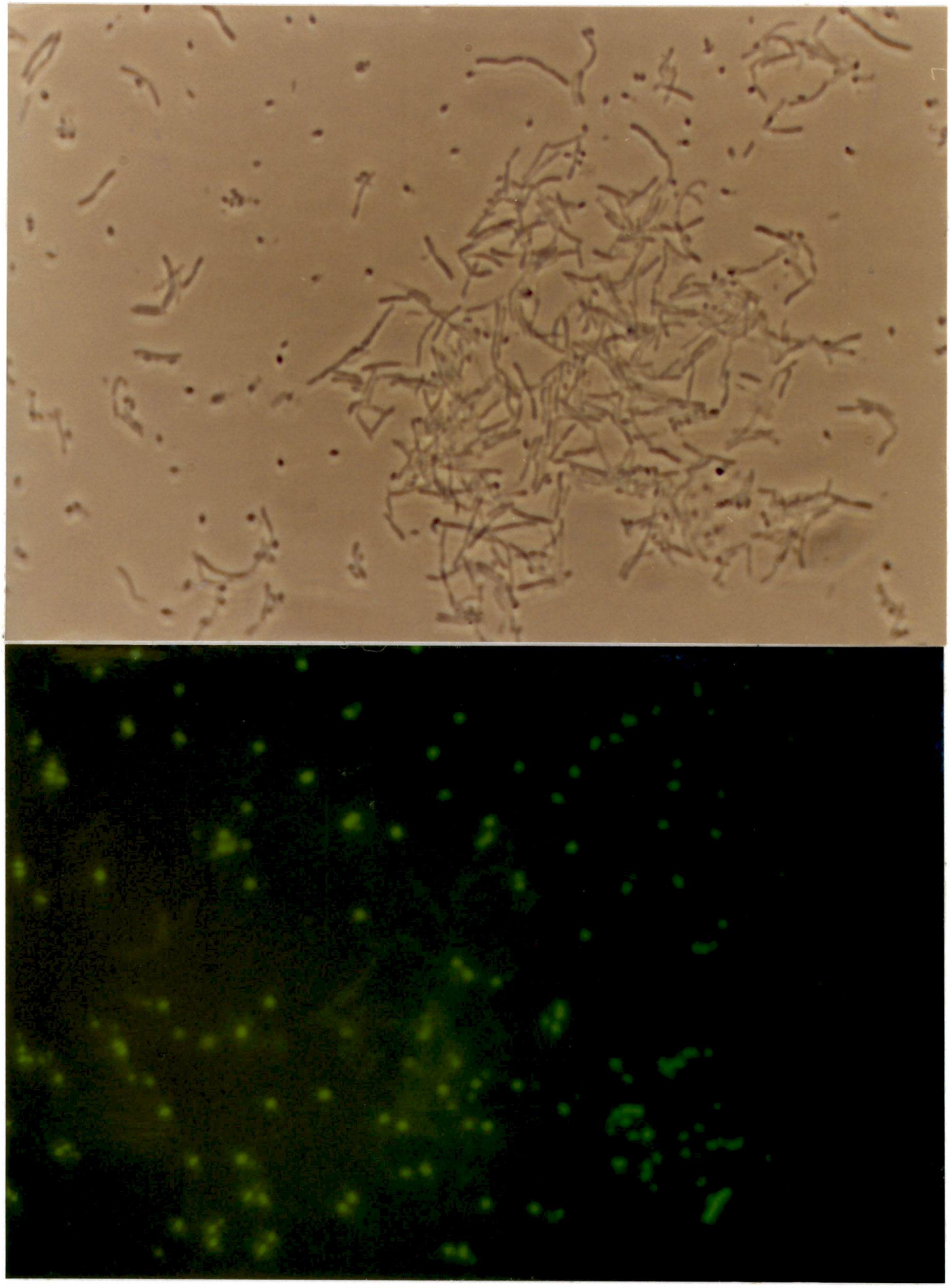


Figure 13: Micrographs of in situ hybridization of a mixture of *Hyphomicrobium* M3 and reference strains with fluorescently labeled *Hyphomicrobium* probe showing mismatch discrimination. Various concentrations of formamide were used in the hybridization and the wash buffers while keeping the temperature constant at 37°C. 13a - 13b: *Hyphomicrobium* M3 and *Escherichia coli* observed under visible light at 10% formamide. 13c-13d: *Hyphomicrobium* M3 and *Sphingomonas* strain A8AN3 under visible and UV light at 10% formamide respectively. 13e-13g: *Hyphomicrobium* M3 and *Zoogloea ramigera* under visible, UV at 10%, UV at 30% formamide respectively. 13h-13l: *Hyphomicrobium* M3 and *Bradyrhizobium japonicum* under visible, UV at 10%, 30%, 45% and 70% respectively. The enlarged view of 13c and 13d are shown on the next page.



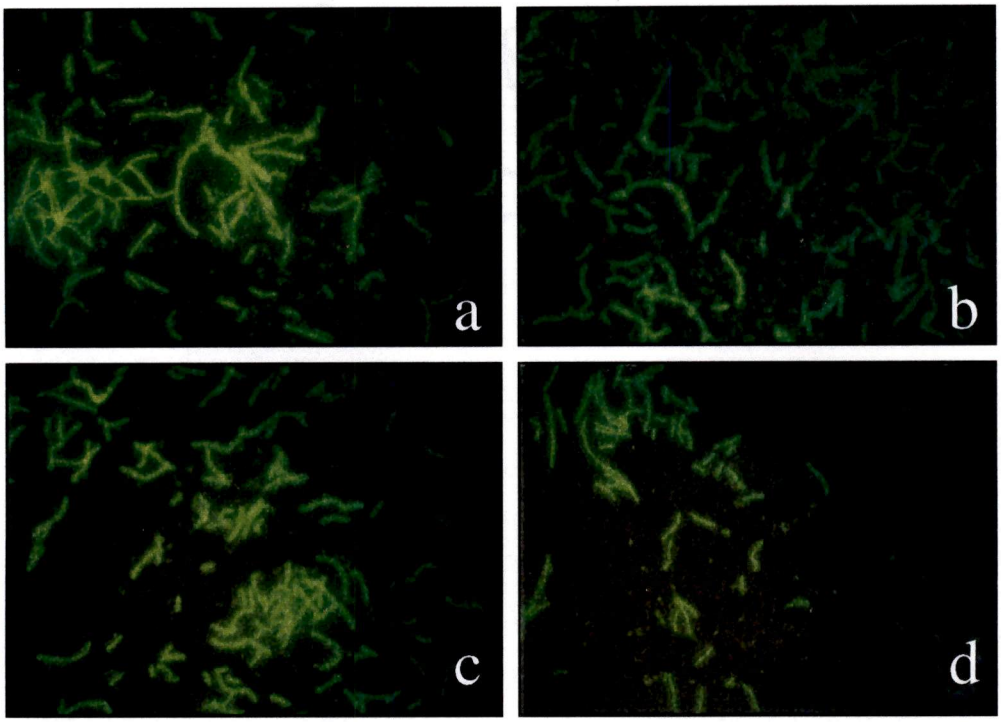


Figure 14: Micrographs of in situ hybridization of a mixture of *Hyphomicrobium* M3 and *Sphingomonas capsulata* with fluorescently labeled *Hyphomicrobium* probe. Various concentrations [10% (a), 30% (b), 45% (c) and 70% (d)] of formamide were used in the hybridization and the wash buffers while keeping the temperature constant at 37°C to check the specificity of the probe in discriminating single mismatch in the target region of *Sphingomonas capsulata.*

Table 11: Hybridization of genomic DNA of control strains with the *Hyphomicrobium* probe targeting the 5′ GCT GC(C/G) CAT TGT CAC CGC C 3′ region of the 16S rRNA at various stringency levels.

Strain	**% Formamide concentration**			
	10	**30**	**45**	**70**
Hyphomicrobium M3	+	+	+	+
Hyphomicrobium vulgare (ATCC 27500)	+	+	+	+
Sphingomonas capsulata (ATCC 14666)	+	+	+	+
Bradyrhizobium japonicum (USDA 110)	+	+	+/-	-
Zoogloea ramigera (ATCC 25935)	+/-	-	-	-
Sphingomonas A8AN3	-	-	-	-
Escherichia coli	-	-	-	-

CHAPTER V

DISCUSSION

The efficacy of 16S rRNA analysis and fluorescent in situ hybridization in identifying organisms from a mixed microbial population was demonstrated. This was accomplished by retrieving 16S rDNA sequences from the total DNA extracted from activated sludge. Initial studies suggested that there were difficulties in optimizing conditions for extraction of total DNA from a complex environment, amplifying of 16S rRNA genes from the total DNA to obtain a clone library and screening of clones bearing the 16S rDNA inserts. Comparative sequence analysis of randomly chosen 16S rDNA clones with the reference sequences in the RDP database revealed considerable microbial diversity in activated sludge which was also suggested by the restriction patterns of the 16S rRNA molecules, although these two methods were not readily comparable. Evolutionary distances between organisms in the activated sludge were determined by the phylogenetic tree constructed from the aligned sequences using the distance matrix method. Designing of a fluorescently labeled oligonucleotide probe based on comparative analysis of retrieved sequences and its application in distinguishing *Hyphomicrobium* spp., a causative organism in filamentous bulking was also accomplished.

The development of a set of oligonucleotide probes will allow in rapid identification and enumeration of both culturable and uncultivable organisms directly in activated sludge particularly those with a potential to cause problems for the wastewater

treatment process. Since these methods do not necessarily require prior cultivation to characterize organisms, they can also be used in studying the bacterial populations of a community existing in any natural environment.

DNA Extraction and Purification:

The DNA extraction and purification procedure for sediments from Ogram was slightly modified on a trial-and-error basis to increase the extraction efficiency of DNA from sludge samples. The priority was to lyse all the bacteria in order to avoid bias in the composition of purified DNA. Detection of 16S rDNA by PCR suggested that the harsh shearing effects of bead-beating (Erb et al., 1993) did not result in excessive DNA shearing. Interference of humic acids in DNA extractions is well documented (Chandler et al., 1997, Ogram et al., 1987, Tebbe et al., 1993). The brown color in the sample, presumably humic acids that co-purify with extracted nucleic acids, could not be totally eliminated even after various steps of precipitation and phenol-chloroform-isoamyl alcohol extraction followed by purification of DNA via the Elu-quick method. The impurities in the sample were also detected by absorbance at 260nm and 280nm wavelengths. The spectral data were higher than that observed for pure DNA. Also, there could be other unidentified contaminants in the sludge samples that interfere with the purification process. Decreases in DNA concentrations by additional purification methods suggests a need for a protocol that involves minimal additional steps but still enhances the purity of the DNA. Possible degradation of DNA in the sludge over time cannot be ruled out as a cause of the observed problems with 16S rDNA cloning(Table 2).

PCR Amplification of 16S rDNA:

The quantity and quality of the PCR product is enhanced by providing optimal conditions for the amplification of a specific template. In the beginning stages, the best buffer conditions for the amplification of 16S rDNA by PCR could not be predicted. The use of the PCR optimizer kit helped to determine the optimal conditions for amplification.

One of the potential problems in the construction of 16S rDNA libraries is the contamination of template DNA or the reagents in the PCR reaction mixture because small amounts of DNA can be amplified by PCR. Universal primers, used to eliminate bias towards selection of specific groups, could amplify 16S rDNA sequence from any eubacteria. In spite of maintaining sterile conditions, PCR products were noticed from samples without added template DNA. This contamination may be from the enzyme preparation used for amplification as the manufacturer could not guarantee the complete purity of the enzyme from *Escherichia coli* hosting the modified *Taq* polymerase gene. The absence of 16S rDNA sequences from the host organism used in manufacturing *Ampli*Taq DNA polymerase, when compared with the library sequences from the sludge suggested that this was not the source of PCR products observed in controls. However, *Ampli*Taq DNA polymerase LD, used upon manufacturer recommendation, eliminated the amplification of such DNAs. It is also important to note that the contaminating DNA introduced in the laboratory during the procedure may be a minor component of mixtures containing sludge DNA, and therefore, may not be represented in the library of 16S rDNA sequences.

The DNA template was diluted to overcome the hindrance of the amplification of undiluted template DNA by humic materials (Erb et al., 1993, Tebbe et al., 1993). But at the same time, the amplification was reduced at higher dilutions because of the lower concentrations of DNA template. PCR products cloned from 1:10 and 1:100 dilutions of sludge DNA generated a large number of white colonies on LB agar with X-gal and ampicillin but not all the white colonies contained inserts. The poor cloning efficiency could be due to insertion of low molecular weight PCR products or primer-dimer (template independent duplex PCR product consisting of the primers used for amplification) formation (Figure 3) during ligation. The problem of primer-dimer and amplification of non-target sequence was overcome empirically by diluting the synthesized primer to 20ng/μl. In spite of these efforts, the reproducibility of successful PCR amplification and cloning from sludge DNA was poor. Inefficient PCR amplification from DNA extracts could also be due to unknown impurities (Pillai et al., 1991). Poor reproducibility due to low copy number of the target DNA cannot be ruled out. It should also be noted that PCR amplification is biased away from the high DNA G+C content (Suzuki et al., 1996). Quantification of the proportion of organisms belonging to high G+C Gram-positive bacteria may be effected by this bias. However, the problems of lysing thick cell-walled, Gram-positive bacteria faced by Suzuki et al., (1996) were not evident as the library contained organisms from low G+C Gram-positive bacteria.

Restriction fragment length polymorphism analysis of a PCR amplified 16S rDNA:

Restriction fragment length polymorphism analysis of 16S rDNAs performed with *Cfo*I, *Hae*III an *Taq*I restriction endonucleases revealed the diversity of microbes in the activated sludge. The species that produced similar bands on electrophoretic separation were thought to be closely related. This was later suggested by sequence analysis as determined by the dendrogram (Figure 8). However, it was not determined which restriction patterns were associated with each 16S rDNA sequence. Although extensive purification of PCR products is not necessary for a satisfactory RFLP analysis (Yoon et al., 1997), inefficient separation of low molecular weight DNA suggested a need for further purification of PCR products amplified from the sludge sample to enhance the discriminating power of this technique. Practical difficulties in differentiating similar, but variant, banding patterns for the closely related strains with minimum sequence divergence, and the lack of reference strains, limited the interpretation of RFLP results to simple observation of microbial population shifts. No information was obtained about the identity of the strains. RFLP alone may not be an effective tool for accurate and reliable identification of microbial populations from complex environments with unknown populations. This technique may be more useful in cases where the restriction fragment patterns of target species are known.

Phylogenetic Analysis:

The identification of the sludge populations represented in the 16S rDNA library must be inferred with caution as the RDP database consists of only approximately 3,600

small subsunit (SSU) prokaryotic rRNA sequences (Maidak et al., 1997). All these sequences are obtained from cultured bacteria. It has been suggested that only 1-15% of the total bacterial populations in activated sludge are culturable, and many of these organisms are not expected to be present in the existing databases. Although some of the 16S rDNA sequences were similar to sequences in the published RDP database, none of the sequences were identical to those of known reference strains. The phylogram (Figure 8) indicated high microbial diversity with clusters of organisms belonging to the α , β, γ, and δ subclasses of the Proteobacteria, the Cytophagales /Flavobacter/ Flexibacter group and the low G+C Gram positive bacteria (Table 6). The dominance of the α , β, and γ subclasses of the Proteobacteria in sludge samples from aeration tanks of a two-stage system with a high-load first stage and a low-load second stage has been reported (Wagner et al., 1993). Members of the β subclass were present in approximately equal proportions in the high- and low-load basins. Members of the α subclass were more prevalent in the low load basin, whereas members of the gamma subclass were more common in the high load basin. The dominance of the β subclass of Proteobacteria in January compared to the more numerous members of the α subclass in April and November indicated a shift in the bacterial community in the Eastman activated sludge, possibly due to changes in organic loading. Interestingly, 10 of the 64 sequences analyzed were identified as *Hyphomicrobium* spp (α subclass of Proteobacteria) which is associated with low F/M (organic loading) conditions (Jenkins et al., 1986). The shift in the microbial community was also suggested by RFLP analysis (Figure 6). The application of fluorescent probes for in situ detection of Gram-negative filamentous

bacteria in activated sludge is relatively new (Bradford et al., 1997, Reyes et al., 1997, Wagner et al., 1993, Wagner et al., 1994). Although, these researchers have developed a variety of oligonucleotide probes for Proteobacteria, 16S rDNA probes for *Hyphomicrobium* spp. have not been previously reported. *Hyphomicrobium* spp. have been observed in sewage treatment plants (Meyers et al., 1986, Holm et al., 1996) and their presence in soils, groundwater, freshwater ponds and lakes, marine samples and hypersaline lakes has also been reported. Previous studies have shown that *Hyphomicrobium vulgare* characteristically grows in clumps (rosettes), often adhering to the walls of the culture vessel. *Hyphomicrobium*-like bacterium are commonly present in the natural environment and utilize methanol under denitrifying conditions (Amaral et al., 1995). *Hyphomicrobium* spp. are competitive under low nutrient conditions (Bergey's Manual of Determinative Bacteriology 1994). Based on the distance matrix trees, the morphological characteristics of organisms observed under electron microscopy, and observations by Eastman Chemical Company scientists *Hyphomicrobium* spp. are important in this activated sludge community especially given the occurrence of methanol as major component of the influent wastewater.

Sequence Analysis of 16 S rDNA from *Hyphomicrobium* Species.

Although, approximate phylogenetic characterization was done by partial 16S rDNA sequence by other researchers (Schmidt et al., 1991), the necessity for complete sequencing of the approximately 1500 bp 16S rDNA to obtain reliable information for phylogenetic characterization has also been stressed. Therefore, it was felt that complete

sequencing would improve the match between *Hyphomicrobium* M3 and previously sequenced *Hyphomicrobium* spp.. However, the similarity ranking of the complete 16S rDNA was further reduced to 68.5% with *Hyphomicrobium*-like organism (US353) and 64% with *Hyphomicrobium vulgare* as compared to over 70% similarity with both strains when the16S rDNA was partially sequenced. The deep branches in the phylogenetic distance tree (Figure 8), in addition to nucleotide substitution per 100 bases as determined by the DISTANCES tool of GCG (Table 9 and Appendix G), and lower Similarity_Rank result (Appendix B) suggests that *Hyphomicrobium* M3 is not identical to *Hyphomicrobium vulgare* and *Hyphomicrobium*-like organism. Therefore, it may be an entirely different species related to US353 and *Hyphomicrobium vulgare*.

Microscopic Examination of Sludge:

The presence of *Hyphomicrobium* spp. in the 16S rDNA libraries was further validated by the appearance of budding bacteria attached to the filaments as observed by electron microscopy. The production of prosthecae from one pole of the cell distinguishes *Hyphomicrobium* and *Hyphomonas* from *Pedomicrobium,* which produces prosthecae from several sites (Harder et al.,). The ability of *Hyphomicrobium* M3 to utilize methylamine hydrochloride and methanol, and the Similarity_Rank and Align Sequence results suggest that the prosthecae-forming organism visible in the sludge sample under study is a *Hyphomicrobium* spp.

Fluorescent In-Situ Hybridization:

Hyphomicrobium species have been isolated in mineral medium without added organic carbon, or with methanol (Aa et al., 1996), methylamine hydrochloride, or chloromethane (Doronina et al., 1996) as sole carbon-energy sources. *Hyphomicrobium* M3 was grown on ATCC 656 medium with methylamine hydrochloride. Exponential growth is preferable for efficient fluorescent in situ hybridization as the intensity of fluorescence of bacteria is stronger for exponentially growing cells than for cells in stationary phase (Delong et al., 1989). However, due to the slow growth of *Hyphomicrobium* M3 samples were simply taken for probing experiments when the medium became turbid.

The binding of the fluorescent *Hyphomicrobium* probe to the target sequence in the pure culture of *Hyphomicrobium* M3 previously isolated by Eastman scientists and to *Hyphomicrobium vulgare* (ATCC 27500) (Figure 12) suggested the accessibility of fixed cells for in situ hybridization.

The probe was tested for its specificity under various mismatches in the target site (Figure 13). Theoretically, a single mismatch in a 17mer oligonucleotide reduces the melting point of the hybrid molecule by 11°C. Single mismatch probes are commonly species-specific. Although clear microscopic discrimination between species was possible in the presence of higher number of mismatches at higher stringency levels, it was found that differentiating species having a single mismatch in the target region was practically impossible with the method employed. Even though most species could be discriminated (Table 9) from *Hyphomicrobium* by using stringent hybridization and washing

conditions, *Sphingomonas capsulata*, the strain with a single mismatch, was strongly fluorescent at all formamide concentrations (Figure 14). A probe made more specific for *Hyphomicrobium* M3 (without degeneracy) would have two mismatches in the target sequence of *Sphingomonas capsulata.* This might reduce the intensity of fluorescence from *Sphingomonas capsulata* and could make the probe more specific. However, the probe was made degenerate to encompass the observed variation in sequences seen for the library clones, *Hyphomicrobium* M3 and the known RDP strains. In some cases the species specificity is not the most important consideration.

Development of species-specific probes can be difficult, since in some cases, the probe has to be complementary to the most variable regions of the rRNA molecules. These regions have the highest mutation frequency, and back mutations in the probe target site (Amann et al., 1996) are possible. Several methods such as use of molecular beacons to discriminate one mismatch oligonucleotide targets (Schofield et al., 1997), use of competitor probes (Manz et al., 1992), fluorescent hybridization probes in combination with flow cytometry (Amann et al., 1990b, Lange et.al., 1996, Wallner, 1996), bleaching of fixed cells before hybridization and sodium borohydride reduction to avoid autofluorescence, use of fluors with emission wavelengths that do not coincide with autofluorescence, (Delong et al., 1989), use of multiple probes (Alfrieder et al., 1996, Amann et al., 1995b, Lee et al.,1993) or oligonucleotides labeled with multiple fluors (Amann et al., 1990) have been suggested as methods to enhance weak fluorescence and/or increase probe specificity.

Probing of Activated Sludge:

In some cases it may be important to confirm that the 16S rDNA libraries are representative of the microbial community. Perhaps, this can be confirmed by repeating the procedures (extraction of DNA, PCR amplification, cloning, sequencing and analysis) for the sludge sample. However, this method is expensive, time consuming and may simply result in a series of sequences consistently skewed by the problems discussed earlier. In addition to biases involved in 16S rDNA analysis, phylogenetic trees obtained by 16S rDNA amplification reflect the number of 16S rDNA gene copies (which varies from 1 to 14 copies per cell) present in the sludge samples and not the relative proportions of different types of organisms. Hence, one should be careful in estimating the relative abundance of microorganisms in the community when using 16S rDNA analysis. Fluorescently labeled probes (developed based on the library sequences) for the target organisms can be used to confirm the legitimacy of the phylogenetic classification of organisms, verify the presence of these organisms in the sludge, and establish their relative abundance.

The *Hyphomicrobium* spp. probe was used to probe activated sludge obtained from the Eastman Chemical Company wastewater treatment plant. Positive hybridization was observed to small filaments attached to the floc edges and to other large filaments (Figure 12a-12C). Autofluorescence (excitation of some photosynthetic pigments at approximately the same wavelength of the fluorochrome) and very thick flocs reduced the image clarity within the flocs.

CHAPTER VI

CONCLUSION

Wastewater treatment plants are susceptible to periodic sludge quality problems. The limitations of pure culture techniques has precluded a deeper understanding of the role of various microorganisms in the activated sludge treatment of wastewater. The capability of 16S rDNA analysis to overcome the limits of classic culture techniques allows characterization of microbial populations in activated sludge suffering from periodic settling-compaction problems. The appearance of *Hyphomicrobium* spp., a well known component of the activated sludge communities (Meyers & Meyers, 1986; Holm et al., 1996) in the 16S rDNA library as determined by comparative sequence analysis was evaluated by designing a fluorescently labeled oligonucleotide probe for in situ monitoring of the sludge. A phylogenetic placement of this organism, not identical to any of the reference strains in the RDP, was made. The study of *Hyphomicrobium* spp. in the context of 16S rDNA library construction, allowed independent confirmation of the legitimacy of the libraries for identifying important groups of organisms via fluorescent in situ hybridization (based on their detection in sludge), electron microscopy (based on their distinctive morphology), and isolation (based on their specific growth requirements) and sequencing of a pure culture of *Hyphomicrobium* spp. previously isolated.

BIBLIOGRAPHY

Aa, K., Olsen, R.A.: The use of various substrates and substrate concentrations by a Hyphomicrobium sp. isolated from soil: Effect on growth rate and growth yield. Microbial Ecology 31: 67-76 (1996).

Alfreider, A., Pernthaler, J., Amann, R., Sattler, B., Glockner, F., Wille, A., Psenner, R.: Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake by in situ hybridization. Applied and Environmental Microbiology 62(6): 2138-2144 (1996).

Amann, R.I., Krumholz, L., Stahl, D.A.: Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic and environmental studies in microbiology. Journal of Bacteriology 172(2): 762-770 (1990a).

Amann, R.I., Binder, B.J., Olson, R.J., Chisholm, S.W., Devereux, R., Stahl, D.A.: Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Applied and Environmental Microbiology 56(6): 1919-1925 (1990b).

Amann, R.I., Ludwig, W., Schleifer, K.: Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiological Reviews 59(1): 143-169 (1995a).

Amann, R.I.: Fluorescently labeled, rRNA-targeted oligonucleotide probes in the study of microbial ecology. Molecular Ecology 4: 543-554 (1995b).

Amann, R., Ludwig, W., Schulze, R., Spring, S., Moore, E., Schleifer, K.: rRNA-targeted oligonucleotide probes for the identification of genuine and former pseudomonads. System. Appl. Microbiol. 19: 501-509 (1996).

Amaral, J.A., Archambault, C., Richards, S.R., Knowles, R.: Denitrification associated with Groups I and II methanotrophs in a gradient enrichment system. FEMS Microbiology Ecology 18: 289-298 (1995).

Applegate, B.M., Matrubutham, U., Sanseverino, J., Sayler, G.S.: Biodegradation genes as marker genes in microbial ecosystems. In: Molecular Microbial Ecology Manual. Ed. A.D.L. Akkermans, J.D. van Elsas and F.J.de Brujin. Kluwer, Netherlands. 6.1.8: 1-14 (1995).

Aarmeda, B.W., Lorenz, M.G., Krumbein, W.E.: Protection of sediment-adsorbed transforming DNA against enzymatic inactivation. Applied and Environmental Microbiology 46(2): 417-420 (1983).

Avaniss-Aghajani, E., Jones, K., Chapman, D., Brunk, C.: A molecular technique for identification of bacteria using small subunit ribosomal RNA sequences. BioTechniques 17: 144-149 (1994).

Balkwill, D.L., Labeda, D.P., Casida, L.E. Jr.: Simplified procedures for releasing and concentrating microorganisms from soil for transmission electron microscopy viewing as thin-sectioned and frozen-etched preparations. Canadian Journal of Microbiology 21: 252-262 (1975).

Balkwill, D.L., Ruckinsky, T.E., Casida, L.E. Jr.: Release of microorganisms from soil with respect to transmission electron microscopy viewing and plate counts. Antonie van Leeuwenhoek 43: 73-87 (1977).

Becker, J. M., Caldwell, G.A., Zachgo, E.A.: Biotechnology- A Laboratory Course. Academic Press, Inc. (1990).

Bradford, D., Hugenholtz, P., Sevoiur, E.M., Cunningham, M.A., Stratton, H., Seviour, R.J., Blackall, L.L.: 16S rRNA analysis of isolates obtained from gram-negative, filamentous bacteria micromanipulated from activated sludge. System. Appl. Microbiol. 19: 334-343 (1996).

Britschgi, T.B., Giovannoni, S.J.: Phylogenetic analysis of a natural marine bacterioplankton population by rRNA gene cloning and sequencing. Applied and Environmental Microbiology 57: 1707-1713 (1991).

Bruns, T.D., Szaro, T.M.: Rate and mode differences between nuclear and mitochondrial small-subunit rRNA genes in mushrooms. Mol. Biol. Evol. 9(5): 836-855 (1992).

Busse, H., El-Banna, T., Oyaizu, H., Auling, G.: Identification of xenobiotic-degrading isolates from the beta subclass of the Proteobacteria by a polyphasic approach including 16S rRNA partial sequencing. International Journal of Systematic Bacteriology 42: 19-26 (1992).

Campbell, L., Carpenter, E.J., Iacano, V.J.: Identification and enumeration of marine chroococcoid Cyanobacteria by immunofluorescence. Applied and Environmental Microbiology 46(3): 553-559 (1983).

Cardarelli-Leite, P., Blom, K., Patton, C.M., Nicholson, M.A., Steigerwalt, A.G., Hunter, S.B., Brenner, D.J., Barrett, T.J., Swaminathan, B.: Rapid identification of Campylobacter species by restriction fragment length polymorphism analysis of a PCR-amplified fragment of the gene coding for 16S rRNA. Journal of Clinical Microbiology 34: 62-67 (1996).

Chandler, D.P., Schreckhise, R.W., Smith, J, L., Bolton Jr. H.: Electroelution to remove humic compounds from soil DNA and RNA extracts. Journal of Microbiological Methods 28: 11-19 (1997).

Clayton, R.A., Sutton, G., Hinkle, Jr. P.S., Bult, C., Fields, C.: Intraspecific variation in small subunit rRNA sequences in GenBank: Why single sequences may not adequately represent prokaryotic taxa. International Journal of Systematic Bacteriology 45(3): 595-599 (1995).

DeLong, E.F., Wickham, G.S., Pace, N.R.: Phylogenetic strains: Ribosomal RNA-based probes for the identification of single cells. Science 243: 1360-1363 (1989).

Doronina, N.V., Sokolov, A.P., Trotsenko, Y.A.: Isolation and initial characterization of aerobic chloromethane-utilizing bacteria. FEMS Microbiology Letters 142: 179-183 (1996).

Eberl, Leo, Schulze, R., Ammendola , A., Geisenberger, O., Erhart, R., Sternberg, C., Molin, S., Amann, R.: Use of green fluorescent protein as a marker for ecological studies of activated sludge communities. FEMS Microbiology Letters 149: 77-83 (1997).

Elsas van, J.D., Mantynen, V., Wolters, A.C.: Soil DNA extraction of the fate of Mycobacterium chlorophenolicum strain PCP-1 in different soils by 16S ribosomal RNA gene sequence based most-probable number PCR and immunofluorescence. Biol. Fertil. Soils 24: 188-195 (1997).

Erb, R.W., Wagner-Dobler, I.: Detection of polychlorinated biphenyl degradation genes in polluted sediments by direct DNA extraction and polymerase chain reaction. Applied and Environmental Microbiology 59(12): 4065-4073 (1993).

Farrelly, V., Rainey, F.A., Stackebrandt, E.: Effect of genome size and rrn gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. Applied and Environmental microbiology 61(7): 2798-2801 (1995).

Ganczarczyk, J.J.: Activated sludge process: Theory and practice. Pollution engineering and technology 23: (1983)

Giovannoni, S.J., DeLong, E.F., Olsen, G.J., Pace, N. R.: Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial cells. Journal of Bacteriology 170: 720-726 (1988).

Gobel, U.B.: Phylogenetic amplification for the detection of uncultured bacteria and the analysis of complex microbiota. Journal of Microbiological methods 23: 117-128 (1995).

Gray, J.P., Herwig, R. P.: Phylogenetic analysis of the bacterial communities in marine sediments. Applied and Environmental Microbiolgy 62: 4049-4059 (1996).

Haddad, A., Camacho, F., Durand, P., Cary, S. C.: Phylogenetic characterization of the epibiotic bacteria associated with the hydrothermal vent polychaete Alvinella pompejana. Applied and Environmental Microbiology 61: 1679-1687 (1995).

Harder, W., Attwood, M.M.: Biology, physiology and biochemistry of Hyphomicrobia. Adv. Microbial Physiol. 17: 303-359 (1978).

Hillis, D. M., Huelsenbeck, J. P., Cunningham C.W.: Application and accuracy of molecular phylogenies. Science 264; 671-677 (1994).

Holm, N. C., Gliesche, C.G, Hirsch, P.: Diversity and structure of Hyphomicrobium populations in a sewage treatment plant and its adjacent receiving lake. Applied and Environmental Microbiology 62(2): 522-528 (1996).

Horan, N.J.: Biological waste water treatment systems: Theory and operations. John Wiley & Sons, Chichester, England (1990).

Innis, M. A., Gelfand, D. H., Sninsky, J.J., White, T.J.: PCR Protocols: A guide to methods and applications. Academic Press, Inc. San Diego, CA (1990).

Jannasch, H.W., Jones, G. E.: Bacterial populations in sea water as determined by different methods of enumeration. Limnol. Oceanogr. 4 (2): 128-139 (1959).

Jenkins, D., et. al. Manual on the causes and control of activated sludge bulking and foaming. Pretoria, SA: Water Research Commission (1986).

Junkins, R., Deeny, K., Eckhoff, T.: The activated sludge process: Fundamentals of operation. Ann Arbor Science Publishers, Ann Arbor, MI (1983).

Kampfer, P., Erhart, R., Beimfohr, C., Bohringer, J., Wagner, M., Amann, R.: Characterization of bacterial communities from activated sludge: Culture-dependent numerical identification versus in situ identification using group- and genus- specific rRNA-targeted oligonucleotide probes. Microbial Ecology 32: 101-121 (1996).

Kjelleberg, S., Albertson, N., Flardh,K., Holmquist, L., Jouper-Jaan, A., Marouga, R., Ostling, J., Svenblad, B., Weichart, D.: How do non-differentiating bacteria adapt to starvation? Antonie von Leeuwenhock 63: 333-341 (1993).

Lane, D.J., Pace, B., Olsen, G.J., Stahl, D. A., Sogin, M. L., Pace, N. R.: Rapid determination of 16S ribosomal RNA sequences for phylogenetic analysis. Proc. Natl. Acad. Sci 82: 6955-6959 (1985).

Lange, M., Guillou, L., Vaulot, D., Simon, N., Amann, R., Ludwig, W., Medlin, L.K.: Identification of the class Prymnesiophyceae and the genus Phaeocystis with ribosomal RNA-targeted nucleic acid probes detected by flow cytometry. J. Phycol. 32: 858-868 (1996).

Langendijk, P.S., Schut, F., Jansen, G.J., Raangs, G.C., Kamphuis, G.R., Wilkinson, M.H.F., Welling, G.W.: Quantitative Fluorescence in situ hybridization of Bifidobacterium spp. With genus-specific 16S rRNA targeted probes and its application in fecal samples. Applied and Environmental Microbiology 61(8): 3069-3075 (1995).

Lee, N.M., Welander, T.: The effect of different carbon sources on respiratory denitrification in biological wastewater treatment. Journal of Fermentation and Bioengineering 82(3): 277-285 (1996).

Lee, S., Bollinger, J., Bezdicek, D., Ogram, A.: Estimation of the abundance of an uncultured soil bacterial strain by a competitive quantitative PCR method. Applied and Environmental Microbiology 62(10): 3787-3793 (1996).

Lee, S.H.: Malone, C., Kemp, P.F.: Use of multiple 16S rRNA targeted fluorescent probes to increase signal strength and measure cellular RNA from natural planktonic bacteria. Marine Ecology Progress Series 101: 193-201 (1993).

Liesack, W., Weyland, H., Stackebrandt, E.: Potential risks of gene amplification by PCR as determined by 16S rDNA analysis of a mixed-culture of strict barophilic bacteria. Microbial Ecology 21: 191-198 (1991).

Maidak, B.L., Olsen, G.J., Larsen, N., Overbeek,R., McCaughey, M.J.: Woese, C.R.: The RDP (Ribosomal Database Project) Nucleic Acid Research 25(1): 109-110 (1997).

Maniatis, T., Fritsch, E.F., Sambrook, J.: Molecular Cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y (1989).

Manz, W., Amann, R., Ludwig, W., Wagner, M., Schleifer, K.: Phylogenetic oligodeoxynucleotide probes for the major subclasses of Proteobacteria: Problems and Solutions. System. Appl. Microbiol. 15: 593-600 (1992)

Martinez-Murcia, A.J., Acinas, S.G., Rodriguez-valera, F.: Evaluation of prokaryotic diversity by restrictase digestion of 16S rDNA directly amplified from hypersaline environments. FEMS Microbiology Ecology 17: 247-256 (1995).

Meyers, A.J., Meyers, C.D.: Hyphomicrobium-mediated sludge bulking in an industrial wastewater treatment system. Presented at the 86th General Meeting of the American Society for Mcrobiology, March 23-28 (1986).

More, M.I., Herrick, J.B., Silva, M.C., Ghiorse, W.C., Madsen, E. L.: Quantitative cell lysis of indigenous microorganisms and rapid extraction of microbial DNA from sediment. Applied and Environmental Microbiology 60(5): 1572-1580 (1994).

Moyer, C.L., Dobbs, F,C., and Karl, D. M.: Estimation of diversity and community structure through RFLP distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. Applied and Environmental Microbiology 60: 871-879 (1994).

Mullis, K.F., Faloona, S., Scharf, R., Saiki, G., Horn: Specific enzymatic amplification of DNA invitro: The polymerase chain reaction. Cold Spring Harb. Symp.Quant. Biol. 51: 263-273 (1986).

Nubel, U., Engelen, B., Felske, A., Snaidr, J., Wieshuber, A., Amann, R., Ludwig, W., Backhaus, Horst.: Sequence heterogeneities of genes encoding 16S rRNAs in Paenibacillus polymyxa detected by temperature gradient gel electrophoresis. Journal of Bacteriology 178: 5636-5643 (1996).

Ogram, A., Sayler, G. S., Barkay, T.: The extraction and purification of microbial DNA from sediments. Journal of Microbiological Methods 7: 57-66 (1987).

Olsen, G.J., Lane, D. J., Giovannoni, S.J., Pace, N. R.: Microbial ecology and evolution: A ribosomal RNA approach. Ann. Rev. Microbiol. 40: 337-365 (1986).

Pace, N. R.: New perspective on the natural microbial world: molecular microbial ecology. ASM News 62(9): 463-470 (1996).

Pillai. S.D., Josephson, K.L., Bailey, R.L., Gerba, C.P., Pepper, I. L.: Rapid method for processing soil samples for polymerase chain reaction amplification of specific gene sequences. Applied and Environmental Microbiology 57(8): 2283-2286 (1991).

Pitulle, C., Dsouza, L., Fox, G. E.: A low molecular weight artificial RNA of unique size with multiple probe target regions. System. Appl. Microbiol. 20: 133-136 (1997).

Polz, M.F., Cavanaugh, C.M.: A simple method for quantification of uncultured microorganisms in the environment based on in vitro transcription of 16S rRNA. Applied and Environmental Microbiology 63(3): 1028-1033 (1997).

Poulsen, L.K., Ballard, G., Stahl, D. A.: Use of rRNA fluorescence in situ hybridization for measuring the activity of single cells in young and established biofilms. Applied and Environmental Microbiology 59(5): 1354-1360 (1993).

Priest, F.G., Ramos-Cormenzana, A., Tindall, B.J.: Bacterial Diversity and Systematics. FEMS Symposium No.75. Plenum Press, NY (1994).

Promega. Technical Manual 16. Promega , Madison, WI (1992).

Promega Corporation manual: Tips for optimizing PCR and PCR-related protocols (1995).

Poulsen, L.K., Ballard, G., Stahl, D.A.: Use of rRNA fluorescence in situ hybridization for measuring the activity of single cells in young and established biofilms. Applied and Environmental Microbiology 59(5): 1354-1360 (1993).

Reyes, F.L., Ritter, W., Raskin, L.: Group-specific small-subunit rRNA hybridization probes to characterize filamentous foaming in activated sludge systems. Applied and Environmental Microbiology 63(3): 1107-1117 (1997).

Reysenbach, A., Giver, L.J., Wickham, G. S., Pace, N. R.: Differential amplification of rRNA genes by polymerase chain reaction. Applied and Environmental Microbiology 58(10): 3417-3418 (1992).

Rice, J., Sleigh, M.A., Burkill, P.H., Tarran, G.A., David O'Connor, C., Zubkov, M.V.: Flow cytometric analysis of characteristics of hybridization of species-specific fluorescent oligonucleotide probes to rRNA of marine nanoflagellates. Applied and Environmental Microbiology 63(3): 938-944 (1997).

Ross, J., Boon, P.I., Sharma, R., Beckett, R.: Variations in the fluorescence intensity of intact DAPI-stained bacteria and their implications for rapid bacterial quantification. Letters in Applied Microbiology 22: 283-287 (1996).

Sayler, G. S., Shields, M. S., Tedford, E. T., Breen, A., Hooper, S. W., Sirotkin, K. M., Davis, J.W.: Application of DNA-DNA colony hybridization to the detection of catabolic genotypes in environmental samples. Applied and Environmental Microbiology 49(5): 1295-1303 (1985).

Sayler, G. S., Layton, A. C.: Environmental application of nucleic acid hybridization. Annu. Rev. Microbiol 44: 625-648 (1990).

Schleifer, K.H., Stackebrandt, E.: Molecular systematics of prokaryotes. Ann. Rev. Microbiol. 37: 143-187 (1983).

Schmidt, T. M., DeLong, E.F., Pace, N. R.: Analysis of a marine picoplankton community by 16S rRNA gene cloning and sequencing. Journal of Bacteriology 173(14): 4371-4378 (1991).

Schofield, P., Pell, A.N., Krause, D.O.: Molecular Beacons: Trial of a Fluorescence-based solution hybridization technique for ecological studies with ruminal bacteria. Applied and Environmental Microbiology 63(3): 1143-1147 (1997).

Schuppler, M., Mertens, F., Schon, G., Gobel, U.B.: Molecular characterization of nocardioform actinomycetes in activated sludge by 16S rRNA analysis. Microbiology 141: 513-521 (1995).

Shin, Y.K., Hiraishi, A., Sugiyama, J.: Molecular systematics of the genus Zoogloea and emendation of the genus. Int. J. Syst. Bact. 43: 826-831 (1993).

Siering, P.L., Ghiorse, W.C.: Development and application of 16S rRNA-targeted probes for detection of iron- and magnesium-oxidizing sheathed bacteria in environmental samples. Applied and Environmental Microbiology, 63(2): 644-651 (1997).

Snaidr, J., Amann, R., Huber, I., Ludwig, W., Schleifer, K.: Phylogenetic analysis and in situ identification of bacteria in activated sludge. Applied and Environmental Microbiology 63 (7): 2884-2896 (1997).

Spring, S., Amann, R., Ludwig, W., Schleifer, K., Petersen, N.: Phylogenetic diversity and identification of nonculturable Magnetotactic bacteria. System. Appl. Microbiol. 15: 116-122 (1992).

Stackebrandt, E., Fischer, A., Roggentin, T., Wehmeyer, U., Bomar, D., Smida, J.: A phylogenetic survey of budding, and/or prosthecate, non-phototrophic eubacteria: membership of Hyphomicrobium, Hyphomonas, Pedomicrobium, Filomicrobium, Caulobacter and 'dichotomicrobium' to the alpha-subdivision of purple non-sulfur bacteria. Arch. Microbiol. 149 (6): 547-556 (1988).

Stackebrandt, E., Goodfellow, M.: Nucleic acid techniques in bacterial systematics (1991).

Stahl, D.A.: Application of phylogenetically based hybridization probes to microbial ecology. Molecular Ecology 4: 535-542 (1995).

Staley, J.T., Fuerst, J.A.: Budding and/or appendaged bacteria. Bergey's Manual of Bacteriology (Section 21): 1890-1904 (1994)

Suzuki, M. T., Giovannoni, S.J.: Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. Applied and Environmental Microbiology 62(2): 625-630 (1996).

Suzuki, M. T., Rappe, M. S., Haimberger, Z.W., Winfield, H., Adair, N., Strobel, J., Giovannoni, S.J.: Bacterial diversity among small-subunit rRNA gene clones and cellular

isolates from the same seawater sample. Applied and Environmental Microbiology 63(3): 983-989 (1997).

Tebbe, C.C., Vahjen, W.: Interference of humic acids and DNA extracted directly from soil in detection and transformation of recombinant DNA from bacteria and a yeast. Applied and Environmental Microbiology 59(8): 2657-2665 (1993).

Torsvik, V., Sorheim, R., Goksoyr, J.: Total bacterial diversity in soil and sediment communities - a review. Journal of Industrial Microbiology 17: 170-178 (1996).

Van de Peer, Y., & De Wachter, R.: Treecon: a software package for the construction and drawing of evolutionary trees. Comput Appl. Biosci 9: 177-182 (1993).

Van de Peer, Y., Jansen, J., Peter de Rijk and Rupert de Wachter.: Database on the structure of small ribosomal subunit RNA. Nucleic Acids Research 25(1): 111-116 (1997).

Voytek, M.A., Ward, B.B.: Detection of ammonium-oxidizing bacteria of the beta-subclass of the class Proteobacteria in aquatic samples with the PCR. Applied and Environmental Microbiology 61(4): 1444-1450 (1995).

Wagner, M., Amann, R., Lemmer, H., Schleifer, K.: Probing activated sludge with oligonucleotides specific for Proteobacteria: Inadequacy of culture dependent methods for describing microbial community structure. Applied and Environmental Microbiology 59(5): 1520-1525 (1993).

Wagner, M., Amann, R., Kampfer, P., Assmus, B., Hartmann, A., Hutzler, P., Springer, N., Schleifer, K.: Identification and in situ detection of gram-negative filamentous bacteria in activated sludge. Syst. Appl. Microbiol. 17: 405-417 (1994).

Wagner, M., Erhart, R., Manz, W., Amann, R., Lemmer, H., Wedi, D., Schleifer, K.: Development of an rRNA-targeted oligonucleotide probe specific for the genus Acinetobacter and its application for in situ monitoring in activated sludge. Applied and Environmental Microbiology 60(3): 792-800 (1994).

Wagner, M., Rath, G., Koops, H., Flood. J., Amann, R.: In situ analysis of denitrifying bacteria in sewage treatment plants. Wat. Sci. Tech. 34(1-2): 237-244 (1996).

Wallner, G., Steinmetz, I., Bitter-suremann, D., Amann, R.: Combination of rRNA-targeted hybridization probes and immuno-probes for the identification of bacteria by flow cytometry. System. Appl. Microbiol. 19: 569-576 (1996).

Wanner, J., Grau, P.: Identification of filamentous microorganisms from activated sludge: a compromise between wishes, needs and possibilities. Wat. Res. 23(7): 883-891 (1989).

Ward, D.M., Bateson, M.M., Weller, R., Ruff-Roberts, A.L.: Ribosomal RNA analysis of microorganisms as they occur in nature. Advances in Microbial Ecology 12: 219-287 (1992).

Weisburg, W.G., Barns, S.M., Palletier, D.A., Lane, D.J.: 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology 173: 697-703 (1991).

Weiss, P., Schweitzer, B., Amann, R., Simon, M.: Identification in situ and dynamics of bacteria on limnetic organic aggregates (Lake Snow). Applied and Environmental Microbiology 62(6): 1998-2005 (1996).

Weller, R., Ward, D. M.: Selective recovery of 16S rRNA sequences from natural microbial communities in the form of cDNA. Applied and Environmental Microbiology, 55(7): 1818-1822 (1989).

Wills, C.: Phylogenetic analysis and molecular evolution. Biocomputing - informatics and genome projects 175-201 (1994).

Woese, C.R., Fox, G.E.: Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc. Natl. Acad. Sci 74: 5088-5090 (1977).

Woese, C.R., Maniloff, J., Zablen, L.B.: Phylogenetic analysis of the mycoplasmas. Proc. Natl. Acad. Sci 77: 494-498 (1980).

Woese, C.R.: Bacterial Evolution. Microbiological Reviews 51(2): 221-271 (1987).

Woese, C.R., Maloy, S., Mandelco and Raj, H.D.: Phylogenetic placement of the *Spirosomaceae*. System. Appl. Microbiol 13: 19-23 (1990).

Yoon, J.H., Lee, S.T., Kim, S., Kim, W,Y., Goodfellow, M., Park, Y.: Restriction fragment length polymorphism analysis of PCR-amplified 16S ribosomal DNA for rapid identification of Saccharomonospora strains. International Journal of Systematic Bacteriology 47(1): 111-114 (1997).

Zimmermann, R.A., Dahlberg, A.E.: Ribosomal RNA: structure, evolution, processing and function in protein biosynthesis. CRC Press, Inc. (1996).

APPENDICES

Appendix A: Partial 16S rDNA sequences of the clones of January, April and November sludge samples.

jan1

```
  1 ATCSTCMTAG CAATAGGCAG aGGGACAGGT GTTGCATGGC WGTCGTCAGC
 51 TCRTGTCGTG AGATGTWGGG TTAAGTCCCG CAACGAGCGC AACCCTCGCC
101 ATTAGTWGCC ATCATTCAGT TGGGCACTCT AGTGGGACTG CCGGTGATAA
151 GCCGGAGGAA GGTGGGGATG ACGTCAAGTC ATCATGGCCC TTATGGGCTG
201 GGATACACAC GTGCTACAAT GGCGGTGACA ATGCGCAGCC ACTCAGCAAT
251 GAGGAGCTAA TCGCAAAAAG CCGTCTCAGT TCGGATTGGG GTCTGCAACT
301 CGACCCCATG AAGTCGGAAT CGCTAGTAAT CGCGCATCAG CACGGCGCGG
351 TGAATACGTT CCCGGGCMTT GTACACACCG CCCGTCACAC CATGGGAGTT
401 GGCTTTACCC GAAGGTAGTG CGCTAACCGC AAGGAGGCAG CTAACCACGG
451 TAAGTCAGVG
```

jan2

```
  1 NGTCGTCAGM TCGTGTCGTG AGATGKKGGG TTAAGTCCCG CAACGAGCGC
 51 RACCCTTGTC ATTAGTKGNT ACGAAAGGGC ACTcTAATGA GACTGCCGGT
101 GACAAACCGG AGGAAGGTGG GGATGACGTC AAGTCcTCAT GGCCCTTATA
151 GGTGGGGCTA CACACGTCAT ACAATGGCCG GTACAAAGGG TAGCCAACCC
201 GCGAGGGGGA GCCAATCCCA CAAAGCCGGT CGTAGTCCGG ATCGCAGTCT
251 GCAACTCGAC TGCGTGAAGT CGGAATCGCT AGTAATCGTG GATCAGCATG
301 TCACGGTGAA TACGTTCCCG GGTCTTGTAC ACACCGCCCG TCACACCATG
351 GGAGcGGGTT CTGCCAGAAG TAGTTAGCCT AACCGCAAGG GGGGCGATTA
401 CCACGGCAGG TTCGTGACTG G
```

jan3

```
  1 NTKNTSGGTC GGMTTGCCGA TCAGWGACGC AGCTAAACGC AGTAAGCCCT
 51 CCCGCNTGGG GAGTACGGTC GCAAGATTNA AACTCAAAGG AATTGACGGG
101 GGCCCGCACA AGCGGTGGAG CATGTGGTTT AATTCGAAGC AACGCGAAGA
151 ACCWTACCAG CTCTTGACAT TCACTGACKG TTCCCAGAGA TGGGATCGTC
201 CTAGCAATAG GCAGTGGGAC AGGTGCTGCA TGGCTGTCGT CAGCTCGTGT
251 CGTGAGATGT TGGGTTAAGT CCCGCAACGA GCGCAACCCT TGTCATTAGT
301 TGCTACGAAA GGGCACTMTA ATGAGACTGC CGGTGACAAA CCGGAGGAAG
351 GTGGGGATGA CGTCAAGTCC TCATGGCCCT TATAGGTGGG GCTACACACG
401 TCATACAATG GCCGGTACAA AGGGTAGCCA ACCCGCGAGG GGGAGCCAAT
451 CCCACAAAGC CGGTCGTAGT CCGGATCGCA GTCTGCAACT CGAMTGCGTG
501 AAGTCGGAAT CGSTAGTAAT CGTGGATCAG CATGTCACGG TGAATACGTT
551 CCCGGGCCTT GTACACACCG CCCGTCACAC CATGGGAGTG GGTTGCTCCA
601 GAAGTAGCTA GTCTAACCGC AAGGGGGACG TTACCACGGA GTGWTCATGA
```

jan4

```
  1 AATTCAAAGG AATTGACGGG ACCCGCACAA GCGGTGGATG ATGTGGTTTA
 51 ATTCGATGCA ACGCGAAAAA CCTTACCCAC CTTTGACATG CACGGAACCC
101 TTTGGAAACA GAGGGGTGCT CGAAAGAGAG CCGTAACACA GGTGCTGCAT
```

151 GGCTGTCGTC AGCTCGTGTC GTGAGATGTT GGGTTAAGTC CCGCAACGAG
201 CGCAACCCTT GTCATTAGTT GCTACGAAAG GGCACTCTAA TGAGACTGCC
251 GGTGACAAGC CGGAGGAAGG TGGGGATGAC GTCAAGTCCT CATGGCCCTT
301 ATAGGTGGGG CTACACACGT CATACAATGG CTGGTACAGA GGGTTGCCAA
351 CCCGCGAGGG GGAGCCAATC CCATAAAACC AGTCGTAGTC CGGATCGCAG
401 TCTGCAACTC GACTGCGTGA AGTCGGAATC GCTAGTAATC GCGGATCAGC
451 ATGTCGCGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
501 ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA AGGAGGGCGA
551 TTACCACGGC AGGTTCGTGA CT

jan5

1 NTWGACATCC CGGGACGGTT TCCAGAGRTG GGATCcTCCT AGCAATAGGC
51 CCGGAGACAG GTGTTGCAWG GSNGTCGTCA GCTYGTGTYG CGAGAWGWTG
101 GGTTAAGTCC CGWAACGAGS GCAACCCTTG TCCTTAGTTG CCAGCACTTc
151 GGGTGGGAAC TCTAAGGAGA cTGCCGGTGA CAAACCGGAG GAAGGTGGGG
201 ACGAcGTCAA GTCATCATGG CCCTtACGGC MTGGGCcACA CACGTGcTAC
251 AATGGMTGGT ACAGAGGGTC GCGAAGCCGC GAGGTGGAGC TAATCCCAAA
301 AAACCGGTCG TAGTCCGGAT CGGAGTcTGC AACTCGACTC CGTGAAGTCG
351 GAATCGCTAG TAATCGCGAA TCAGAATGTC GCGGWGAGTA CGTTCCCGGG
401 CCTTGTACAC ACCGCCCGTC ACACCATGGG AGTGGGTWGC AAAAGAAGTG
451 GGTAGTCTAA CSTTCGGGAG GGCGCTCACC ACTTTGTGAT CATG

jan6

1 NGCAGCWAAA CGCRTTAAGC ATCCCCGCNT GGGGAGWACG GCCGCAAGGC
51 TAAAACTCAA AGGAATTGAC GGGGGCCCGC ACAAGCGGTG GAGCATGNGG
101 TTTAATTNGA AGCAACGCGA AGAACCTTAC CAGCTCTTGA CATTCACTGA
151 CTGTTCCCAG AGATGGGTTC GTCCTAGCAA TAGGCAGTGG GACAGGTGCT
201 GCATGGCTGT CGTCAGCTCG TGCCGTGAGA TGTTGGGTTA AGTCCCGCAA
251 CGAGCGCAAC CCTCGCCATT AGTTGCCATC ATTAAGTTGG GCACTCTAGT
301 GGGACTGCCG GTGATAAGCC GGAGGAAGGT GGGGATGACG TCAAGTCATC
351 ATGGCCCTTA TGGGCTGGGC TACACACGTG CTACAATGGC GATGACAGTG
401 GGCAGCTACA CAGCAATGTG ATGCTAATCT CTAAAAGCCG TCTCAGTTCG
451 GATTGGGGTC TGCAACTCGA CCCCATGAAG TCGGAATCGC TAGTAATCGC
501 GCATCAGCAC GGCGCGGTGA ATACGTTCCC GGGCCTTGCA CACACCGCCC
551 GTCACACCAT GGGAGTTGGT CATACCCTAA AACGGTGCGC TAACCGCAAG
601 GAGGCAGCCG GCCACGGTAT GGTCAGCGAC TGGG

jan7

1 NGTTGAAACT CAAAGGAATT GAYGGGACCC GCACAAGCGG TGGATGAKTG
51 GTNKAAWTCG KGCAACGCGA AAAACNTTAC CCACSTTTGA MATGTACGGA
101 ACCCTTTGGA GACAGAGGGG TGCTCGAAAG AGAGCCGTAA CACAGGTGCT
151 GCATGGCWGT CGTCAGCTMG WGTCGTGAGA TGTTGGGTTA AGTCCCGCAA
201 CGAGCGCAAC CMTTGTCATT AGTTGMTANG AAAGGGCACT cTAATGAGAC
251 TGCCGGTGAC AAGCCGGAGG AAGGTGGGGA CGACGTCAAG TCCTCATGGC
301 CCTKATAGGT GGGGMTACAC ACGTCATACA ATGGCTGGTA CAGAGGGTTG
351 CCAACCCGCG AGGGGGAGCC AATCCCATAA AACCAGTCGT AGTCCGGATC
401 GCAGTCTGCA ACTCGAcTGC GTGAAGTCGG AATCGCTAGT AATCGCGGAT

451 CAGCATGTcG CGGCGAATAC GTTCCCGGGT cTTGTACACA CCGCCCGTCA
501 CACCATGGGA GCGGGTTCTG CCAGAAGTAG TTAGCCTAAC CGCAAGGAGG
551 GCGATTACCA CGGCAGGTT

jan8

1 ANGAATTGAC GGGGGCNTGC ACAAGCSGKW GGAGCATGTG GTTTAACTCG
51 AAGCAACGCG CAGAACCTTA CCRGCGTWWG ACATCCTCAT CGCGATTTCC
101 AGAGADGGAT TTCTTCAGTT CGGCTGGATG AGWGACAGGT GCTGCAtGGC
151 TGTCGTCAGC TCGTGTCGaG AGATGTTGGG TTAAGTCCCG CAACGAGCGC
201 AACCcTCGTC CTTAGTTGCC ATCATTTAGT TGGGCACTCT AAGGAAACCG
251 CCGGTGATAA GCCGGAGGAA GGTGGGGAWG ACGTCAAGTC cTCATGGCCC
301 TTACACGCTG GGCTACACAC GtGCTACAAT GGCGGTGACA GTGGGCAGCC
351 ACCTCGCGAG AGGGAGCTAA TCCCAAAAAG CCGTCTCAGT TCGGATTGTT
401 CTCTGCAACT CGAGAGCATG AAGGCGGAAT CGcTAGTAAT CGCGGATCAG
451 CATGCCGCGG TGAATACGTT CCCAGGCCTT GTACACACCG CCCGTCACAC
501 CATGGGAGTT GGATTCAcTC GAAGGCGTTG AGCTAACCCG CAAGGGAGGC
551 AGGCGACCAC AGTGGTTTAG CGACT

jan9

1 TGTGCCGAAG TTAACGCATT AAGCATCCCG CMTGGGGAGT ACGGTCGCAA
51 GATTGAAACT CAAAGGAATT GGCGGGGGCC CGCACAAGCG GTGGAGCATG
101 TGGTWTAACT MGATGCAAAG CGAAGAACCT TACCAAGGCT TGACATACTG
151 GCTCAAGCTA ATGTGAAAGC ATTTTGTGTW NTAAGGCTTG CCTWACATAG
201 TCAGTACAGG TGCTGCATGG CWGTCGTCAG CCCGTGCCGT GAGGTGTCCG
251 GTTAAGTCCG GTAACGAGCG CAACCCCTAT GTTTAGTTGC CAGCACGTAA
301 GGTGGGAACT MTAAAGAGAC TGCCGGCGAC AAGCCGGAGG AAGGCGGGGA
351 CGACGTCAAG TCATCATGGC CCTTATGCTT TGGGCTACAC ACATGCTACA
401 ATGGCGGATA CAATGGGTTG CCAAACTGCG AAGTGGAGCC AATCCCATCA
451 AAGTCCGTCT CAGTTCGGAT TGCAGGCTGC AACTcGCCTG CATGAAGTTG
501 GAATCGtTAG TAATCGTGGA TCAGCACTGC CACGGTGAAT ACGTTCCCGG
551 GCCTTGCACA CACCGCCCGT CACACCACGA AAGTCGGTTC TACCCGAAGT
601 GGTTGAGCTA ACCCGCAAGG GAGGCAGGCT CCCTAAGGTA GGGCTGGTAA
651 WT

jan10

1 AACKGGTTGT NGGGTCTTTA CTGACTCAGT AACGAAGCTA ACGCGTGAAG
51 TTGACCGCCT GGGGAGTACG GCCGCAAGGT TGAAACTCAA AGGAATTGAC
101 KGGGACCCGC ACAAGCGGTG GATGAWGTGG TTTAATTCGA TGCAACGCGA
151 AAAACCTTAC CCACCTKTGA CATGTACGGA ACCCTTTGGA GACAGAGGGG
201 TGcTCGAAAG AGAGCCGTAA CACAGGTGMT GCATGGCTGT cGTCAGCTCG
251 TGTCGTGAGA TGTTGGGTTA AGTCCCGCAA CGAGCGCAAC CCTTGTCATT
301 AGTTGCTACG AAGGGGCACT cTAATGAGAC TGCCGGTGAC AAGCCGGAGG
351 AAGGTGGGGA TGACGTCAAG TCCTCATGGC CCTTATAGGT GGGGMTACAC
401 ACGTCATACA ATGGMTGGTA CAGAGGGTTG CCAACCCGCG AGGGGGAGCC
451 AATCCCATAA AACCAGTBGT AGTCCGGATC GCAGTCTGCA ACTCGACTGC
501 GTGAAGTCGG AATCGCTAGT AATCGCGGAT CAGCATGTCG CGGTGAATAC
551 GTTCCCGGGT CTWGTACACA CCGCCCGTCA CACCATGGGA GCGGGTTCTG
601 CCAGAAGTAG TTAGCCTAAC CGCAAGGAGG GCGATTACCA CGGCAGGTTC

651 GTGA

jan11

1 AAAGGGAGCC TGGACACAGG KGMWGCATGG CKGTMGTCAG CTMGWGTCGT
51 GAGATGWTGG GTWAAGTCCC GCAARCGAGC GCAACCSTTA TCCTTAGTTG
101 MTACGCAAGG GCASTMTAAG GAGACTGCCG GTGACAAACC GGAGGAAGGT
151 GGGGATGACG TCAAGTCCTC ATGGCCCTTA TGSGCAGGGC TTCACACGTC
201 ATACAATGGT CGGTACAGAG GGTCGCCAAC CCGCGAGGGG GAGCTAATCC
251 CATAAAGCCA GTCGTAGTCC GGATCGCAGT CTGCAACTCG ACTGCGTGAA
301 GTCGGAATCG CTAGTAATCG CGGATCAGAA TGTCGCGGTG AATACGtTCC
351 CGSGTCTTGT ACACACCGCC CGTCACACCA TGGGAGcGGG TTCTGCCAGA
401 AGTAGTTAGC CTAACCGCAA GGAGGGCGAT TACCACGGCA GGT

jan12

1 NTWTGTTGGG TCTTAGCTGA NTCAGTAAAC GAAGMTAACG CGTGAAGTTG
51 ACCGCCTGGG GAGTACGGCC GCAAGGTTGA AACTCAAAGG AATTGACGGG
101 GACCCGCAAC AAGCGGWGGA TGATGTGGTT TAATTCGATG CAACGCGAAA
151 AACCTTACCC ACCTTTGACA TGTACGGAAC CCTTTGGAGA CAGAGGGGTG
201 CTCGAAAGAG AGCCGTAACA CAGGTGCTGC ATGGCTGTCG TCAACTCGTG
251 TCGTGAGATG WTGGGTTAAG TCCCGCAACG AGCGCAACCC TTGTCATTAG
301 TTGCTACGAA AGGGCACTMT AATGAGACTG CCGGTGACAA GCCGGAGGAA
351 GGTGGGGATG ACGTCAAGTC CTCATGGCCC TTATAGGTGG GGCTACACAC
401 GTCATACAAT GGCTGGTACA GAGGGTTGCC AACCCGCGAG GGGGAGCCAA
451 TCCCATAAAA CCAGTCGTAG TCCGGATCGC AGTCTGCAAC TCGACTGCGT
501 GAAGTCGGAA TCGCTAGTAA TCGCGGATCA GCATGTCGCG GTGAATACGT
551 TCCCGGGTCT TGTACACACC GCCCGTCACA CCATGGGAGC GGGTTCTGCC
601 AGAAGTAGTT AGCCTAACCG CAAGGAGGGC GATTACCACG GCAGGTTCGW
651 GANT

jan13

1 NKGNTNGAAA GAGAGCCGTN ACACAGGTGC WGMATGGCKG TCGTCAGCTC
51 GTGTCVWGAG ATGTTGGGAT AAGTCCCGCA ACGAGSGCAA CCSTNGTCAT
101 TAGTTGCWAC GRAAGGGCAC TMTAATGAGA NTGCCGGTGA CAAACCGGAG
151 GAAGGTGGGG AWGACGTCAA GTCCTCATGG CCCTTATAGG TGGGGCTACA
201 CACGTCATAC AATGGCTGGT ACAGAGGGTT GCCAACCCGC GAGGGGGAGC
251 TAATCCCATA AAACCAGTCG TAGTCCGGAT CGCAGTCTGC AACTCGACTG
301 CGTGAAGTCG GAATCGCTAG TAATCGCGGA TCAGCATGTc GCGGTGAATA
351 CGTTCCCGgG TCTTGTACAC ACCGCCCGTC ACACCATGGG AGcGGGTTCT
401 GCCAGAAGTA GTTAGCCTAA CCGCAAGGAG GGCGATTACC ACGGCAGGTT

jan14

1 GCNGTAAACG ATGAATGCCA GCCGTCGGGA AGMTTGCTTT TCSGGTGGMG
51 CRGMTAACGC TTTAAGCATT CCGCCTGGGG AGTACGGTCG CAAGATTAAA
101 ACTCAAAGGA ATTGACGGGG GCCCGCACAA GCGGTGGAGC ATGTGGTTTA
151 ATTCGAAGCA ACGCGCAGAA CCTTACCGGC TSTTGACATG CCACGACGAC
201 TTCCAGAGAT GGATTTCTTC CCTTCGGGGA CGTGGACACA GGWGCWGCAT

251 GGCWGTCGTC AGCTCGWGTC GTGAGATGWT GGGTWAAGTC CCGCAACGAG
301 CGCAACCCTC GCCTMTAGTT GCCAGCATTC AGTTGGGCAC TCTAGAGGGA
351 CTGCCGGTGA TAAGCCGAGA GGAAGGTGGG GATGACGTCA AGTCCTCATG
401 GCCCTTACGG GCCGGGCTAC ACACGTGCTA CAATGGTGGT GACAGAGGGA
451 TAATCCCTAA AAGCCATCTC AGTTCAGATC GGGGTCTGCA ACTCGACCCC
501 GTGAAGGTGG AATCGCTAGT AATCGTGGAT CAGCATGCCA CGGTGAATAC
551 GTTCCCGGGC STTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTTA
601 CCCGAAGGCG MTGCGCTAAC CCGCAAGGGA GGCAGGCGAC CACGGTAGGT
651 CAGCGA

jan15

1 CAACGCGAAA AACVTTACHT GCGCTTGACA TGMCAGGAAC CCYGAAGAGA
51 TTTGGGGGWG CCCGAAAGGG AGCMTGGACA CAGGWGBTGC ATGGCTGTMG
101 TCAGCTCGWG TCGTGAGATG TTGGGWTAAG TCCCGCAACG RGCGCAACCM
151 TTATCMTTAG TTGCTACGCA AGGGCAHTCT AAGGAGACTG CCGGTGACAA
201 ACCGGAGGAA GGWGGGGATG ACGTCAAGTC CTCATGGCCC TTATGCGCAG
251 GGCTTCACAC GTCATACAAT GGTCGGTACA GAGGGTMGCC AACCCGCGAG
301 GGGGAGCTAA TCCCATAAAG CCAGTCGTAG TCCGGATCGC AGTCTGCAAc
351 TCGACTGCGT GAAGTCGGAA TCGCTAGTAA TCGCGGATCA GAATGTCGCG
401 GTGAATACGT TCCCGGGTCT TGTACACACC GCCcGTCACA CCATGGGAGC
451 GGGTTCTGCC AGAAGTAGTT AGCcTAACCG CAAGGAGGGC GATTACCACG
501 GCRAGGTT

jan18

1 CGGGGGCCCG CACAAGCGGT GGAGCATGTG GTWTAATTCG AAGCAACGCG
51 AAGAACCWWA CCAGCCCTWG ACATCCCGGG ACGGTTTCCA GAGATGGGAT
101 CCTCCTAGCA ATAGGCCCGG AGACAGGTGT TGCATGGCKG TMGTCAGCTC
151 GWGTCGTGAG ATGWTGGGTT AAGTCCCGCA ACGAGCGCAA CCCTCGCTGT
201 TAGWTGCCAT CATTTAGTTG GGCACTMTAA CGGGACCGCC GGTGATAAGC
251 CGGAGGAAGG TGGGGATGAC GTCAAGTCAT CAWGGCCCTW ACGGGCTGGG
301 CTACACACGT GYTACAAWGG CGACCATAAA GGTTTAATCC CCAAAAGTSG
351 TCTCAGTTCA GATTGTCCTC TGCAACTCGA GGGCATGAAG TCGGAATCGY
401 TAGTAATCGC AGATCAGCAC GcTGCGGTGA ATACGTTCCC GGGCCTTGTA
451 CACACCGCCC GTCACACCAT GGGAGTTGTT ATACCCGAAG GCAGTGCGCC
501 AACCGCAAGG AGGCAGCWGA CCACGGTGAG GT

jan19

1 NTTCCCACGC CCTTAAAACG GAKGRTAANT TSGMTGKTGG GMGAAACACA
51 GCCAGCGGCC AAGCGAAARG CATTAAGTTA TCCACCTGGG GAGTACGACC
101 GCAAGGTTGA AACTTCAAAG GAATTGACGG GGGCCCGCAC AAGCGGAGGA
151 GCATGTGGTT TAATTCGATG ATACGCGAGG AACCTTACST GGGNTTGAAA
201 GTTAGNGACC GACTCCGAAA AGAGTCTTTC AGAAATGACG CGAAACTAGG
251 TGCTGCATGG CTGTCGTCAG CTCGTGCCGT GAGGTGTTGG GTTAAGTCCC
301 GCAACGAGCG CAACCCCTAT TgTTAGTTGC CATCAGGTTA TGCTGGGGAC
351 TCTAAGAAGA CTGCCCGCGC AAGCGGTGAG GAAGGTGGGG ATGACGTCAA
401 GTCATCACGG CCCTTACGTC CAGGGCTACA CACGTGCTAC AATGGCCGGT
451 ACAATGGGCA GCCACATGGC AACATGGAGC TAATCTTTAA AACCGGTCTC
501 AGTTCGGATT GAGGTSTGCA ACTCGACCTC ATGAAGCTGG ATTCGCTAGT

551 AATCGCAAAT CAGCAATgTT GCGGTGAATA CGTTCCCGGG CCTTGTACAC
601 ACCGCCCGTC AAGCCATRGA AGTCGGGAGT ACCTAAGGTC CATAACCGCA
651 AGGAGTGGCC TAGGTAAAAC TGTGANT

jan20

1 ACAAGCGGTG GGGCATGTGG TTWAATTSGA SGCAASGCGG AAGACCTTAC
51 CKGGGCTAGA AAAKGCAGGA ACSTGGGAGA AGTCTBGGGG TGCTCTTCGG
101 AGAGCCTGTA GTTAGGWGCT GCATGGCTGT CGTCAGCTCG TGTCGTGAGA
151 TGKTGGGTTA RGTCCCGCAA CGAGCGCAAC CCcTATCACT AGTTACCAGC
201 GGGTCATGCC GGGGACTGTA GTGAGACCGC CGATATTCAA ATCGGAGGAA
251 GGTGGGGATG ACGTCAGGTC ATCATGGCCC TTATGTCCAG GGCTACACAC
301 GTGCTACAAT GGTCGGTACA AAGCGCTGCA AACCCGCGAG GGGGAGCCAA
351 TCGCAAAAAA CCGACCTCAG TACAGATAAG AGTSTGCAAC TCGACTCCTT
401 GAAGTCGGAA TCGCTAGTAA TCGCTGATCA GCAGGCAGCG GTGAATACGt
451 TCCCGGGCCT tGTACACACC GCCCGTCACA CCATGGGAGT CATTWGCTCC
501 AGAAGCGCCT GCGCCAACCG TAAGGAGGCA GGTTCCAAGG ATGAATGTAA
551 ST

jan21

1 NCTSGAAAGA GAGCMGTAAC ACAGGTGGST GCATGGCTGT CGTCAGCTCG
51 TGTMGWGAGA TGKKGGGNTA AGTCCCGCAA CGAGCGCAAC CCTKGTCATT
101 AGKTGCTACG AAAGGGCACT NTAATGAGAC TGCCGGaGAC AAGCCGGAGG
151 AAGGTGGGGA CGACGTCAAG TCcTCATGGC CCTKATAGGT GGGGSTACAC
201 ACGTCATACA ATGGCTGGTA CAGAGGGTTG CCAACCCGCG AGGGGGAGCC
251 AATCCCATAA AACCAGTCGT AGTCCGGATC GCAGTCTGCA ACTCGACTGC
301 GTGAAGTCGG AATCGcTAGT AATCGCGGAT CAGCATGTCG CGGTgaATAC
351 GTTCCCGGGT CTtGTACACA CCGCCCGTCA CACCATGGGA GCGGtTCTGC
401 CAGAAGTAGT TAGCCTAACC GCAAGGAGGG CGATTACCAC GGCAGGTTCG
451 TGACT

jan23

1 SGGCCGCAAG GTTGAAACTC AAAGGAATWG ACGGGGACCC GCACAAGCGG
51 TGGATGATGT GGTTTAATTC GATGCAACGC GAAAAACCTT ACCCACCTTT
101 GACATGGCAG GAAGTTTCCA GAGATGGATT CGTGCTCGAA AGAGAACCTG
151 CACACAGGTG CTGCATGGCT GTCGTCAGCT CGTGTCGTGA GATGTTGGGT
201 TAAGCCCGCA ACGAGCGCAA CCCTTGTCAT TAGTTGCTAC GAAAGGGCAC
251 TCTAATGAGA CTGCCGGTGA CAAACCGGAG GAAGGTGGGG ATGACGTCAA
301 GTCCTCATGG CCCTTATAGG TGGGGCTACA CACGTCATAC AATGGCCGGT
351 ACAAAGGGTA GCCAACCCGC GAGGGGGAGC CAATCCCACA AAGCCGGTCG
401 TAGTCCGGAT CGCAGTCTGC AACTCGACTG CGTGAAGTCG GAATCGGTAG
451 TAATCGTGGA TCAGCATGTC ACGGTGAATA CGTTCCCGGG TCTTgTACAC
501 ACCGCCCGTC ACACCATGGG AGCGGGWTCT GCCAGAAGTA GTTAGCCTAA
551 CCGCAAGGGG GGCGATTACC ACGGCAGGTT CGWGACT

apr1

1 GGACGGGCTN TGCKGGTTTC AGTAACGGAA GCTAACGGCG TGAAGTTGAC

51 CGCCTGGGGA GTACSGCCCG CAAGGTTGAA ACTCCAAAGG GAATTGACGG
101 GGACCCGCAC AAGCGGTGGA TGATGTGGTT TAATTSGATG CAACGCGAAA
151 AACCTTACYT ACCCTTGACA TGCCAGGAAC TTGCCAGAGA TGGCTTGGTG
201 CTCGAAAGAG AACCTGGACA CAGGTGCTGC ATGGCCGTCG TCAGCTCGTG
251 TCGTGAGATG TTGGGTTAAG TCCCGCAACG AGCGCAACCC TTATCATTAG
301 TTGCTACGCA AGGGCACTCT AATGAGACTG CCGGTGACAA ACCGGAGGAA
351 GGTGGGGATG ACGTCAGGTC ATCATGGCCC TTATGGGTAG GGCTACACAC
401 GTCATACAAT GGCCGGTACA GAGGGCTGCC AACCCGCGAG GGGGAGCTAA
451 TCTCAGAAAA CCGGTCGTAG TCCGGATCGC AGTCTGCAAC TCGACTGCGT
501 GAAGTCGGAA TCGCTAGTAA TCGCGGATCA GCTTGCCGCG GTGAATACGT
551 TCCCGGGTCT TGTACATACC GCCCGTCACA CCATGGGAGC GGGTTCTGCC
601 AGAAGTAGTT AGCCTAACCG CAAGGAGGGC GATTACCACG GCAGGTTCGT
651 GACTGGGKGV A

apr2

1 GGGTCTTTAA CTGACTTCCA GTAACGAAGC TAACGCGTGA AGTTGACCGC
51 CTGGGGAGTA CGGCCGCAAG GTTGAAACTC AAAGGAATTG ACGGGGGCCC
101 GCACAAGCGG TGGATGATGT GGTTTAATTC GATGCAACGC GAAAAACCTT
151 ACCCACCTTT GACATGGCAG GAAGTTTCCA GAGATGGATT CGTGCTCGAA
201 AGAGAACCTG CACACAGGTG CTGCATGGCT GTCGTCAGCT CGTGTCGTGA
251 GATGTTGGGT TAAGTCCCGC AACGAACGCA ACCCTTGCCA TTAGTTGCTA
301 CGAAAGGGCA CTCTAATGGG ACTGCCGGTG ACAAACCGGA GGAAGGTGGG
351 GATGACGTCA AGTCCTCATG GCCCTTATAG GTGGGGCTAC ACACGTCATA
401 CAATGGCTGG TACAGAGGGT TGCCAACCCG CGAGGGGGAG CTAATCCCAT
451 AAAGCCAGTC GTAGTCCGGA TCGCAGTCTG CAACTCGACT GCGTGAAGTC
501 GGAATCGCTA GTAATCGCGG ATCAGAATGT CGCGGCGAAT ACGTTCCCGG
551 GTCTTGTACA CACCGCCCGT CACACCATGG GAGCGGGTTC TGCCAGAAGT
601 AGTTAGCCTA ACCGCAAGGA GGGCGTTACC ACGGCAGGKT CGTGACTGGG
651 KGMA

apr3

1 GTTCAATTCG ATGCAAcGCG AAAAACCTTA CCTGCGCTTG ACATGCCAGG
51 AACCCCGAAG AGATTTGGGG GTGCCCGAAA GGGAGCCTGG ACACAGGTGC
101 TGCATGGCTG TCGTCAGCTc GTGTCGTGAG ATGTKGGGTT AAGTCCCGCA
151 ACGAGCGCAA CCCTTATCCT TAGTTGcTAC GCAAGGGCAC TCTAAGGAGA
201 CTGCCGGTGA CAAACCGGAG GAAGGTGGGG ATGACGTCAA GTCCTCATGG
251 CCCTTATGCG CAGGGCTTCA CACGTCATAC AATGGTCGGT ACAGAGGGTC
301 GCCAACCCGC GAGGGGGAGC TAATCCCATA AAGCCAGTCG TAGTCCGGAT
351 CGCAGTcTGC AACTcGAcTG CGTGAAGTCG GAATCGCTAG TAATCGCGGA
401 TCAGAATGTC GCGGTGAATA CGTTCCCGGG TCTTGTACAC ACCGCCCGTC
451 ACACCATGGG AGCGGGTTCT GCCAGAAGTA GTTAGCCTAA CCGCAAGGAG
501 GGCGATTACC ACGGCAGGTT CGTGACT

apr4

1 ATTWGAAGGG GCTTAGTGGS GCAGGSTAAA CGSGATTAAA GTTGACCGCC
51 TGGGGRGTAC CGGCCGCAAG GTTAAAACTT CAAAGGAATT GACGGGGGGC
101 CCGCACAAGC GGTGGAGCAT GWGGTTTAAT TCGGATGCAA CGCGAAGAAC
151 CTTAACCAGG TBTTGACATC CTCGGAACTT TCCAGAAATG GATTGGTGCC
201 TTCGGGAACC GAGAGACAGG TGCTGCATGG CTGTCGTCAG CTCGTGTCGT

251 GAGATGTTGG GTTAAGTCCC GTAACGAGCG CAACCCTTGT CCTTAGTTGC
301 CAGCATTTCG GATGGGAACT MTAAGGAGAC CGCCGGTGAC AAACCGGAGG
351 AAGGTGGGGA TGACGTCAAG TCATCATGGC CCTTATGGCC TGGGSTACAC
401 ACGTGCTACA ATGGCTGGTA CAGAGGGCTG CAAAAGCGCG AGCTGAAGCT
451 AATCCTACAA AGCCAGTYGT AGTCCGGATT GGAGTCTGCA ACTCGACTCC
501 ATGAAGTCGG AATCGCTAGT AATCGCGAAT CAGAATGTCG CGGTGAATAC
551 GTTSCCGGSC CTTGTACACA CCGCCCGTCA CACCATGGGA GWRGGTTGTA
601 CCAGAAGTGG GTAGCCTAAC CGCAAGGAGG CGCTCACCAC GGTATGATTC
651 WTGACTGGGK GVA

apr5

1 SCCCCTTAAA ASGGATGTAT GGSCTTTGGT GTTGGTTCCC CGCAAGGGGA
51 TCAGKGCCCG TAGGAAATCT TGATTAAGSC ATACCCACCC TGGGGGAGKA
101 CGATCGCCAA GGKTGAAACT TCAAAGGAAT TGACGGGGGC CSGCACAAGC
151 GGTGGATCAT GTGGTTTAAT TCGATGCAAC GSGAAGAACC TTACCCGGGS
201 TTGAAGTGCA GGAAGTACAG AGATGAAAAT CGACGGACCC GTAAAGTCGG
251 AATCCTGCAG AGGTGCTGCA TGGCTGTCGT CAGCTCGTGC CGTGAGGTGT
301 TGGGTTAAGT CCCGCAACGA GCGCAACCCC TATYTCCAGT TGCCAGCGGT
351 TTGGCCGGGC ACTCTGGAGA GACTGCCTAC GCAAGTAGAG AGGAAGGTGG
401 GGATGACGTC AAGTCATCAT GGCCCTTACG TCCGGGGSTA CACAMGTGAT
451 ACAATGGATG GCACAGTGGG CGAGGCCGCG AGGCCAAGGT AATCCCCAAA
501 ACCATTCTCA GTTCGGATTG GAGTCTGCAA CTCGACTCCA TGAAGGTGGA
551 ATCGYTAGTA ATCGCGGATC AGCACGCCGC GGTGAATACG TTCCCGGGCC
601 TTGTACACAC CGCCCGTCAA GCCATGGGAG CCGGGGGCGC CCGAAGCCGG
651 TAGTGATACC GTCGAAGGCG AAACTGTGAT

apr6

1 GGGGTTSTTA ACTGAACTTC CAGTAAACGA AAGCTTAACG CGGTGAAAGT
51 TGACCSGCCT GGGGAGTACG GCCGCCAAGG TTGAAACTNC AAAGGAATTG
101 AACGGGGACC CGCACAAGCG GTGGATGATG TGGTTTAATT CGATGCAACG
151 CGAAAAACCT TACCCACCTT TGACATGGCA GGAAGTTTCC AGAGATGGAT
201 TCGTGCTCGA AAGAGAACCT GCACACAGGT GCTGCATGGC TGTCGTCAGC
251 TCGTGTCGTG AGATGTTGGG TTAAGTCCCG CAACGAGCGC AACCCTTGCC
301 ATTAGTTGCT ACGAAAGGGC ACTCTAATGG GACTGCCGGT GACAAACCGG
351 AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATA GGTGGGGCTA
401 CACACGTCAT ACAATGGCTG GTACAGAGGG TTGCCAACCC GCGAGGGGGA
451 GCTAATCCCA TAAAGCCAGT CGTAGTCCGG ATCGCAGTCT GCAACTCGAC
501 TGCGTGAAGT CGGAATCGCT AGTAATCGCG GATCAGAATG TCGCGGTGAA
551 TACGTTCCCG GGTCTTGTAC ACACCGCCCG TCACACCATG GGAGCGGGTT
601 CTGCCAGAAG TAGTTAGCCT AACCGCAAGG AGGGCGATTA CCACGGCAGG
651 TTCGTGANTG GGKGN

apr10

1 RTGaGADGTK GGGTKAAGTC CCGCAaCGAG SGCAACCCTc GCCAKKAGTt
51 GCCATCAtTA AGtKGGGCAC TWTAGTGGGA MTGCCGGWGA TAAGCCGGAG
101 GAAGGTGGRG ATGACGTCAA GTCATCATGG CCCTtATGGG STGGGATACA
151 CASGTGGTAC AAWGGcGGtG ACAATGGGCa GCGaGGcgGS AATGCCRAGM
201 TRATSTCAAA AaGCCGTMTC AGTTSGGATK GGGCTYTGCA AGTCGAGCCC
251 ATGAAGTSGG AATCGGTAGT AATMGTGGAT CaGCACGCCa cGNTGaATAc

301 GTTCcSGGGC CttGtACACA CCGCCCGTCA CACCATGGGA GTTGGMTTTA
351 CCCGAAGACG GTGCGCTAAC CAGCAATGGA GGCAGCCGGC CACGGTAAGT
401 C

apr11

1 GGATAANTTC GCTGTTGGCG ATACANCAGC CAGCGGCCAA GCGAAAGCAT
51 TAAGTTATCC CACCTGGGGG AGTACGACCG CAAGGTTGAA ACTCAAAGGA
101 ATTGACGGGG GCCCGCACAA GCGGAGGAGC ATGTGGTTTA ATTCGATGAT
151 ACGCGAGGAA CCTTACCTGG GCTTGAAAGT TAGCGACCGA CTCCGAAAAG
201 AGTCTTTCAG AAATGACGCG AAACTAGGTG CTGCATGGCT GTCGTCAGCT
251 CGTGCCGTGA GGTGTTGGGT TAAGTCCCGC AACGAGCGCA ACCCCTATTC
301 TTAGTTGCCA TCAGGTTATG CTGGGGACTC TAAGAAGACT GCCCGCGCAA
351 GCGGTGAGGA AGGTGGGGAT GACGTCAAGT CATCACGGCC CTTACGTCCA
401 GGGCTACACA CGTGCTACAA TGGCCGGTAC AATGGGCAGC CACATGGCAA
451 CATGGAGCTA ATCTTTAAAA CCGGTCTCAG TTCGGATTGA GGTCTGCAAC
501 TCGACCTCAT GAAGCTGGAT TCGCTAGTAA TCGCAAATCA GCAATGTTGC
551 GGTGAATACG TTCCCGGGCC TTGTACACAC CGCCCGTCAA GCCATGGAAG
601 TCGGGAGTAC CTAAAGTCCA TAACCGCAAG GATGGCCTAG GTAAAACTGT
651 GATGGGNNMA

apr12

1 CCCGTTAAAA ACCGGATTGG GATGGTTAAG CCNGTTCGGG AATAAGCTTG
51 GTTAATTTCG GGTGGCCGCA GGCTTAAACG CATTAAGCAT CCCCGCCTGG
101 GGAGTAACGG CCGCAAGGTT AAAAACTTCA AAGGAATTTG ACGGGGGCCC
151 GCACAAGCGG TGGGAGCATG TGGTTTAATT NGACGCAACG GCGAAGAACC
201 TTACCAGCTN TTGACATTCA CTGATCGCCT GGAGAGATCC GGGAGTCCTA
251 GCAATAGGCA GTGGGACAGG TGCTGCATGG CTGTCGTCAG CTCGTGTCGT
301 GAGATGTTGG GTTAAGTCCC GCAACGAGCG CAACCCTCGC CATTAGTTGC
351 CATCATTTAG TTGGGCACTC TAGTGGGACT GCCGGTGATA AGCCGGAGGA
401 AGGTGGGGAT GACGTCAAGT CATCATGGCC CTTACGGGCT GGGCTACACA
451 CGTGCTACAA TGGCGGTGAC AATGCGCAGC CACCTAGCAA TAGGGAGCTA
501 ATCGCAAAAA GCCGTCTCAG TTCAGATTGG AGTCTGCAAC TCGACTCCAT
551 GAAGTCGGAA TCGCTAGTAA TCGCGCATCA GCATGGCGCG GTGAATACGT
601 TCCCGGGCCT TGTACACACC GCCCGTCACA CCATGGGAGT TGGTTTTACC
651 CGAAGACGGT GCGCTAACCC GCAAGGGAGG CAGCCGGCCA CGGTAAGTCC
701 AGCGACTGGG NGC

apr13

1 NGGACCCGCA CAAGCGGTGG AtGATGTGGA TTAATTCGAt GCAACGCGAA
51 AAACCTTACC TACCCTtGAC ATGCCAGGAA CCTTGCTGAG AGGTGAGGGT
101 GCCCGAAAGG GAGCCTGGAC ACAGGKGCTG CATGGCWGTS GTCAGCTCGT
151 GTCGTGAGAT GTTGGGTTAA GTCCCGCAAC GAGCGCAACC CTTGCCATTA
201 ATtGCCATCA TTTAGTTGGG CACTTTAATG GGACTGCCGG TGACAAACCG
251 GAGGAAGGTG GGGATGACGT CAAGTCCTCA TGGCCCTTAT GGGTAGGGCT
301 TCACACGTCA TACAATGGTC GGTACAGAGG GTTGCCAAGC CGCGAGGTGG
351 AGCGAATCTC ACAAAGCCGA TCGTAGTCCG GATTGCAGTC TGCAACTCGA
401 CTGCATGAAG TCGGAATCGC TAGTAATCGC GGATCAGCAT GTCGCGGTGA
451 ATACGTTCCC GGGTCTTGTA CACACCGCCC GTCACACCAT GGGAGTGGGT

501 TTCACCAGAA GCAGGTAGCC TAACCGCAAG GAGGGCGCTT ACCACGGTGA
551 GATCATGA

apr14

1 TKTAAAMGAT GAGWANTTAA AGGTTGGTAG GGGAACCTAT CAGTCTTGAa
51 GCTAACACGA TAAGTAATTC SGCCTGGAAG TACGGCCGCA AGGTTGAAAC
101 TCAAAtGAAT TGACGGGGGG CCCGCACAAG CGGTGGAGCA TGWGGCTTAA
151 TTCGATGCAA CGCGAAGAAC CTTACCtACC CTTGACATCC TGCGAATCCC
201 GATGAGAGTT GGGAGTGCCG AAAGGAGCGC AGAGACAGGT GCTGCATGGC
251 TGTSGTCAGC TCGTGTTGTG AGATGTTGGG TTAAGTCCcG TAACGAGCGC
301 AACCCTTGTC CTTAGTTGCC ATCATTTAGT TGGGGACTCT AAGGAGACTG
351 CCGGTGATGA ACCGGAGGAA GGCGGGGACG ACGTCAAGTC ATCATGGCCT
401 TTATGGGTAG GGSTACACAC GTGCTACAAT GGGGcGTACA GAGAGTCGCG
451 AACCCGCGAG GGGGAGCCAA TCTcGTAAAG CGTCTYGTAG TCCGGATTGG
501 GGTCTGCAAC TCGACTCCAT GAAGTTGGAA TCGCTAGTAA TCGCGAATCA
551 GCATTGTCGC GGTGAATACG TTCCCcGGGCC TTGTACACAC CGCCCGTCAC
601 GTCATGGGAG TGGATGGTAC CAGAAGCGGG TAGCTGAACC GAAAGGAAGG
651 CGCTTGCCAC GGTATGATTC ATGA

apr15

1 TGKGGKTTAA TTCGAAGCAA CGCGAAGAAC cTtACCRGCC CKTGACATCC
51 cGGGAcGGTT TCCAGAGATG GGATCMTCgT AGCAATAGGC CCGGAGACAG
101 GTGTtGCATG GCTgTCGTCA GCTcGtGTCG TGAGATGaTG GGTTAAGTCC
151 CGCAACGAGc GCAACCcTCG GWGWKAGTTG CCATCATTtA GTtGGGCACT
201 STAACGGGAC CGCcGGTGAT AAGCCGGAGG AAGGTGGGGA CGACGTCAAG
251 TCaTCAtGGC CCtTACGGGg tGGGVTACAc ACGtGCTACA ATGGCGACCA
301 TaAAGGttTA ATCCCCAAAA GTgGTgTCAG TTCAGAWTGT CCTSTGCAAC
351 TMGAGGGCAT GAAGTCGGAA TCGTTaGTAA TCGCAGATCA GCACGCTGCG
401 GTGAATAcGT TCCCGGGCCT tGTACACACC GCCCGTCACA CCATGGGAGT
451 TGGtTNTACC CGAAGGCAGT GCGCCAACCG CAAGGAGGCA GCTGACCACg
501 GTAGGTCAWG A

apr16

1 TCGATGATAC GcGAGGAACC TTACSKGGGM TTGAAAGTta GCGACCGACT
51 CCGAAAAGGT ctTTCAGAAA TGAcGCGAAA CTaGGTGCTG CATGGCtGTC
101 GTCAGCTCGT gCCGtGAGGT GKTGGGTTAA GTCCCGCAAC GAGCGCAACC
151 CCTATTCTTA GttGCCATCA GGWTATGcTG GGGAcTcTAa GAAGACtGCC
201 CGCGCAAGCG GTGAGGAAGG TGGGGAtGAc GTCAAGTCAT CACGGCCCTt
251 ACGTSCAGGG MTACACACGT GGtACAATGG CCGgWACAaT GGGCAGCCAC
301 aTGGCAACAT GGAGcTAATC TTtAAAACCG GTCTCAGTTC GGATTGAGGT
351 cTGCAACTCG ACCTCAtGAG GCTgGATTCG CTAGTAATCG CAAATCAGCA
401 ATGTTGCGGT GAATACGTTC CCGGGCCTtG TACACACCGC CCGTCAAGCC
451 ATGGAAGTCG GGAGTACcTA AAGTCCATAA CCGCAAGGAT GGCCTAGGTA
501 AAAStGKGAS N

apr17

1 NCAGTGGGAC AGGTGgtGCA TGGcTGTcGT CAGCTCGTGT cGTGAGATGT

51 TGGGTTAAGT CCCGCAACGA GCGCNACCCT TGTCATTAGT tGgTACGAaA
101 GGGCACTgTA ATGAGASTGC CGGTGACAAA CCGGAGGAAG GTGGGGAtGA
151 cGTCAAGTCC TCaTGGCCCT tATAGGTGGG GCKACACACG TCRTACAATG
201 GCCGGTACAA AGGGTAGCCA ACCCGCGAGG GGGAGCCAAT CCCACAAAGC
251 CGGTCGTAGT CCGGATSGCA GTSTGCAACT gGACTGCGTG AAGTCGGAAT
301 CGCTAGTAAT CGTGGATCAG CATGTCACGG TGAATACGTT CCCGGGCCTt
351 GTACACACCG CCCGTCACAC CATGGGAGTG GGtTGCTCCA GAAGTAGCTA
401 GTCTAACCGC AAGGGGGACG TTACCACGGA GTGaTcATGA CN

apr18

1 GGAGCAKGWG GTTTAATTCG ACGCAACGCG AAGAACCTTA CCAGCTSTTG
51 ACATTCACTG ATCGCCTGGG GAGACCTGGG AATCCTAGCA ATAGGCAGTG
101 GGACAGGTGC TGCATGGCtG TcGTCAGCTc GTGTCGTGGG ATGTTGGGWT
151 AAGTCCCGCA ACGAGCGCAA CCcTCGCCAT TAGTTGCCAT CATTCAGTtG
201 GGCACTMTAG TGGGACTGCC GGTGATAAGC CGGAGGAAGG TGGGGATGAC
251 GTCAAGTCAT CATGGCCCTT ACGGGCTGGG CTACACACGT GCTACAATGG
301 CGGTGACAAT GCGCAGCCAC CTAGCAATAG GGAGcTAaTC GCAAAAAGCC
351 GTCTCAGTTC AGATTGGAGT CTGCAACTCG ACTCCATGAA GTCGGAATCG
401 CTAGTAATCG CGCATCAGCA TGGCGCGGTG AATACGTTCC CGGGCCTtGT
451 ACACACCGCC CGTCACACCA TGGGAGTTGG TCATACCCTA AAACGGTGCG
501 CTAACCGCAA GGAGGCAGCC GGCCACGGTA TGTCCAGCGA CTGGGN

apr19

1 GCGGTGGAGC ATGWGGTTTA ATTTGGACGC AACGCGAAGA ACCTTACCAG
51 CTCTTGACAT GTCCGGACGG TTTCCAGAGA TGGGATCCTC CCAGCAATGG
101 GCCGGAACAC AGGTGCTGCA TGGCTGTCGT CAGCTCGTGT CGTGAGATGT
151 TGGGTTAAGT CCCGCAACGA GCGCAACCCT CGCCATCAGT TGCCATCAWT
201 AAGTTGGGCA CTCTGGTGGG ACTGCCGGTG ATAAGCCGGA GGAAGGTGGG
251 GATGACGTCA AGTCATCATG GCCCTTACGG GCTGGGCTAC ACACGTGTTA
301 CAATGGCGGT GACAATGcGC AGCCACCTCG CGAGAGGGAG cTAATCGCAA
351 AAAGCCGTCT CAGTTCGGAT tGGGCTCTGC AACTCGAGCC CATGAAGTTG
401 GAATCGYTAG TAATCGCGCA TCAGCATGGC GCGGTGAATA CGTTCCCGGG
451 CCTTGTACAC ACCGCCCGTC ACACCATGGG AGTTGGCTTT ACCCGAAGGC
501 GGTGCGCTAA CCGCAAGGAG GCAGCCGGCC ACGGTAAGTC AGCGA

apr20

1 ACGCGNARGA ACYTTACCAG CTCTTGACAT GTCCGGACGG TTTCCaGAGA
51 TGGGATCSTC CTAGCAATAG GCCGGAACAC AGGTGCTGCA TGGCgGTCGT
101 CAGCTCGTGT cgTGAGATGT TGGGTTAAGT CCCGCAACGa GcGCAACCST
151 CGCCATTAGT tGCCATCATT AAGTTGGGCA CTCTAGTGGG ACTGCCGGTG
201 ATAAGCCGGA GGAAGGTGGG GATGACGTCA AGTCATCATG GCCCTTATGG
251 GCTGGGVTAC ACACGtGSTA CAATGGCGGT GACAATGGGC AGCGAGGCAG
301 CAATGCCAAG CTAATCTCAA AAAGCCGTCT CAGTTCGGAT TGGGcTCTGC
351 AACTCGAGCC CATGAAGTCG GAATCGCTAG TAATCGTGGA TCAGCACGCC
401 ACGGTGaATA CGTTCCCGGG CCTtgTACAC ACCGCCCGTC ACACCATGGG
451 AGTTGGcttT ACCCGaAGAC GGTGCGCTAA CCAGCAATGG AGGCAGCCGG
501 CCACGGTAAG TCGAGCGA

apr21

```
  1 AAGGAATKGA SGGGGGCCCG CACAAGCGGT GGAGCATGTG GKTaAATTTV
 51 GAaGCAACGc GAAGAACNgT ACCAGCCCTT GACATCCCGG GAcGGTTTCC
101 AGAGATGGGA TCCTCNTAGC AATAGGCCCG GAGACAGGWG TTGCAWGGMt
151 GTCGTCAGCT CGTGTCGTgA GAtGttGGGT TAAGTCCCGC AAcGAGCGCA
201 ACCCTCGCtG TTAGTtGCCa TCaTTTAGTT GGGCAcTcTA ACGGGACCGC
251 CGGTGaTAAG CCGGAGGAAG GTGGGGATGA CGTCAAGTCA TcATGGCCCT
301 TACGGGcTGG GcTACAcACG TGSTACAATG GcGACCATAA AGGTTtAATC
351 CCCAAAAGTc GTCTCAGTTC AGaTTGTCCT CtGCAACTCG AGGGCAtGAA
401 GTCGGAATCG NTAGTAATCG CAGATCAGCA CGCTGCGGTG AATACGTTCC
451 CGGGCCTTGT ACACACCGCC CGTCACACCA TGGGaGTTGT TNTACCCGAA
501 GGCAGTGCGC CAACCGCAAG GAGGCAGCTG ACCACGGTAG GTCATGAKBG
551 NNGGC
```

apr22

```
  1 GGCATTAAGT KGGGCANTTT AGSGKGACTG CCGGtGACAA ACCGGAGGAA
 51 GGCGGGGAKG ACGTCAAATC ATCATGCCCC AWaTGGCCTG GGctACAcAC
101 GTACTACAAt GGCTGgtACA ACGGGAaGCG AAGcTGCGAA GCGGAGCGAA
151 TCTTAAGAAT CCAGTCTCAG TTCGGATTGC AGGcTGCAAc TCGCCTGCAT
201 GAGGTCGGAa TTGCTAGTAA TCGCGGATCA GCATGCCGCG GTGAATACGT
251 TCCCGGGTcT TGTACACACC GCCCGTCACA CCACGAGAGT TTACAACACC
301 CGAAGTCGGT GAGGTAACCG CAAGGAGCCA GCCGCCGAAG GTGGGGTAGT
```

apr23

```
  1 GGGCAGRACB TTACCAACCc TTGACATGGC GATcGCGGTT CCAGAGATGG
 51 TTCcTTCaGT TCGGCtGGAT CGCACACAGG TGctGCATGG cTGTcGTCaG
101 CTCGtGTCGt GAGatGDTCG gTAAGTCcGG CAAcGAGcGC AACCCACAST
151 TgTAGTTGCC AGCATTCAGT TGGGCAcTgT AGAAGAACTG CCGATGaTAA
201 GTcGGAGGAA GGtGTGGAtG ACGTCAAGTC cTCATGGCCC YKACGGGTtG
251 GGSTACACAc GtGgTACAAT GGTGGtGACA ATGGGTTaAT CCCCAAAAGC
301 CATCTCAGTT cGGATTGGGG TgTGCAACTc GACCCCaTGA AGTCGGAATC
351 GaTaGTAATC GCGTAACAGC AtGACGCGGT GAATAcGTTC CgGGGCCtTG
401 TACACACCGC CSGTCACACC ATGGGAGTTG GRWTCACCCG AAGGSGTGCG
451 CCAACCTCGC AAGAGGGGGC AGCCGACCAC GTGGMTC
```

apr24

```
  1 NGCAGCTAAC GCAGTAAGCC TTCCGCCTGG GGAGTACGGT CGCAAGATTA
 51 AAACTCAAAG GAATTGACGG GGGCCCGCAC AAGCGGTGGA GCATGTGGTT
101 TAATTCGAAG CAACGCGAAG AACCTTACCA GCCCTTGACA TCCCGGGACG
151 GTTTCCAGAG ATGGGATCCT CCTAGCAATA GGCCCGGAGA CAGGTGTTGC
201 ATGGCTGTCG TCAGCTCGTG TCGTGAGATG TTGGGTTAAG TCCCGCAACG
251 AGCGCAACCC TCGCTGTTAG TTGCCATCAT TTAGTTGGGC ACTMTAACGG
301 GACCGCCGGT GATAAGCCGG AGGAAGGTGG GGATGACGTC AAGTCATCAT
351 GGCCCTTACG GGSTGGGCTA CACACGTGCT ACAATGGCGA CCATAAAGGT
401 TTAATCCCCA AAAGTCGTCT CAGTTCAGAT TGTCCTCTGC AACTMGAGGG
451 CATGAAGTCG GAATCGCTAG TAATCGCAGA TCAGCACGCT GCGGTGAATA
501 CGTTCCCGGG CCTTGTACAC ACCGCCCGTC ACACCATGGG AGTTGATTCT
```

551 ACCCGAAGGC AGTGCGCCAA CCGCAAGGAG GCAGCTGACC ACGGTAGGTC
601 CATGANT

apr25

1 GAATTGACGG GGGCCCGCAC AAGCGGTGGA GCATGTGGTT TAATTCGAAG
51 CAACGCGCAG AACYTTACCA ACCCTTGACA TGGGASGTAT GGGCTTCAGA
101 GATGAGGTCC TTCAGTTCGG CTGGCGTCCA CACAGGTGCT GCATGGCTGT
151 CGTCAGCTCG TGTCGTGAGA TGTTGGGTTA AGTCCCGCAA CGAGCGCAAC
201 CCCTATBTTC AGTTGCCATC ATTCAGTTGG GCACTCTGAA GAAACTGCCG
251 GTGACAAGCC GGAGGAAGGC GGGGATGACG TCAAGTCCTC ATGGCCCTTA
301 CGGGTTGGGc TACACACGTG CTACAATGGT GGTGACAATG GGGAGCGAGG
351 CCGCGAGGCC AAGCCAATCT CAAAAAGCCA TCTCAGTTCG GATTGCACTC
401 TGCAACTcGA GTGCATGAAG TTGGAATCGc TAGTAATCGC GCATCAGCAT
451 GGCGCGGTGA ATACGTTCCC GGGCCTTGTA TACACCGCCC GTCACACCAT
501 GGGAGWTGGT TTTACCCGAA GCCGGTGCGC GAACCGCAAG GACGCAGCCG
551 ACCACGGTAA GTCNAGCGAC TGGGN

apr26

1 GGAGTACGCC CSCAAGGTTA AAACTCAAAG GAATTGACGG GGGCCCGCAC
51 AAGCGGtGGA GCATGTGGTT TAATTcGAAG CAACGCGAAG AACCTTACCA
101 GCTCTTGACA TTCACTGACT GTTCCCAGAG atGGGATCGT CCTAGCAATA
151 GGCAGTGGGA CAGGTGcTGC ATGGCTGTCG TCAGcTCGTG TCGTGAGATG
201 TTGGGKTAAG TCCCGCAACG AGCGCAACCC TCGCCATTAG TtGCCATCAT
251 TCAGTTGGGC ACTHTAGTGG GASTGCCGGT GATAAGCCGG AGGAAGGTGG
301 GGATGACGTC AAGTCATCAT GGCCCHTACG GGSTGGGMTA CACAcGTGgT
351 ACAATGGCGA CCATAAAGGT TTAATCCCCA AAAGTcGTCT CAGTTCAGAT
401 TGTCCTcTGC AACTcGAGGG CATGAAGTCG GAATCGNTAG TAATCGCAGA
451 TCAGCACGcT gCGGTGAATA CGTTCCCGgG CCTTGTACAC ACCGCCCGTC
501 ACACCATGGG AGttGGTTYT ACCCGAAGGC AGTGCGCCAA CCGCAAGGAG
551 GCAGCTGACC ACGGTAGGTC CAATGA

apr27

1 MTCAAAGGAA TTGACGGGGG TCCGCACAAG CGGTGGAGCA TGTGGTTTAA
51 TTCGATGATA CGCGAGGAAC CTTACCTGGG CTAGAATGCC CTTGACAGAT
101 TCAGGGATGG ATTTTTTCGC AAGAACAAGG AGCAAGGTGC TGCATGGCTG
151 TCGTCAGCTC GTGCCGTGAG GTGTTGGGTT AAGTCCCGCA ACGAGCGCAA
201 CCCCTATTBT TAGTTGCCAG CATGTCATGG TGGGGACTYT AAGAAGACTG
251 CCTGCGCAAG CAGAGAGGAA GGAGGGGATG ACGTCAAGTC ATCATGGCCC
301 TTACGCCCAG GGcTACACAc GTGCTACAAT GGCGTcTACA AAGTGTTGCC
351 AGTCAGTGAT GACGAGCCAA TCACAAAAAG GACGTCTCAG TTCGGATTGT
401 AGGCTGCAAC TCGCCTGCAT GAAGTTGGAA TCGCTAGTAA TcGTGGATCA
451 GCACTGCCAC GGTGAATACG TTCCCGGGCC TTGCACACAC CGCCCGTCAC
501 ACCACGAAAG TCGTTcTACC CGAAGTGGTT GAGCTAACCC GCAAGGGAGG
551 CAGGCTCCTA AGGTAGGGCT GGTAATT

apr29

1 NCSGATCGGT GACGCAGCTA ACGCAgTAAG CCNTCCCGCC tGGGaGTACG

51 GTCGCAAGAT TAAAACTCAA AGGAATTGAC GGGGGCCCGC ACAAGCGGTG
101 GAGCATGTGG TTTAATTCGA AGCAACGCGA AGAACCTTAC CAGCCCTTGA
151 CATCCCGGGA CGGTTTCCAG AGATGGGATC CTCCTAGCAA TAGGCCCGGA
201 GACAGGTGTT GCATGGCTGT CGTCAGCTCG TGTCGTGAGA TGTTGGGTTA
251 AGTCCCGCAA CGAGCGCAAC CCTCGCTGTT AGTTGCCATC ATTTAGTTGG
301 GCACTCTAAC GGGACCGCCG GTGATAAGCC GGAGGAAGGT GGGGATGACG
351 TCAAGTCATC ATGGCCCTTA CGGGcTGGGc TACACACGTG CTACAATGGC
401 GACCATAAAG GTTTAATCCC CAAAAGTCGT CTCAGTTCAG ATTGTCCTCT
451 GCAACTCGAG GGCATGAAGT CGGAATCGCT AGTAATCGCA GATCAGCACG
501 CTGCGGTGAA TACGTTCCCG GGCCTTGTAC ACACCGCCCG TCACACCATG
551 GGAGTTGTTC TACCCGAAGG CAGTGCGCCA ACCGCAAGGA GGCAGCTGAC
601 CACGGTAGGT CCAATGANT

apr30

1 GTTCAATTCG ATGCAAcGCG AAAAACCTTA CCTGCGCTTG ACATGCCAGG
51 AACCCCGAAG AGATTTGGGG GTGCCCGAAA GGGAGCCTGG ACACAGGTGC
101 TGCATGGCTG TCGTCAGCTc GTGTCGTGAG ATGTKGGGTT AAGTCCCGCA
151 ACGAGCGCAA CCCTTATCCT TAGTTGcTAC GCAAGGGCAC TCTAAGGAGA
201 CTGCCGGTGA CAAACCGGAG GAAGGTGGGG ATGACGTCAA GTCCTCATGG
251 CCCTTATGCG CAGGGCTTCA CACGTCATAC AATGGTCGGT ACAGAGGGTC
301 GCCAACCCGC GAGGGGGAGC TAATCCCATA AAGCCAGTCG TAGTCCGGAT
351 CGCAGTcTGC AACTcGAcTG CGTGAAGTCG GAATCGCTAG TAATCGCGGA
401 TCAGAATGTC GCGGTGAATA CGTTCCCGGG TCTTGTACAC ACCGCCCGTC
451 ACACCATGGG AGCGGGTTCT GCCAGAAGTA GTTAGCCTAA CCGCAAGGAG
501 GGCGATTACC ACGGCAGGTT CGTGACT

apr31

1 CTGGGGAGTA CGTCGCAAGA TTGAAACTYA AAGGAATTGG CGGGGGCCCG
51 CACAAGNGGT GGAGCATGTG GTTTAATTCG ATGCAAAGCG AAGAACCTTA
101 CCAAGGCTTG ACATACTGGC TCAAGCTAAT GTGAAAGCAT TTTGTGTTGT
151 AAGGCTTGCC TTACATAGTC AGTACAGGTG CTGCATGGCT GTCGTCAGCT
201 CGTGCCGTGA GGTGTCCGGT TAAGTCCGGT AACGAGCGCA ACCCCTATGT
251 TTAGTTGCCA GCACGTAAGG TGGGAACTCT AAAGAGACTG CCGGCGACAA
301 GCCGGAGGAA GGCGGGGACG ACGTCAAGTC ATCATGGCCC TTATGCTTTG
351 GGCTACACAC ATGCTACAAT GGCGGATACA ATGGGTTGCC AAACTGCGAA
401 GTGGAGCCAA TCCCATCAAA GTCCGTCTCA GTTCGGATTG CAGGCTGCAA
451 CTCGCCTGCA TGAAGTTGGA ATCGCTAGTA ATcGTGGATC AGCACTGCCA
501 CGGTGAATAC GTTCCCGGGC CTTGCACACA CCGCCCGTCA CACCACGAAA
551 GTCGTTCTAC CCGAAGTGGT TGAGCTAACC CGCAAGGGAG GCAGGCTCCT
601 AAGGTAGGGC TGGTAATT

nov2

1 GGTTAAGTCC CGCAACGAGC GCNACCCCCA TCCTTAGTTG CCATCATTAA
51 GTTGGGCACT NTAAGGAAAC TGCCGGTGAC AAGCCGGAGG AAGGTGGGGA
101 TAACGTCAAG TCTTCATGGC CCTTACGGGT CGGGCNACAC ACGTANTACA
151 ATGGCAGTGA CAATGGGTTG CANAGTCGCA AGGCNGAGCT AATCCTAAAA
201 AANTGCCTCA GTTCGGATTG TCCTCTGCAA CTCGAGGGCA TGAAGNTGGA
251 ATCGCTAGTA ATCGCGCAAC AGCATGGCGC GGTGAATACG TTCCCGGGCC

301 TTGTACACAC CGCCCGTCAC GCCATGGGAG TTGGTTTTGC CCGAAGCCGG
351 TGCGCTAACC GCAAGGAAGC AGCCGACCAC NGTACGATAG CGAGT

nov3

1 NNTNGACATC CCGGTNGAAG GANTCGAGAN ATGGGTCCTT CAGTTNGGAG
51 TGGACCGATG ACAGGTGNTG CANGGCTGTC GTCAGCTCGT GTCGTGAGAT
101 GTTGGGTTAA GTCCCGCAAC GAGCGCAACC CTNGCCCTTA GTTGCCATCA
151 TTCAGATGGG CACTCTAAGG GGACTGCCGG TGATAAGCCG AGAGGAAGGT
201 GGGGATGACG TCAAGTCCTC ATGGCCCTTA CGGGNTGGGC NACACACGTG
251 NTACAATGGT GGTGACAGTG GGCAGCGAGA CCGNGAGGTC GAGCTAATCT
301 CCAAAAGCCA TCTCAGTTCG GATTGCACTC TGCAACTCGA GTGCATGAAG
351 TTGGAATCGC TAGTAATCGT GGATCAGCAT GCCACGGTGA ATACGTTCCC
401 GGGCATNGTA CACACCGCCC GTCACACCAT GGGAGTTGGC TTTACCCGAA
451 GGCGCTGTGC TAACCGCAAG GAGGCAGGCG ACCACGGTAG GTCAGCGACT
501 GGGG

nov4

1 TAAACGATGT CAACTGGTTG TTGGTTCTTA GCTGATTCAG TAACGAAGCT
51 AACGCGTGAA GTTGACCGCC TGGGGAGTAC GGCCGCAAGG TTGAAACTCA
101 AAGGAATTGA CGGGGACCCG CACAAGCGGT GGATGATGTG GTTTAATTNG
151 ATGCAACGCG AAAAACCTTA CCCACCTTTG ACATGTACGG AAACCTTTGG
201 AGACAGAGGG GTGCTCGAAA GAGAGCCGTA ACACAGGTGC TGCATGGCTG
251 TCGTCAGCTC GTGTCGTGAG ATGTTGGGTT AAGTCCCGCA ACGAGCGCAA
301 CCCTTGTCAT TAGTTGCTAC GAAAGGGCAC TCTAATGAGA CTGCCGGTGA
351 CAAGCCGGAG GAAGGTGGGG ATGACGTCAA GTCCTCATGG CCCTTATAGG
401 TGGGGCTACA CACGTCATAC AATGGCTGGT ACAGAGGGTT GCCAACCCGC
451 GAGGGGGAGC CAATCCCATA AAACCAGTCG TAGTCCGGAT CGCAGTCTGC
501 AANTCGACTG CGTGAAGTCG GAATCGCTAG TAATCGCGGA TCAGCATGTC
551 GCGGTGAATA CGTTCCCGGG TNTTGTACAC ACCGCCCGTC ACACCATGGG
601 AGCGGGTTCT GCCAGAAGTA GTTAGCCTAA CCGCAAGGAG GGCGATTACC
651 ACGGCAGGTT CGTGANT

nov5

1 ACCCTAGGTA GTCNAACGGC CGTAAAATGN NGGTAATTGG ACATTTGNGA
51 TAAACTNTAA AGNGTCAGAG CGGAAAGCAT TAAGTATCCC CAANTGGGAA
101 GTACGACCGC AANGTTGAAA CTCAAAGGAA TTGGCGGGGG TCCGCACAAG
151 CGGTGGAGCA TGTGGTTTAA TTNAGATGAT ACGCGAGGAA CCNTACNTGG
201 GATAGAATGA TGGGAGACCG TGGGTGAAAG CTCACTTTGT AGCAATACAN
251 TGCCAGTAAG GTGCTGCATG GCTGTCGTCA GCTCGTGCCG TGAGGTGTTG
301 GGTTAAGTCC CGCNACGANC GCAACCCCCA TCACTAGTTG CCATCANGTA
351 ACGCTGGGAA CTCTAGTGAA ACTGCCGTCG TAAGACGTGA GGAAGGAGGN
401 GATGATGTCC AGTCATCATG GCCTTAAAGC CCAGGGATAC ACACGTGCNA
451 CAATGGAGTG GANAAAGGGN TGCAACACAG NGATGTGAAG NTAATCCCAA
501 AAACCANTTC TCAGTTCAGA TTGGAGTCTG CAACTCGACT CCATGAAGCT
551 GGAATCGCTA GTAATCGTAT ATCAGCAATG ATACGGTGAA TACGTTCCCG
601 GACCTTGCAC ACACCGCCCG TCAAGCCATG GAAGCCGGGT GTACCTAAAG
651 TCGTTAACCG TAAGGAGACG CCTAGGTAAA ACTGGTGAT

nov6

```
  1 NNGCCCTTAA ACTATGGATA CTCGACATTT GCGATAAACT GTAAGTGTCT
 51 GAGCGAAAGC ATTAAGTATC CCACCTGGGA AGTACGACCG CAAGGTTGAA
101 ACTCAAAGGA ATTGGCGGGG GTCCGCACAA GCGGTGGAGC ATGTGGTTTA
151 ATTCGATGAT ACGCGAGGAA CCTTACCTGG GCTAGAATGC TGGGAGACCG
201 TGGGTGAAAG CTCACTTTGT AGCAATACAC TGCCAGTAAG GTGCTGCATG
251 GCTGTCGTCA GCTCGTGCCG TGAGGTGTTG GGTTAAGTCC CGCAACGAGC
301 GCAACCCCCA TCACTAGTTG CCATCAGGTA ACGCTGGGAA CTCTAGTGAA
351 ACTGCCGTCG TAAGACGTGA GGAAGGAGGG GATGATGTCA AGTCATCATG
401 GCCTTTATGC CCAGGGCTAC ACACGTGCTA CAATGGAGTG GACAAAGGGC
451 TGCAACACAG TGATGTGAAG CTAATCCCAA AAACCACTTC TCAGTTCAGA
501 TTGGAGTCTG CAACTCGACT CCATGAAGCT GGAATCGCTA GTAATCGTAT
551 ATCAGCAATG ATACGGTGAA TACGTTCCCG GACCTTGCAC ACACCGCCCG
601 TCAAGCCATG GAAGCCGGGT GTACCTAAAG TCGTTAACCG TAAGGAGACG
651 CCTAGGTAAA ACTGTGACTG GGT
```

nov7

```
  1 CTCAAGCTAA TGAGAAAGCA TTTTGNGTTG TAAGGCTTGC CTTACATAGT
 51 CAGTACAGGN GGNGCATGGC TGTCGTCAGC TCGTGCCGTG AGGTGTCCGG
101 TTAAGTCCCG CAACGAGCGC AACCCTTGTC ATTAGTTGNT ACGAAAGGGC
151 ACTNTAATGA GACTGCCGGT GACAAACCGG AGGAAGGTGG GGATGACGTC
201 AAGTCCTCAT GGCCCTTATA GGTGGGGNTA CACACGTCAT ACAATGGCCG
251 GTACAAAGGG TAGCCAACCC GCGAGGGGGA GCCAATCCCA CAAAGCCGGT
301 CGTAGTCCGG ATCGCAGTCT GCAACTCGAC TGCGTGAAGT CGGAATCGCT
351 AGTAATCGTG GATCAGCATG TCACGGTGAA TACGTTCCCG GGTCTCNTAC
401 ACACCGCCCG TCACACCATG GGAGCGGGTT CTGCCAGAAG TAGTTAGCCT
451 AACCGCAAGG GGGGCGATTA CCACGGCAGG TTCGTGACT
```

nov8

```
  1 TCNTAGCAAT AGGCCCGGAG ACAGGTGTTG CATGGCTGTN GTCAGCTCGT
 51 GTCNTGAGAT GNTGGGNTAA GTCCCGCNAC GAGCGCAACC CTCGCTGTTA
101 GTNGCCATCA TTTAGTTGGG CACTCTAACG GGACCNCCGG TGATAAGCCG
151 GAGGAAGGTG GGGATGACGT CAAGTCATCA TGGCCCTTAC GGGCTGGGCT
201 ACACACGTGC TACAATGGCG ACCATAAAGG TTTAATCCCC AAAAGTCGTC
251 TCAGTTCAGA TTGTCCTCTG CAACTCGAGG GCATGAAGTC GGAATCGNTA
301 GTAATCGCAG ATCAGCACGC TGCGGTGAAT ACGTTCCCGG GCCTNGTACA
351 CACCGCCCGT CACACCATGG GAGTTGTTAT ACCCGAAGGC AGTGCGCCAA
401 CTGCAAGGAG GCAGCTGACC ACGGTAGGTC NATGACTG
```

nov9

```
  1 CACGCCNTAA ACTATGGATA CTTNGACATT TGNGATAAAC TGTAAGNGTC
 51 TGGAGCGAAA GCATTAAGTA TCCCACCTGG GAAGTACGAC CGCAAGGTTG
101 AAACTCAAAG GAATTGGCGG GGGGTCCGCA CAAGCGGTGG AGCATGTGGT
151 TTAATTCGAT GATACGCGAG GAACCTTACN TGGGCTAGAA TGCTGGGAGA
201 CCGTGGGTGA AAGCTCACTT TGTAGCANTA CACTGCCAGT AAGGTGCTGC
251 ATGGCTGTCG TCAGCTCGTG CCGTGAGGTG TTGGGTTAAG TCCCGCAACG
301 AGCGCAACCC CCATCACTAG TTGCCATCAG GTAACGCTGG GAACTCTAGT
```

351 GAAACTGCCG TCGTAAGACG TGAGGAAGGA GGGGATGATG TCAAGTCATC
401 ATGGCCTTTA TGCCCAGGGC TACACACGTG CTACAATGGA GTGGACAAAG
451 GGCTGCAACA CAGTGATGTG AAGCTAATCC CAAAAACCAC TTCTCAGTTC
501 AGATTGGAGT CTGCAACTCG ACTCCATGAA GCTGGAATCG CTAGTAATCG
551 TATATCAGCA ATGATACGGT GAATACGTTC CCGGACCTTG CACACACCGC
601 CCGTCAAGCC ATGGAAGCCG GGTGTACCTA AAGTCGTTAA CCGTAAGGAG
651 ACGCCTAGGT AAAACTGTGA TGGGT

nov12

1 NGACCGCAAG GTTAAAACTC AAAGGAATTG ACGGGGGCCC GCACAAGCAG
51 CGGAGCGTGT GGTTTAATTC GATGCAACGC GAAGAACCTT ACCTAGGTTT
101 GACGTAGTGG TAGTAGTGAA GTGAAAGCGG AACGACCCTT CGGGGAGCCA
151 TTACAGGTGC TGCATGGCTG TCGTCAGCTC GTGTCGTGAG ATGTTGGGTT
201 AAGTCCCGCA ACGAGCGCAA CCNTNGTCGC TAGTTATACG TGTCTAGCGA
251 GACCGCCGGT ATCAAACCGG AGGAAGGTGG GGATGACGTC AAGTCAGCAT
301 GGCCTTTATG CCTAGGGCGA CACACACGCT ACAATGGCTG GTACAATGAG
351 CAGCGAACGG GTGACCTGGA GCGAATNTCA TCAAAGCCAG TCGTAGTTCA
401 GATTGTAGGC TGCAACCCGC CTACATGAAG CCGGAGTTGC TAGTAAACGC
451 AGGTCAGCCA TACTGCGTTG AATACGTTCC CGGGCCTTGT ACACACCGCC
501 CGTCACGTCA TGGGAGTTGG TAACACCTGA AGTCGGTGAC TGAACCGCAA
551 GGACAGAGCT GCCTCAGGGT GGGGCTGTGA CTGGACGAA

nov12 re

1 NGACCGCAAG GTTAAAACTC AAAGGAATTG ACGGGGGCCC GCACAAGCAG
51 CGGAGCGTGT GGTTTAATTC GATGCAACGC GAAGAACCTT ACCTAGGTTT
101 GACGTAGTGG TAGTAGTGAA GTGAAAGCGG AACGACCCTT CGGGGAGCCA
151 TTACAGGTGC TGCATGGCTG TCGTCAGCTC GTGTCGTGAG ATGTTGGGTT
201 AAGTCCCGCA ACGAGCGCAA CCNTNGTCGC TAGTTATACG TGTCTAGCGA
251 GACCGCCGGT ATCAAACCGG AGGAAGGTGG GGATGACGTC AAGTCAGCAT
301 GGCCTTTATG CCTAGGGCGA CACACACGCT ACAATGGCTG GTACAATGAG
351 CAGCGAACGG GTGACCTGGA GCGAATNTCA TCAAAGCCAG TCGTAGTTCA
401 GATTGTAGGC TGCAACCCGC CTACATGAAG CCGGAGTTGC TAGTAAACGC
451 AGGTCAGCCA TACTGCGTTG AATACGTTCC CGGGCCTTGT ACACACCGCC
501 CGTCACGTCA TGGGAGTTGG TAACACCTGA AGTCGGTGAC TGAACCGCAA
551 GGACAGAGCT GCCTCAGGGT GGGGCTGTGA CTGGACGAA

nov14

1 GGAGGARGGA TAAGCACTTT TTGAKTGTCG CAGCTAACGC GTTAAACAGG
51 GCGCMTGTGA AGTACGACCG CAAGGTTAAA ACACAAAGGA ATTGACGGGG
101 ACCCGCACAA GCAGTGGAGT GCCTGGTTTA ATTCGATGAT CAACGAAGAA
151 CCTCACCTGG GCTTGAACTG TGGCTGCTAA TCCTATGAAA GTAGGACTCT
201 TTCGAAGGTG CCACAGAGGT GATGCATGGC TGTCGTAAGC TCGTGTCGTG
251 AGATGTCGGG TTAAGTCCCA CAACGAGCGC AACCCCTTTC AGATGTTACA
301 ATTACTTCTG ATACTGCCCC GCCAAAACGG GGAGGAAGGA GGGGATGACG
351 TCCAGTCATC ATGTCCCTTA TGTCCAGGGC TACACAGACA CTACAATGGC
401 GATAACAATG GGTTGCCAAG CCGCAAGGCG GAGCTAATCC CATCAAAGAT
451 CGTCTCAGTT CAGATTGCAG GCTGCAACTC GCCTGTATGA AGTTGGAATT
501 GCTAGTAATC GGAAATCAGC GTGTTCCGGT GAATACGTTC TCGGGTCTTG
551 TACACACCGC CCGTCAGGGC ATGGGAGTTG CTCATACTCG AAGTCCTACG

601 TTTATCGTGG CCGAAAGTTN ATGGTGGCGA CTGGCTTNAA

nov16

1 NANGATGAAA GCCASTTTTC GGCAAGCANG GNTTTTCGGT GACACACCTA
51 ACGGATTAAG CATTCCGCCT GGGGAGTACG GTCGCAAGAT TAAAACTCAA
101 AGGAATTGAC GGGGGCCCGC ACAAGCGGTG GAGCATGTGG TTTAATTCGA
151 AGCAACGCGC AGAACCTTAC CAACCCTTGA CATGGCGATC GCGGTTCCAG
201 AGATGGTTCC TTCAGTTCGG CTGGATCGCA CACAGGTGCT GCATGGCTGT
251 CGTCAGCTCG TGTCGTGAGA TGTTCGGTTA AGTCCGGCAA CGAGCGCAAC
301 CCACACTTCT AGTTGCCAGC ATTCAGTTGG GCACTCTAGA AGAACTGCCG
351 ATGATAAGTC GGAGGAAGGT GTGGATGACG TCAAGTCCTC ATGGCCCTTA
401 CGGGTTGGGC TACACACGTG CTACAATGGT GGTGACAATG GGTTAATCCC
451 CAAAAGCCAT CTCAGTTCGG ATTGGGGTCT GCAACTCGAC CCCATGAAGT
501 CGGAATCGCT AGTAATCGCG TAACAGCATG ACGCGGTGAA TACGTTCCCG
551 GGCCTTGTAC ACACCGCCCG TCACACCATG GGAGTTGGGT TCACCCGAAG
601 GCGGTGCGCC AACCTCGCAA GAGGGGGCAG CCGACCACGT GGGCTCAGCG
651 ACTGGGKGAA

nov17

1 ACAAGCGGAG GAGCATGTGG TTTAATTSGA TGATACGCGA GGAACCTTAC
51 CTGGGCTTGA AAGTTAGCGA CCGACTCCGA ARAGAGTCTT TCAGAAATGA
101 CGCGAAANTA GGTGCTGCAT GGCTRTCGTC AGCTCGTGCC GTGAGGTGTT
151 GGGTTAAGTC CCGCAACGRG CGCAACCCMT ATTCWTAGTT GCCATCAGGT
201 TATGCTGGGG ACTCTAAGAA GACTGCCCGC GCAAGCGGTG AGGAAGGTGG
251 GGATGACGTC AAGTCATCAC GGCCCTTACG TCCAGGGCTA CACACGTGCT
301 ACAATGGCCG GTACAATGGG CAGCCACATG GCAACATGGA GCTAATCTTT
351 AAAACCGGTC TCAGTTCGGA TTGAGGTSTG CAACTCGACC TCATGAAGCT
401 GGATTCGCTA GTAATCGCAA ATCAGCAATG TTGCGGTGAA TACGTTCCCG
451 GGCCTTGTAC ACACCGCCCG TCAAGCCATG GAAGTCGGGA GTACCTAAAG
501 TCCATAACCG CAAGGAGTGG CCTAGGTAAA ACTTGATGAC T

nov18

1 CGAAACCCCW ATMGTCRGKD GCCAGCAWTC RGKTGGRGAS TSWGACGAGA
51 CCGCCGGTGT TAAACCGGAG GAAGGCRGNG ACGASGTMRA GTMATCRKGG
101 CSTKKAWRTV CRGGGCWGCA CACGTGGTAC AATGCGGCGT ACAAAGGGTT
151 GCCAACCCGT GAGGGGGAAC CAATCCCAAA AAGCGCCGCT CAGTTCGGAT
201 TGCAGGCTGC AACTCGCCTG CATGAAGCTG GAATSGCTAG TAATCGCGGA
251 TCAGGTATGC CGCGGTGAAT GTGTTCCTGA GCVTTGTACA CACCGCCCGT
301 CAAGCCACGA AAGGGGGGGG CATCCCAAGT CGCCGAGCCA ACCGCAAGGG
351 GGCAGTGCCT AAGATGAATC CSTGAKTGGA

nov19

1 TGGTGGGGCT ACACACGTCA TACAATGGCC GGTACAAAGG GTcGCAAACC
51 CGCGAGGGGG AGCCAATCCA TCAAAGCCGG TcGTAGTCCG GATCGCAGTC
101 TGCAACTNGA CTGCGTGAAG TCGGAATCGN TAGTANTCGT GGATCAGCAT
151 GTCACGGTGA ATACGTTCCC GGGTCTTGTA CACACCGCCC GTCACACCAT
201 GGGAGCGGGT CTCGCCAGAA GTAGTTAGCC TAACCGC

nov22

```
  1 NGTGGGCTAC ACACGTGGTA CAATGGCGGT GACAATGCGC AGCCACCTCG
 51 CGAGAGGGAG NTAATCGCAA AAAGCCGTCT CAGTTCGGAT TGGGCTCTGC
101 AACTCGAGCC CATGAAGTTG GAATCGNTAG TAATCGCGCA TCAGCATGGC
151 GCGGTGAATA CGTTCCCGGG CCTTGTACAC ACCGCCCGTC ACACCATGGG
201 AGTTGGCTTT ACCCGAAGAC GGTGCGCTAA CCGCAAGGAG GCAGCCGGCC
251 ACGGTAAGTC AGCGATT
```

Appendix B: Similarity_Rank results for the clones of January, April and November sludge samples.

442 **jan1** Unknown

S_ab	Olis	Short-ID	Full name
0.674	1374	str.Hyp353	str. US-353.
0.658	1315	Hyp.vulgar	Hyphomicrobium vulgare str. MC-750.
0.618	1336	Rhp.roseus	Rhodoplanes roseus str. 941.
0.581	1366	Azr.cauli2	Azorhizobium caulinodans.
0.577	1396	Azr.cauli3	Azorhizobium caulinodans.
0.570	1367	Rhb.galeg2	Rhizobium galegae.
0.569	267	env.PAD53	str. PAD53.
0.568	1397	Rhb.spOK55	Rhizobium sp. str. OK-55.
0.566	1325	Rhp.elegan	Rhodoplanes elegans str. AS130.
0.566	1396	Rhb.galega	Rhizobium galegae str. gal 1261.
0.566	1397	Rhb.sp113	Rhizobium sp. str. 113.
0.561	1354	Ag.rhizogn	Agrobacterium rhizogenes.
0.561	1397	Rhb.spOK50	Rhizobium sp. str. OK-50.
0.559	1392	Ag.tumefa7	Agrobacterium tumefaciens.
0.559	1355	Azs.lipof2	Azospirillum lipoferum str. VPI Sp 59b.
0.559	1402	Rhb.legu11	Rhizobium leguminosarum.
0.557	1297	Azs.amazo2	Azospirillum amazonense str. Y-1.
0.557	1392	Rhb.tropi8	Rhizobium tropici.
0.557	1353	Rhb.legum4	Rhizobium leguminosarum str. C-O5.
0.557	1365	Azs.brasi2	Azospirillum brasilense str. Sp 7.

405 **jan2** Unknown

S_ab	Olis	Short-ID	Full name
0.820	1377	Brch.denit	Brachymonas denitrificans str. AS-P1.
0.785	1411	Id.dechlrt	Ideonella dechloratans.
0.785	1371	Vrv.pardox	Variovorax paradoxus.
0.775	1398	Lptx.disc2	Leptothrix discophora str. SP-6.
0.756	1364	Rub.gelat2	Rubrivivax gelatinosus str. ATH 2.2.1.
0.748	1399	Lptx.disc3	Leptothrix discophora str. SS-1.
0.748	1370	Rub.gelat3	Rubrivivax gelatinosus str. A3.
0.743	1371	Rhf.ferme2	Rhodoferax fermentans str. FR2.
0.743	1371	Rhf.ferme3	Rhodoferax fermentans str. FR3.

0.734 263 env.PAD44 str. PAD44.
0.723 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.723 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.721 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.716 1430 Brd.holmes Bordetella holmesii.
0.706 1395 Rub.gelati Rubrivivax gelatinosus str. ATH 2.2.1.
0.696 1444 Com.testos Comamonas testosteroni str. RH 1104.
0.694 1275 Spt.natans Sphaerotilus natans str. 565.
0.691 1352 Brd.bronc2 Bordetella bronchiseptica str. Dog 71.
0.689 1360 Brd.pperts Bordetella parapertussis.
0.677 1359 Brd.pertus Bordetella pertussis.

---

622 **jan3** Unknown

S_ab Olis Short-ID Full name

0.681 263 env.PAD44 str. PAD44.
0.611 1377 Brch.denit Brachymonas denitrificans str. AS-P1.
0.596 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.595 1430 Brd.holmes Bordetella holmesii.
0.585 1416 Ps.aerugin Pseudomonas aeruginosa str. NIH 18.
0.582 1375 Ps.stutze2 Pseudomonas stutzeri str. 19SMN4.
0.582 1375 Ps.stutz13 Pseudomonas stutzeri str. ST27MN3.
0.580 1366 Ps.stutze6 Pseudomonas stutzeri str. Stanier 222.
0.580 1371 Ps.stutz11 Pseudomonas stutzeri str. LS401.
0.580 1371 Vrv.pardox Variovorax paradoxus.
0.580 1373 Ps.stutz10 Pseudomonas stutzeri str. Stanier 419.
0.580 1374 Ps.stutze7 Pseudomonas stutzeri str. Stanier 224.
0.580 1372 Ps.stutz14 Pseudomonas stutzeri str. ZoBell.
0.580 1367 Ps.stutze8 Pseudomonas stutzeri str. DNSP21.
0.580 1366 Ps.stutzer Pseudomonas stutzeri str. Stanier 221.
0.580 1371 Ps.baleari Pseudomonas balearica str. SP1402.
0.580 1374 Ps.stutze5 Pseudomonas stutzeri str. Stanier 220.
0.577 1369 Ps.stutze4 Pseudomonas stutzeri str. AN11.
0.577 1369 Ps.stutze9 Pseudomonas stutzeri.
0.577 1360 Brd.pperts Bordetella parapertussis.

---

559 **jan4** Unknown

S_ab Olis Short-ID Full name

0.830 1371 Vrv.pardox Variovorax paradoxus.

0.798 1371 Rhf.ferme3 Rhodoferax fermentans str. FR3.
0.798 1371 Rhf.ferme2 Rhodoferax fermentans str. FR2.
0.792 1398 Lptx.disc2 Leptothrix discophora str. SP-6.
0.780 1399 Lptx.disc3 Leptothrix discophora str. SS-1.
0.778 1411 Id.dechlrt Ideonella dechloratans.
0.776 263 env.PAD44 str. PAD44.
0.775 1377 Brch.denit Brachymonas denitrificans str. AS-P1.
0.757 1364 Rub.gelat2 Rubrivivax gelatinosus str. ATH 2.2.1.
0.751 1370 Rub.gelat3 Rubrivivax gelatinosus str. A3.
0.742 1444 Com.testos Comamonas testosteroni str. RH 1104.
0.737 1430 Brd.holmes Bordetella holmesii.
0.733 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.732 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.728 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.717 1395 Rub.gelati Rubrivivax gelatinosus str. ATH 2.2.1.
0.708 1360 Brd.pperts Bordetella parapertussis.
0.699 844 str.Stripa Stripa-derived bacterium
0.699 1352 Brd.bronc2 Bordetella bronchiseptica str. Dog 71.
0.699 1359 Brd.pertus Bordetella pertussis.

---

460 **jan5** Unknown

S_ab Olis Short-ID Full name

0.610 272 env.FIE8 str. FIE8.
0.600 1374 Mbc.whtbur Methylobacter whittenburyi.
0.585 1369 Mbc.luteu2 Methylobacter luteus.
0.572 1414 str.SCB111 str. SCB11.
0.565 1345 str.Thd2 str. Thd2.
0.561 1414 sym.Codaco symbiont of Codakia costata gill
0.561 1377 Mmb.agile Methylomicrobium agile str. A30.
0.561 1362 Mmb.album Methylomicrobium album str. VKM-BG8.
0.554 1392 sym.Andoph symbiont of Anodontia phillipiana (bivalve mollusc)
0.552 1369 Azs.sp3 Azospirillum sp. str. AM-53.
0.550 1385 sym.Solmve symbiont of Solemya velum
0.546 1416 Ps.aerugin Pseudomonas aeruginosa str. NIH 18.
0.546 1383 Mmb.pelagi Methylomicrobium pelagicum.
0.546 1415 Zyb.palmae Zymobacter palmae str. T109.
0.544 272 env.PAD61 str. PAD61.
0.541 1375 Ps.stutze2 Pseudomonas stutzeri str. 19SMN4.
0.541 1375 Ps.stutz13 Pseudomonas stutzeri str. ST27MN3.
0.541 1374 V.carchari Vibrio carchariae.
0.541 1444 Ps.flavesc Pseudomonas flavescens str. B62 (T).

0.539 1371 Ps.stutz11 Pseudomonas stutzeri str. LS401.

---

612 **jan6** Unknown

S_ab Olis Short-ID Full name

0.685 1374 str.Hyp353 str. US-353.
0.670 1315 Hyp.vulgar Hyphomicrobium vulgare str. MC-750.
0.611 1336 Rhp.roseus Rhodoplanes roseus str. 941.
0.596 267 env.PAD53 str. PAD53.
0.575 266 env.PAD54 str. PAD54.
0.574 1341 Rps.viridi Rhodopseudomonas viridis.
0.574 1343 Rps.virid3 Rhodopseudomonas viridis.
0.569 1325 Rhp.elegan Rhodoplanes elegans str. AS130.
0.562 1372 Ag.tumefa4 Agrobacterium tumefaciens.
0.562 1402 Ag.tumefa6 Agrobacterium tumefaciens.
0.562 267 env.PAD26 str. PAD26.
0.560 1316 Rps.spHGJF Rhodopseudomonas sp. str. H. Gest HG/JF.
0.560 1349 Mag.spAMB1 Magnetospirillum sp. str. AMB-1.
0.560 1316 Rdb.marin2 Rhodobium marinum.
0.559 1370 Mag.magne2 Magnetospirillum magnetotacticum.
0.559 1419 Ag.tumefac Agrobacterium tumefaciens.
0.556 1371 Ag.tumefa2 Agrobacterium tumefaciens.
0.554 1340 Rhb.ciceri Rhizobium ciceri str. UPM-Ca7.
0.552 1396 Azr.cauli3 Azorhizobium caulinodans.
0.552 1368 Ag.tumefa3 Agrobacterium tumefaciens str. ICPB TT111.

---

538 **jan7** Unknown

S_ab Olis Short-ID Full name

0.717 1371 Rhf.ferme3 Rhodoferax fermentans str. FR3.
0.717 1371 Rhf.ferme2 Rhodoferax fermentans str. FR2.
0.716 1371 Vrv.pardox Variovorax paradoxus.
0.697 1398 Lptx.disc2 Leptothrix discophora str. SP-6.
0.690 1411 Id.dechlrt Ideonella dechloratans.
0.682 1399 Lptx.disc3 Leptothrix discophora str. SS-1.
0.680 1364 Rub.gelat2 Rubrivivax gelatinosus str. ATH 2.2.1.
0.677 1370 Rub.gelat3 Rubrivivax gelatinosus str. A3.
0.660 1395 Rub.gelati Rubrivivax gelatinosus str. ATH 2.2.1.
0.654 263 env.PAD44 str. PAD44.
0.649 1430 Brd.holmes Bordetella holmesii.

0.647 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.643 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.643 1377 Brch.denit Brachymonas denitrificans str. AS-P1.
0.638 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.630 1444 Com.testos Comamonas testosteroni str. RH 1104.
0.619 1352 Brd.bronc2 Bordetella bronchiseptica str. Dog 71.
0.619 1360 Brd.pperts Bordetella parapertussis.
0.619 1379 Rcy.tenui2 Rhodocyclus tenuis str. 3760.
0.615 1391 Rcy.purpur Rhodocyclus purpureus str. 6770.

---

540 **jan8** Unknown

S_ab Olis Short-ID Full name

0.850 267 env.PAD26 str. PAD26.
0.846 169 Spg.spB712 Sphingomonas sp. str. B712.
0.800 1283 Spg.rosa Sphingomonas rosa.
0.754 1319 Rhz.subfa2 Rhizomonas suberifaciens.
0.752 1343 Spg.capsu2 Sphingomonas capsulata.
0.730 1333 Spg.adhae3 Sphingomonas adhaesiva.
0.729 266 env.PAD23 str. PAD23.
0.720 1345 Spg.yanoi6 Sphingomonas yanoikuyae str. B1.
0.715 239 str.B274 str. B274.
0.715 1325 Spg.macgol Sphingomonas macrogoltabidus.
0.711 1360 Spg.spSS86 Sphingomonas sp. str. SS86.
0.704 1320 Spg.yanoi5 Sphingomonas yanoikuyae.
0.700 1346 Spg.yanoi2 Sphingomonas yanoikuyae.
0.700 1309 Spg.terrae Sphingomonas terrae.
0.696 1329 Spg.capsul Sphingomonas capsulata.
0.689 1348 Zym.mobili Zymomonas mobilis subsp. mobilis.
0.689 1281 Spg.yanoi7 Sphingomonas yanoikuyae.
0.674 1329 Rdb.orient Rhodobium orientis str. MB312.
0.672 1360 Blb.natatr Blastobacter natatorius.
0.669 1352 Blb.BF14 Blastobacter sp. str. BF14 ?.

---

623 **jan9** Unknown

S_ab Olis Short-ID Full name

0.465 200 L.homohioc Lactobacillus homohiochii.
0.449 216 L.kefir2 Lactobacillus kefir.
0.430 1382 Sln.lactic Selenomonas lacticifex str. VB4b.

0.425 1334 str.srb str. TD3.
0.419 1316 Rdb.marin2 Rhodobium marinum.
0.419 1316 Rps.spHGJF Rhodopseudomonas sp. str. H. Gest HG/JF.
0.417 1447 Frm.balear Ferrimonas balearica str. PAT.
0.417 1435 She.algaBr Shewanella alga str. BrY.
0.416 1365 Azs.brasi2 Azospirillum brasilense str. Sp 7.
0.416 1404 Dmn.tiedje Desulfomonile tiedjei str. DCB-1.
0.416 1348 Mlb.GK118 Methylobacterium sp. str. GK118.
0.416 1348 B.fastidio Bacillus fastidiosus.
0.414 1466 Dsv.desulf Desulfovibrio desulfuricans.
0.413 1435 She.putre2 Shewanella putrefaciens.
0.411 1353 Mlb.rhode2 Methylobacterium rhodesianum.
0.411 1340 Myp.bullat Mycoplana bullata.
0.411 1355 Azs.lipof2 Azospirillum lipoferum str. VPI Sp 59b.
0.409 1349 Mlb.GK101 Methylobacterium sp. str. GK101.
0.408 1396 Azr.cauli3 Azorhizobium caulinodans.
0.408 1347 Mlb.zatma2 Methylobacterium zatmanii str. MA1/5.

---

626 **jan10** Unknown

S_ab Olis Short-ID Full name

0.776 1371 Vrv.pardox Variovorax paradoxus.
0.748 1371 Rhf.ferme3 Rhodoferax fermentans str. FR3.
0.748 1398 Lptx.disc2 Leptothrix discophora str. SP-6.
0.748 1371 Rhf.ferme2 Rhodoferax fermentans str. FR2.
0.720 1411 Id.dechlrt Ideonella dechloratans.
0.716 1399 Lptx.disc3 Leptothrix discophora str. SS-1.
0.714 1377 Brch.denit Brachymonas denitrificans str. AS-P1.
0.707 263 env.PAD44 str. PAD44.
0.704 1364 Rub.gelat2 Rubrivivax gelatinosus str. ATH 2.2.1.
0.701 1370 Rub.gelat3 Rubrivivax gelatinosus str. A3.
0.693 1444 Com.testos Comamonas testosteroni str. RH 1104.
0.690 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.682 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.668 1430 Brd.holmes Bordetella holmesii.
0.666 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.666 1395 Rub.gelati Rubrivivax gelatinosus str. ATH 2.2.1.
0.665 844 str.Stripa Stripa-derived bacterium
0.663 267 env.PAD58 str. PAD58.
0.660 1379 Rcy.tenui2 Rhodocyclus tenuis str. 3760.
0.645 1352 Brd.bronc2 Bordetella bronchiseptica str. Dog 71.

---

420 **jan11** Unknown

S_ab	Olis	Short-ID	Full name
0.676	1391	Rcy.purpur	Rhodocyclus purpureus str. 6770.
0.669	1387	Rcy.tenuis	Rhodocyclus tenuis str. 2761.
0.669	1427	Brd.bronch	Bordetella bronchiseptica str. S-1.
0.669	1385	Rcy.tenui3	Rhodocyclus tenuis str. SW18.
0.667	1398	Lptx.disc2	Leptothrix discophora str. SP-6.
0.667	1411	Id.dechlrt	Ideonella dechloratans.
0.667	1371	Vrv.pardox	Variovorax paradoxus.
0.664	1364	Rub.gelat2	Rubrivivax gelatinosus str. ATH 2.2.1.
0.662	1370	Rub.gelat3	Rubrivivax gelatinosus str. A3.
0.660	1399	Lptx.disc3	Leptothrix discophora str. SS-1.
0.648	1379	Rcy.tenui2	Rhodocyclus tenuis str. 3760.
0.645	1352	Brd.bronc2	Bordetella bronchiseptica str. Dog 71.
0.643	1395	Rub.gelati	Rubrivivax gelatinosus str. ATH 2.2.1.
0.643	1371	Rhf.ferme3	Rhodoferax fermentans str. FR3.
0.643	1430	Brd.holmes	Bordetella holmesii.
0.643	1360	Brd.pperts	Bordetella parapertussis.
0.643	1371	Rhf.ferme2	Rhodoferax fermentans str. FR2.
0.640	1407	Ral.eutrop	Ralstonia eutropha str. 335 (R.Y. Stanier).
0.626	1350	Brd.avium	Bordetella avium.
0.626	1448	sym.Critsp	symbiont of Crithidia sp.

620 **jan12** Unknown

S_ab	Olis	Short-ID	Full name
0.789	1371	Vrv.pardox	Variovorax paradoxus.
0.761	1371	Rhf.ferme3	Rhodoferax fermentans str. FR3.
0.761	1371	Rhf.ferme2	Rhodoferax fermentans str. FR2.
0.749	263	env.PAD44	str. PAD44.
0.748	1398	Lptx.disc2	Leptothrix discophora str. SP-6.
0.740	1411	Id.dechlrt	Ideonella dechloratans.
0.731	1399	Lptx.disc3	Leptothrix discophora str. SS-1.
0.727	1377	Brch.denit	Brachymonas denitrificans str. AS-P1.
0.711	1364	Rub.gelat2	Rubrivivax gelatinosus str. ATH 2.2.1.
0.708	1444	Com.testos	Comamonas testosteroni str. RH 1104.
0.706	1370	Rub.gelat3	Rubrivivax gelatinosus str. A3.
0.679	1430	Brd.holmes	Bordetella holmesii.
0.676	1387	Rcy.tenuis	Rhodocyclus tenuis str. 2761.
0.676	844	str.Stripa	Stripa-derived bacterium

0.674 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.670 267 env.PAD58 str. PAD58.
0.668 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.668 1275 Spt.natans Sphaerotilus natans str. 565.
0.666 1395 Rub.gelati Rubrivivax gelatinosus str. ATH 2.2.1.
0.663 1274 Lptx.disco Leptothrix discophora str. SS-1.

---

422 **jan13** Unknown

S_ab Olis Short-ID Full name

0.754 1371 Vrv.pardox Variovorax paradoxus.
0.709 1371 Rhf.ferme3 Rhodoferax fermentans str. FR3.
0.709 1371 Rhf.ferme2 Rhodoferax fermentans str. FR2.
0.709 1411 Id.dechlrt Ideonella dechloratans.
0.701 1398 Lptx.disc2 Leptothrix discophora str. SP-6.
0.692 1399 Lptx.disc3 Leptothrix discophora str. SS-1.
0.692 1391 Rcy.purpur Rhodocyclus purpureus str. 6770.
0.690 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.690 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.687 1364 Rub.gelat2 Rubrivivax gelatinosus str. ATH 2.2.1.
0.685 1370 Rub.gelat3 Rubrivivax gelatinosus str. A3.
0.673 1444 Com.testos Comamonas testosteroni str. RH 1104.
0.671 1395 Rub.gelati Rubrivivax gelatinosus str. ATH 2.2.1.
0.669 263 env.PAD44 str. PAD44.
0.668 1377 Brch.denit Brachymonas denitrificans str. AS-P1.
0.668 1430 Brd.holmes Bordetella holmesii.
0.664 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.645 1407 Ral.eutrop Ralstonia eutropha str. 335 (R.Y. Stanier).
0.645 1379 Rcy.tenui2 Rhodocyclus tenuis str. 3760.
0.637 1448 sym.Critsp symbiont of Crithidia sp.

---

622 **jan14** Unknown

S_ab Olis Short-ID Full name

0.659 1397 Rhb.sp113 Rhizobium sp. str. 113.
0.658 1344 Aqb.spirit Aquabacter spiritensis str. SPL-1.
0.654 1360 Rhb.legum6 Rhizobium leguminosarum.
0.654 1397 Rhb.spOK55 Rhizobium sp. str. OK-55.
0.651 1359 Myp.dmorph Mycoplana dimorpha.
0.651 1396 Rhb.galega Rhizobium galegae str. gal 1261.

0.651 1356 Rhb.legum8 Rhizobium leguminosarum.
0.650 1402 Rhb.legu11 Rhizobium leguminosarum.
0.650 1350 Rhb.legu12 Rhizobium leguminosarum.
0.650 1364 Rhb.etl182 Rhizobium etli str. TAL 182.
0.650 1394 Bar.bacill Bartonella bacilliformis.
0.650 1397 Rhb.spOK50 Rhizobium sp. str. OK-50.
0.650 1360 Bar.vinso4 Bartonella vinsonii subsp. berkhoffii str. 93-C01.
0.650 1402 Ag.vitis3 Agrobacterium vitis.
0.650 1372 Ag.vitis2 Agrobacterium vitis.
0.650 1387 Bar.vinso3 Bartonella vinsonii subsp. vinsonii.
0.648 1344 Bru.canis Brucella canis.
0.648 1366 Rhb.galeg3 Rhizobium galegae.
0.646 1384 Rhb.legu13 Rhizobium leguminosarum str. RCR 3644.
0.646 1371 Ag.vitis Agrobacterium vitis.

---

481 **jan15** Unknown

S_ab Olis Short-ID Full name

0.642 1411 Id.dechlrt Ideonella dechloratans.
0.642 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.638 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.638 1391 Rcy.purpur Rhodocyclus purpureus str. 6770.
0.638 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.634 1430 Brd.holmes Bordetella holmesii.
0.634 265 env.PAD37 str. PAD37.
0.628 1399 Lptx.disc3 Leptothrix discophora str. SS-1.
0.627 263 env.PAD44 str. PAD44.
0.622 1371 Vrv.pardox Variovorax paradoxus.
0.622 1352 Brd.bronc2 Bordetella bronchiseptica str. Dog 71.
0.617 1398 Lptx.disc2 Leptothrix discophora str. SP-6.
0.617 1379 Rcy.tenui2 Rhodocyclus tenuis str. 3760.
0.615 1364 Rub.gelat2 Rubrivivax gelatinosus str. ATH 2.2.1.
0.615 1360 Brd.pperts Bordetella parapertussis.
0.613 1407 Ral.eutrop Ralstonia eutropha str. 335 (R.Y. Stanier).
0.610 267 env.PAD58 str. PAD58.
0.609 1370 Rub.gelat3 Rubrivivax gelatinosus str. A3.
0.609 1448 sym.Critsp symbiont of Crithidia sp.
0.607 1359 Brd.pertus Bordetella pertussis.

---

506 **jan18** Unknown

S_ab	Olis	Short-ID	Full name
0.542	1343	Rps.virid3	Rhodopseudomonas viridis.
0.542	1341	Rps.viridi	Rhodopseudomonas viridis.
0.534	1346	Thb.sp051	Thiobacillus sp. str. THI051.
0.520	1368	Srh.fredi2	Sinorhizobium fredii.
0.520	1407	Srh.fredi5	Sinorhizobium fredii.
0.518	1359	Myp.dmorph	Mycoplana dimorpha.
0.518	1367	Srh.xinjia	Sinorhizobium xinjiangensis.
0.514	1356	Rhb.loti2	Rhizobium loti.
0.514	1395	Rhb.loti5	Rhizobium loti.
0.512	1362	Rhb.loti3	Rhizobium loti str. 3F6g2.
0.512	1329	Rdb.orient	Rhodobium orientis str. MB312.
0.512	1340	Rhb.ciceri	Rhizobium ciceri str. UPM-Ca7.
0.512	1401	Srh.melil7	Sinorhizobium meliloti.
0.512	1322	R.fulvum2	Rhodospirillum fulvum str. 1360.
0.510	1360	Srh.terang	Sinorhizobium teranga.
0.510	1360	Rhb.sp7834	Rhizobium sp.
0.508	1374	str.Hyp353	str. US-353.
0.508	1364	Srh.fredi3	Sinorhizobium fredii.
0.508	1362	Srh.saheli	Sinorhizobium saheli.
0.508	1325	Rhp.elegan	Rhodoplanes elegans str. AS130.

638 **jan19** Unknown

S_ab	Olis	Short-ID	Full name
0.503	1420	Sph.heparn	Sphingobacterium heparinum str. HIM 762-3.
0.492	1376	Sph.sprvo2	Sphingobacterium spiritivorum.
0.487	271	env.PAD36	str. PAD36.
0.484	1343	env.agg58	str. agg58.
0.480	1406	Msc.arenar	Microscilla arenaria str. HJ-1.
0.475	1367	Sph.thalpo	Sphingobacterium thalpophilum.
0.475	1380	Rik.mifusu	Rikenella microfusus.
0.472	1362	Msc.furves	Microscilla furvescens str. TV-2.
0.470	1379	Cy.ferment	Cytophaga fermentans.
0.467	1371	Flx.canada	Flexibacter canadensis.
0.461	271	env.PAD30	str. PAD30.
0.456	1384	F.columna2	Flavobacterium columnare str. RR-1a.
0.455	1403	Prv.disien	Prevotella disiens.
0.453	1347	Msc.marina	Microscilla marina str. SIO-8.
0.450	1412	Rht.marinu	Rhodothermus marinus.
0.450	1412	Rht.marin2	Rhodothermus marinus.

0.450 269 env.PAD48 str. PAD48.
0.448 1385 F.ameridie Flavobacterium ameridies.
0.447 1351 Blt.sp6 Blattabacterium sp.
0.447 1392 Flx.aggre1 Flexibacter aggregans str. Lewin NN-3.

---

526 **jan20** Unknown

S_ab Olis Short-ID Full name

0.530 1416 Pol.spPl49 Polyangium sp. str. Pl 4943.
0.510 1442 Pol.cellul Polyangium cellulosum subsp. ferrugineum str. SMP 4
0.496 1373 Con.apicul Chondromyces apiculatus str. Cm a2.
0.480 271 env.PAD12 str. PAD12.
0.475 1393 Con.crocat Chondromyces crocatus str. Cm c6.
0.472 265 env.FIE4 str. FIE4.
0.458 758 env.PVB46 str. PVB_46.
0.443 1389 Nc.mobilis Nitrococcus mobilis.
0.439 1346 Acbt.capsl Acidobacterium capsulatum str. 161.
0.438 265 env.FIE20 str. FIE20.
0.435 1371 Ard.aquaeo Arhodomonas aquaeolei str. HA-1.
0.428 1387 env.PVB3_5 str. PVB_3, PVB_5.
0.426 1411 Arm.eucren Aeromonas eucrenophila.
0.426 1388 env.PVB25 str. PVB_25.
0.426 1345 str.Thd2 str. Thd2.
0.426 1385 env.PVB47 str. PVB_47.
0.424 1372 Arm.eucre2 Aeromonas eucrenophila.
0.424 1378 Chr.vinosm Chromatium vinosum.
0.424 1435 Arm.ichthi Aeromonas ichthiosmia.
0.424 1410 Arm.veroni Aeromonas veronii.

---

428 **jan21** Unknown

S_ab Olis Short-ID Full name

0.701 1371 Vrv.pardox Variovorax paradoxus.
0.694 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.694 1398 Lptx.disc2 Leptothrix discophora str. SP-6.
0.694 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.689 1411 Id.dechlrt Ideonella dechloratans.
0.678 1399 Lptx.disc3 Leptothrix discophora str. SS-1.
0.678 1430 Brd.holmes Bordetella holmesii.
0.673 1371 Rhf.ferme2 Rhodoferax fermentans str. FR2.

0.673 1371 Rhf.ferme3 Rhodoferax fermentans str. FR3.
0.671 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.664 1364 Rub.gelat2 Rubrivivax gelatinosus str. ATH 2.2.1.
0.657 1370 Rub.gelat3 Rubrivivax gelatinosus str. A3.
0.645 1377 Brch.denit Brachymonas denitrificans str. AS-P1.
0.643 1352 Brd.bronc2 Bordetella bronchiseptica str. Dog 71.
0.643 1360 Brd.pperts Bordetella parapertussis.
0.640 1379 Rcy.tenui2 Rhodocyclus tenuis str. 3760.
0.638 1395 Rub.gelati Rubrivivax gelatinosus str. ATH 2.2.1.
0.635 263 env.PAD44 str. PAD44.
0.633 1359 Brd.pertus Bordetella pertussis.
0.626 1350 Brd.avium Bordetella avium.

---

571 **jan23** Unknown

S_ab Olis Short-ID Full name

0.823 1377 Brch.denit Brachymonas denitrificans str. AS-P1.
0.778 1371 Vrv.pardox Variovorax paradoxus.
0.762 1411 Id.dechlrt Ideonella dechloratans.
0.751 1444 Com.testos Comamonas testosteroni str. RH 1104.
0.744 1398 Lptx.disc2 Leptothrix discophora str. SP-6.
0.741 263 env.PAD44 str. PAD44.
0.720 1399 Lptx.disc3 Leptothrix discophora str. SS-1.
0.716 1364 Rub.gelat2 Rubrivivax gelatinosus str. ATH 2.2.1.
0.711 1370 Rub.gelat3 Rubrivivax gelatinosus str. A3.
0.711 844 str.Stripa Stripa-derived bacterium
0.697 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.697 1371 Rhf.ferme2 Rhodoferax fermentans str. FR2.
0.697 1371 Rhf.ferme3 Rhodoferax fermentans str. FR3.
0.694 1430 Brd.holmes Bordetella holmesii.
0.678 1395 Rub.gelati Rubrivivax gelatinosus str. ATH 2.2.1.
0.676 1360 Brd.pperts Bordetella parapertussis.
0.669 1350 Brd.avium Bordetella avium.
0.667 1352 Brd.bronc2 Bordetella bronchiseptica str. Dog 71.
0.667 1275 Spt.natans Sphaerotilus natans str. 565.
0.665 1359 Brd.pertus Bordetella pertussis.

---

635 **apr1** Unknown

S_ab Olis Short-ID Full name

0.920 264 env.FIE1 str. FIE1.
0.820 1370 Rub.gelat3 Rubrivivax gelatinosus str. A3.
0.817 1364 Rub.gelat2 Rubrivivax gelatinosus str. ATH 2.2.1.
0.795 1398 Lptx.disc2 Leptothrix discophora str. SP-6.
0.786 1399 Lptx.disc3 Leptothrix discophora str. SS-1.
0.781 1411 Id.dechlrt Ideonella dechloratans.
0.743 1395 Rub.gelati Rubrivivax gelatinosus str. ATH 2.2.1.
0.706 1274 Lptx.disco Leptothrix discophora str. SS-1.
0.690 1371 Vrv.pardox Variovorax paradoxus.
0.687 1275 Spt.natans Sphaerotilus natans str. 565.
0.660 1377 Brch.denit Brachymonas denitrificans str. AS-P1.
0.655 267 env.PAD58 str. PAD58.
0.643 1444 Com.testos Comamonas testosteroni str. RH 1104.
0.642 265 env.PAD37 str. PAD37.
0.635 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.635 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.630 1407 Ral.eutrop Ralstonia eutropha str. 335 (R.Y. Stanier).
0.624 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.620 1430 Brd.holmes Bordetella holmesii.
0.619 1371 Rhf.ferme3 Rhodoferax fermentans str. FR3.

---

633 **apr2** Unknown

S_ab Olis Short-ID Full name

0.815 1444 Com.testos Comamonas testosteroni str. RH 1104.
0.788 1371 Vrv.pardox Variovorax paradoxus.
0.782 1377 Brch.denit Brachymonas denitrificans str. AS-P1.
0.779 263 env.PAD44 str. PAD44.
0.728 1398 Lptx.disc2 Leptothrix discophora str. SP-6.
0.725 1411 Id.dechlrt Ideonella dechloratans.
0.711 1371 Rhf.ferme3 Rhodoferax fermentans str. FR3.
0.711 1371 Rhf.ferme2 Rhodoferax fermentans str. FR2.
0.695 1399 Lptx.disc3 Leptothrix discophora str. SS-1.
0.694 1364 Rub.gelat2 Rubrivivax gelatinosus str. ATH 2.2.1.
0.690 1370 Rub.gelat3 Rubrivivax gelatinosus str. A3.
0.654 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.646 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.645 1274 Lptx.disco Leptothrix discophora str. SS-1.
0.645 1395 Rub.gelati Rubrivivax gelatinosus str. ATH 2.2.1.
0.643 1430 Brd.holmes Bordetella holmesii.
0.640 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.637 1407 Ral.eutrop Ralstonia eutropha str. 335 (R.Y. Stanier).

0.630 1275 Spt.natans Sphaerotilus natans str. 565.
0.627 1433 Zoo.ramig6 Zoogloea ramigera.

---

626 **apr3** Unknown

S_ab Olis Short-ID Full name

0.740 1315 Hyp.vulgar Hyphomicrobium vulgare str. MC-750.
0.727 1374 str.Hyp353 str. US-353.
0.631 1316 Rdb.marin2 Rhodobium marinum.
0.631 1316 Rps.spHGJF Rhodopseudomonas sp. str. H. Gest HG/JF.
0.620 1336 Rhp.roseus Rhodoplanes roseus str. 941.
0.613 1325 Rhp.elegan Rhodoplanes elegans str. AS130.
0.613 1332 Rm.vanniel Rhodomicrobium vannielii str. EY33.
0.610 1331 R.rubrum3 Rhodospirillum rubrum.
0.607 1386 Rps.palus2 Rhodopseudomonas palustris str. ATH 2.1.6.
0.604 1370 Mag.magne2 Magnetospirillum magnetotacticum.
0.604 1371 Ag.vitis Agrobacterium vitis.
0.602 1365 Rhb.sp7836 Rhizobium sp.
0.602 1365 Rhb.sp7854 Rhizobium sp.
0.601 1370 Bdr.sp9980 Bradyrhizobium sp.
0.601 1349 Mag.spAMB1 Magnetospirillum sp. str. AMB-1.
0.599 1369 Bdr.sp9520 Bradyrhizobium sp.
0.599 1372 Bdr.sp0689 Bradyrhizobium sp.
0.599 1396 Azr.cauli3 Azorhizobium caulinodans.
0.597 1376 Mag.gryphi Magnetospirillum gryphiswaldense str. MSR-1.
0.597 1346 Thb.sp051 Thiobacillus sp. str. THI051.

---

629 **apr4** Unknown

S_ab Olis Short-ID Full name

0.593 1415 sym.Thya.f symbiont of Thyasira flexuosa gill
0.579 1374 Leg.anisa Legionella anisa.
0.579 1385 sym.Solmve symbiont of Solemya velum
0.572 1414 sym.Codaco symbiont of Codakia costata gill
0.572 1374 Mbc.whtbur Methylobacter whittenburyi.
0.572 1392 sym.Andoph symbiont of Anodontia phillipiana (bivalve mollusc)
0.572 1373 Leg.steigw Legionella steigerwaltii.
0.571 1376 Leg.cherri Legionella cherrii.
0.569 1390 Osp.kriegi Oceanospirillum kriegii.
0.569 1376 Leg.brunen Legionella brunensis.

0.569 1369 Mbc.luteu2 Methylobacter luteus.
0.568 1387 Mlc.capsu4 Methylococcus capsulatus str. Bath.
0.568 1369 Azs.sp3 Azospirillum sp. str. AM-53.
0.564 1379 Mlc.capsu3 Methylococcus capsulatus str. Texas.
0.561 1327 sym.Solmre symbiont of Solemya reidi gill
0.560 1362 Mmb.album Methylomicrobium album str. VKM-BG8.
0.558 1367 Leg.stheln Legionella sainthelensi.
0.558 1444 Ps.flavesc Pseudomonas flavescens str. B62 (T).
0.558 1379 Cox.burnet Coxiella burnetii str. Q177.
0.556 1365 Leg.pneum6 Legionella pneumophila str. Chicago 2.

---

644 **apr5** Unknown

S_ab Olis Short-ID Full name

0.461 282 env.OS_M Octopus Spring microbial mat DNA Type M
0.454 271 env.FIE12 str. FIE12.
0.446 1412 Rht.marinu Rhodothermus marinus.
0.446 1412 Rht.marin2 Rhodothermus marinus.
0.436 1474 Peb.propio Pelobacter propionicus str. OttBd1.
0.435 269 env.PAD48 str. PAD48.
0.434 265 env.PAD8 str. PAD8.
0.433 460 E.coli3 Escherichia coli.
0.432 229 env.OS-9 str. OSIII-9.
0.431 362 env.OS_E Octopus Spring microbial mat DNA Type E
0.429 1379 Mlc.capsu3 Methylococcus capsulatus str. Texas.
0.427 1371 Ard.aquaeo Arhodomonas aquaeolei str. HA-1.
0.422 1371 Flx.canada Flexibacter canadensis.
0.422 1387 Mlc.capsu4 Methylococcus capsulatus str. Bath.
0.419 1382 Acb.semini Actinobacillus seminis.
0.418 1420 Spi.aurant Spirochaeta aurantia str. J1.
0.418 1277 Blp.wadswr Bilophila wadsworthia.
0.418 1359 Thb.caldus Thiobacillus caldus str. KU.
0.415 1381 H.actin522 Haemophilus actinomycetemcomitans.
0.413 1390 Pas.mair89 Pasteurella mairii.

---

645 **apr6** Unknown

S_ab Olis Short-ID Full name

0.792 1444 Com.testos Comamonas testosteroni str. RH 1104.
0.787 263 env.PAD44 str. PAD44.

0.778 1371 Vrv.pardox Variovorax paradoxus.
0.764 1377 Brch.denit Brachymonas denitrificans str. AS-P1.
0.730 1411 Id.dechlrt Ideonella dechloratans.
0.722 1398 Lptx.disc2 Leptothrix discophora str. SP-6.
0.713 1371 Rhf.ferme3 Rhodoferax fermentans str. FR3.
0.713 1371 Rhf.ferme2 Rhodoferax fermentans str. FR2.
0.702 1399 Lptx.disc3 Leptothrix discophora str. SS-1.
0.699 1364 Rub.gelat2 Rubrivivax gelatinosus str. ATH 2.2.1.
0.695 1370 Rub.gelat3 Rubrivivax gelatinosus str. A3.
0.667 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.659 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.659 1430 Brd.holmes Bordetella holmesii.
0.657 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.651 1395 Rub.gelati Rubrivivax gelatinosus str. ATH 2.2.1.
0.645 265 env.PAD37 str. PAD37.
0.645 1407 Ral.eutrop Ralstonia eutropha str. 335 (R.Y. Stanier).
0.643 1360 Brd.pperts Bordetella parapertussis.
0.637 1433 Zoo.ramig6 Zoogloea ramigera.

---

360 **apr10** Unknown

S_ab Olis Short-ID Full name

0.522 1374 str.Hyp353 str. US-353.
0.506 1315 Hyp.vulgar Hyphomicrobium vulgare str. MC-750.
0.483 1414 Bdr.japon5 Bradyrhizobium japonicum.
0.475 1369 Bdr.sp9520 Bradyrhizobium sp.
0.475 1368 Bdr.sp9514 Bradyrhizobium sp.
0.475 1370 Bdr.sp9980 Bradyrhizobium sp.
0.475 1372 Bdr.sp0689 Bradyrhizobium sp.
0.475 1370 Bdr.sp9966 Bradyrhizobium sp.
0.472 1406 Bdr.sp129 Bradyrhizobium sp. str. 129.
0.472 1364 Bdr.japoni Bradyrhizobium japonicum.
0.467 1394 Bdr.japon4 Bradyrhizobium japonicum.
0.461 1357 Blb.denitr Blastobacter denitrificans.
0.461 1365 Azs.brasi2 Azospirillum brasilense str. Sp 7.
0.458 1294 Azs.brasi7 Azospirillum brasilense str. Sp 81.
0.456 1325 Thb.novell Thiobacillus novellus.
0.456 1355 Azs.lipof2 Azospirillum lipoferum str. VPI Sp 59b.
0.456 1357 Osp.pusill Oceanospirillum pusillum.
0.453 1359 Bdr.japon2 Bradyrhizobium japonicum.
0.450 1316 Rdb.marin2 Rhodobium marinum.
0.450 1316 Rps.spHGJF Rhodopseudomonas sp. str. H. Gest HG/JF.

635 **apr11** Unknown

S_ab	Olis	Short-ID	Full name
0.539	1420	Sph.heparn	Sphingobacterium heparinum str. HIM 762-3.
0.528	1343	env.agg58	str. agg58.
0.518	1376	Sph.sprvo2	Sphingobacterium spiritivorum.
0.515	1406	Msc.arenar	Microscilla arenaria str. HJ-1.
0.515	1362	Msc.furves	Microscilla furvescens str. TV-2.
0.507	1380	Rik.mifusu	Rikenella microfusus.
0.493	1367	Sph.thalpo	Sphingobacterium thalpophilum.
0.491	1371	Flx.canada	Flexibacter canadensis.
0.485	1403	Prv.disien	Prevotella disiens.
0.483	271	env.PAD36	str. PAD36.
0.480	1392	Flx.aggre1	Flexibacter aggregans str. Lewin NN-3.
0.479	1385	Msc.serice	"Microscilla sericea" str. SIO-7.
0.476	1384	F.columna2	Flavobacterium columnare str. RR-1a.
0.474	1412	Rht.marin2	Rhodothermus marinus.
0.474	1412	Rht.marinu	Rhodothermus marinus.
0.472	1347	Msc.marina	Microscilla marina str. SIO-8.
0.471	1379	Cy.ferment	Cytophaga fermentans.
0.469	1385	F.ameridie	Flavobacterium ameridies.
0.466	1374	Wee.virosa	Weeksella virosa.
0.466	1378	Bac.putred	Bacteroides putredinis str. 1 Ando.

676 **apr12** Unknown

S_ab	Olis	Short-ID	Full name
0.661	1374	str.Hyp353	str. US-353.
0.649	1315	Hyp.vulgar	Hyphomicrobium vulgare str. MC-750.
0.592	267	env.PAD53	str. PAD53.
0.571	266	env.PAD54	str. PAD54.
0.567	1332	Rm.vanniel	Rhodomicrobium vannielii str. EY33.
0.567	1316	Rdb.marin2	Rhodobium marinum.
0.567	1316	Rps.spHGJF	Rhodopseudomonas sp. str. H. Gest HG/JF.
0.564	1406	Bdr.sp129	Bradyrhizobium sp. str. 129.
0.561	1364	Bdr.japoni	Bradyrhizobium japonicum.
0.556	1372	Bdr.sp0689	Bradyrhizobium sp.
0.554	267	env.PAD26	str. PAD26.
0.554	269	env.PAD48	str. PAD48.

0.553 1336 Rhp.roseus Rhodoplanes roseus str. 941.
0.552 1370 Bdr.sp9980 Bradyrhizobium sp.
0.552 1386 Rps.palus2 Rhodopseudomonas palustris str. ATH 2.1.6.
0.552 1369 Bdr.sp9520 Bradyrhizobium sp.
0.551 216 Thb.acido2 Thiobacillus acidophilus.
0.550 1394 Bdr.japon4 Bradyrhizobium japonicum.
0.549 1343 Rps.virid3 Rhodopseudomonas viridis.
0.549 1341 Rps.viridi Rhodopseudomonas viridis.

---

543 **apr13** Unknown

S_ab Olis Short-ID Full name

0.753 1379 Rcy.tenui2 Rhodocyclus tenuis str. 3760.
0.753 1374 Azc.indige Azoarcus indigens str. VB32 (T).
0.746 1410 Tha.aromat Thauera aromatica str. K172, clone K1.
0.746 1381 Zoo.ramig4 Zoogloea ramigera str. 235 (N.C. Dondero).
0.738 1442 Zoo.ramig7 Zoogloea ramigera.
0.729 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.729 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.727 1391 Rcy.purpur Rhodocyclus purpureus str. 6770.
0.722 1379 Zoo.ramig1 Zoogloea ramigera.
0.705 1363 Azc.toluly Azoarcus tolulyticus str. Tol-4.
0.703 1368 Azc.BH72 Azoarcus sp. str. BH72.
0.703 1420 Azc.evansi Azoarcus evansii str. KB740.
0.703 1376 Nss.brien2 Nitrosospira briensis str. C-128.
0.702 1360 Azc.denit4 Azoarcus denitrificians str. Td-17.
0.696 1367 Azc.S5b2 Azoarcus sp. str. S5b2.
0.694 265 env.PAD37 str. PAD37.
0.694 1400 Nss.multi3 Nitrosospira multiformis str. C-71.
0.694 1360 Azc.denit2 Azoarcus denitrificians str. Td-1.
0.682 267 env.PAD58 str. PAD58.
0.681 1360 Azc.denit8 Azoarcus denitrificians str. Td-3.

---

638 **apr14** Unknown

S_ab Olis Short-ID Full name

0.550 1390 Osp.kriegi Oceanospirillum kriegii.
0.524 1367 Leg.stheln Legionella sainthelensi.
0.522 1345 str.Thd2 str. Thd2.
0.519 1376 Leg.brunen Legionella brunensis.

0.519 1368 Leg.jordan Legionella jordanis.
0.517 1384 Leg.cincin Legionella cincinnatiensis.
0.514 1457 Leg.lytica Legionella lytica.
0.514 1362 Mmb.album Methylomicrobium album str. VKM-BG8.
0.511 1362 Leg.wdswrt Legionella wadsworthii.
0.511 1374 Mbc.whtbur Methylobacter whittenburyi.
0.511 1383 Mmb.pelagi Methylomicrobium pelagicum.
0.511 1377 Mmb.agile Methylomicrobium agile str. A30.
0.506 1384 Leg.jorda2 Legionella jordanis.
0.506 1360 Leg.feele2 Legionella feeleii str. 691-WI-H.
0.505 1373 Leg.steigw Legionella steigerwaltii.
0.505 1374 Leg.anisa Legionella anisa.
0.505 1365 Leg.pneum6 Legionella pneumophila str. Chicago 2.
0.505 1387 Mlc.capsu4 Methylococcus capsulatus str. Bath.
0.505 1379 Mlc.capsu3 Methylococcus capsulatus str. Texas.
0.503 1369 Mbc.luteu2 Methylobacter luteus.

---

482 **apr15** Unknown

S_ab Olis Short-ID Full name

0.548 1343 Rps.virid3 Rhodopseudomonas viridis.
0.548 1341 Rps.viridi Rhodopseudomonas viridis.
0.527 1329 Rdb.orient Rhodobium orientis str. MB312.
0.517 1336 Rhp.roseus Rhodoplanes roseus str. 941.
0.517 1346 Thb.sp051 Thiobacillus sp. str. THI051.
0.512 1353 Spg.spSYK6 Sphingomonas sp. str. SYK6.
0.510 1360 Rhb.sp7834 Rhizobium sp.
0.510 1360 Srh.terang Sinorhizobium teranga.
0.508 1374 str.Hyp353 str. US-353.
0.508 1347 Mlb.zatma2 Methylobacterium zatmanii str. MA1/5.
0.506 1359 Myp.dmorph Mycoplana dimorpha.
0.506 1407 Srh.fredi5 Sinorhizobium fredii.
0.506 1368 Srh.fredi2 Sinorhizobium fredii.
0.504 1339 Mlb.spF15 Methylobacterium sp. str. F15.
0.504 1367 Srh.xinjia Sinorhizobium xinjiangensis.
0.504 1340 Rhb.ciceri Rhizobium ciceri str. UPM-Ca7.
0.504 1349 Mlb.GK101 Methylobacterium sp. str. GK101.
0.502 1371 Ag.tumefa2 Agrobacterium tumefaciens.
0.502 1372 Ag.tumefa4 Agrobacterium tumefaciens.
0.502 1322 R.fulvum2 Rhodospirillum fulvum str. 1360.

---

487 **apr16** Unknown

S_ab	Olis	Short-ID	Full name
0.507	1420	Sph.heparn	Sphingobacterium heparinum str. HIM 762-3.
0.480	1343	env.agg58	str. agg58.
0.472	1406	Msc.arenar	Microscilla arenaria str. HJ-1.
0.468	1380	Rik.mifusu	Rikenella microfusus.
0.466	1376	Sph.sprvo2	Sphingobacterium spiritivorum.
0.450	1367	Sph.thalpo	Sphingobacterium thalpophilum.
0.446	1403	Prv.disien	Prevotella disiens.
0.441	1371	Flx.canada	Flexibacter canadensis.
0.437	1363	Csb.indthe	Chryseobacterium indoltheticum.
0.437	1386	Cy.difflue	Cytophaga diffluens str. Lewin LIM-1.
0.435	1412	Rht.marinu	Rhodothermus marinus.
0.435	1412	Rht.marin2	Rhodothermus marinus.
0.435	1384	F.columna2	Flavobacterium columnare str. RR-1a.
0.435	1378	Bac.putred	Bacteroides putredinis str. 1 Ando.
0.435	1394	Emb.brevi2	Empedobacter brevis.
0.431	1373	Cyb.marinu	Cyclobacterium marinus str. WH-A; Raj.
0.429	1209	Csb.mening	Chryseobacterium meningosepticum.
0.429	1362	Msc.furves	Microscilla furvescens str. TV-2.
0.429	1372	Ppm.catoni	Porphyromonas catoniae.
0.429	1385	Msc.serice	"Microscilla sericea" str. SIO-7.

424 **apr17** Unknown

S_ab	Olis	Short-ID	Full name
0.679	1377	Brch.denit	Brachymonas denitrificans str. AS-P1.
0.625	1427	Brd.bronch	Bordetella bronchiseptica str. S-1.
0.623	1416	Ps.aerugin	Pseudomonas aeruginosa str. NIH 18.
0.623	1430	Brd.holmes	Bordetella holmesii.
0.608	1371	Vrv.pardox	Variovorax paradoxus.
0.608	1444	Com.testos	Comamonas testosteroni str. RH 1104.
0.604	1460	Ps.aerugi3	Pseudomonas aeruginosa.
0.599	1371	Ps.baleari	Pseudomonas balearica str. SP1402.
0.599	1371	Ps.stutz11	Pseudomonas stutzeri str. LS401.
0.597	1375	Ps.stutze2	Pseudomonas stutzeri str. 19SMN4.
0.597	1375	Ps.stutz13	Pseudomonas stutzeri str. ST27MN3.
0.594	1374	Ps.stutze3	Pseudomonas stutzeri str. AN10.
0.594	1366	Ps.stutze6	Pseudomonas stutzeri str. Stanier 222.
0.594	1366	Ps.stutzer	Pseudomonas stutzeri str. Stanier 221.

0.594 1371 Rhf.ferme3 Rhodoferax fermentans str. FR3.
0.594 1374 Ps.stutze5 Pseudomonas stutzeri str. Stanier 220.
0.594 1369 Ps.stutze4 Pseudomonas stutzeri str. AN11.
0.594 1369 Ps.stutze9 Pseudomonas stutzeri.
0.594 1372 Ps.stutz14 Pseudomonas stutzeri str. ZoBell.
0.594 1367 Ps.stutze8 Pseudomonas stutzeri str. DNSP21.

---

521 **apr18** Unknown

S_ab Olis Short-ID Full name

0.702 1315 Hyp.vulgar Hyphomicrobium vulgare str. MC-750.
0.691 1374 str.Hyp353 str. US-353.
0.582 1386 Rps.palus2 Rhodopseudomonas palustris str. ATH 2.1.6.
0.580 1396 Azr.cauli3 Azorhizobium caulinodans.
0.572 1336 Rhp.roseus Rhodoplanes roseus str. 941.
0.570 1406 Bdr.sp129 Bradyrhizobium sp. str. 129.
0.570 1369 Bdr.sp9520 Bradyrhizobium sp.
0.570 1372 Bdr.sp0689 Bradyrhizobium sp.
0.568 1370 Bdr.sp9980 Bradyrhizobium sp.
0.568 1366 Azr.cauli2 Azorhizobium caulinodans.
0.568 1364 Bdr.japoni Bradyrhizobium japonicum.
0.568 1316 Rdb.marin2 Rhodobium marinum.
0.568 1316 Rps.spHGJF Rhodopseudomonas sp. str. H. Gest HG/JF.
0.568 1370 Bdr.sp9966 Bradyrhizobium sp.
0.566 1394 Bdr.japon4 Bradyrhizobium japonicum.
0.566 1365 Rhb.sp7836 Rhizobium sp.
0.566 1365 Rhb.sp7854 Rhizobium sp.
0.564 1342 Rps.palus7 Rhodopseudomonas palustris.
0.564 1359 Bdr.japon2 Bradyrhizobium japonicum.
0.562 1370 Mag.magne2 Magnetospirillum magnetotacticum.

---

527 **apr19** Unknown

S_ab Olis Short-ID Full name

0.723 1374 str.Hyp353 str. US-353.
0.719 1315 Hyp.vulgar Hyphomicrobium vulgare str. MC-750.
0.638 1325 Rhp.elegan Rhodoplanes elegans str. AS130.
0.617 1396 Azr.cauli3 Azorhizobium caulinodans.
0.615 1342 Rps.palus7 Rhodopseudomonas palustris.
0.615 1414 Bdr.japon5 Bradyrhizobium japonicum.

0.615 1394 Bdr.japon4 Bradyrhizobium japonicum.
0.609 1341 Rps.viridi Rhodopseudomonas viridis.
0.609 1343 Rps.virid3 Rhodopseudomonas viridis.
0.607 1366 Azr.cauli2 Azorhizobium caulinodans.
0.605 1357 Blb.denitr Blastobacter denitrificans.
0.605 1406 Bdr.sp129 Bradyrhizobium sp. str. 129.
0.605 1364 Bdr.japoni Bradyrhizobium japonicum.
0.603 1359 Bdr.japon2 Bradyrhizobium japonicum.
0.598 1373 Rps.palust Rhodopseudomonas palustris str. GH.
0.596 1404 Zoo.ramig5 Zoogloea ramigera.
0.596 1360 Rhb.loti4 Rhizobium loti.
0.596 1359 Rhb.huakui Rhizobium huakuii.
0.596 1337 Zoo.ramig2 Zoogloea ramigera.
0.596 1370 Bdr.sp9980 Bradyrhizobium sp.

---

494 **apr20** Unknown

S_ab Olis Short-ID Full name

0.719 1374 str.Hyp353 str. US-353.
0.676 1315 Hyp.vulgar Hyphomicrobium vulgare str. MC-750.
0.652 1414 Bdr.japon5 Bradyrhizobium japonicum.
0.640 1394 Bdr.japon4 Bradyrhizobium japonicum.
0.634 1406 Bdr.sp129 Bradyrhizobium sp. str. 129.
0.626 1357 Blb.denitr Blastobacter denitrificans.
0.623 1342 Rps.palus7 Rhodopseudomonas palustris.
0.623 1364 Bdr.japoni Bradyrhizobium japonicum.
0.623 1373 Rps.palust Rhodopseudomonas palustris str. GH.
0.617 1370 Bdr.sp9980 Bradyrhizobium sp.
0.615 1372 Bdr.sp0689 Bradyrhizobium sp.
0.615 1370 Bdr.sp9966 Bradyrhizobium sp.
0.615 1369 Bdr.sp9520 Bradyrhizobium sp.
0.605 1359 Bdr.japon2 Bradyrhizobium japonicum.
0.601 1368 Bdr.sp9514 Bradyrhizobium sp.
0.595 1341 Rps.viridi Rhodopseudomonas viridis.
0.595 1343 Rps.virid3 Rhodopseudomonas viridis.
0.591 1325 Rhp.elegan Rhodoplanes elegans str. AS130.
0.587 1344 Afp.cleve Afipia clevelandensis.
0.585 1351 Anc.aquati Ancylobacter aquaticus.

---

528 **apr21** Unknown

S_ab	Olis	Short-ID	Full name
0.581	1343	Rps.virid3	Rhodopseudomonas viridis.
0.581	1341	Rps.viridi	Rhodopseudomonas viridis.
0.561	1336	Rhp.roseus	Rhodoplanes roseus str. 941.
0.559	1346	Thb.sp051	Thiobacillus sp. str. THI051.
0.551	1359	Myp.dmorph	Mycoplana dimorpha.
0.551	1407	Srh.fredi5	Sinorhizobium fredii.
0.551	1368	Srh.fredi2	Sinorhizobium fredii.
0.549	1367	Srh.xinjia	Sinorhizobium xinjiangensis.
0.547	1325	Rhp.elegan	Rhodoplanes elegans str. AS130.
0.545	1401	Srh.melil7	Sinorhizobium meliloti.
0.545	1340	Rhb.ciceri	Rhizobium ciceri str. UPM-Ca7.
0.545	1374	str.Hyp353	str. US-353.
0.545	1344	Bru.canis	Brucella canis.
0.542	1356	Rhb.loti2	Rhizobium loti.
0.542	1360	Srh.terang	Sinorhizobium teranga.
0.542	1395	Rhb.loti5	Rhizobium loti.
0.542	1360	Rhb.sp7834	Rhizobium sp.
0.542	1364	Srh.fredi3	Sinorhizobium fredii.
0.542	1329	Rdb.orient	Rhodobium orientis str. MB312.
0.540	1362	Rhb.loti3	Rhizobium loti str. 3F6g2.

335 **apr22** Unknown

S_ab	Olis	Short-ID	Full name
0.731	1358	B.viscosus	Bacillus viscosus.
0.713	1401	Pae.valid3	Paenibacillus validus.
0.707	1399	Pae.plymy3	Paenibacillus polymyxa.
0.696	1384	Pae.macer2	Paeniacillus macerans.
0.678	1354	Pae.durum	Paenibacillus durum.
0.669	1329	Pae.alvei2	Paenibacillus alvei.
0.654	1380	Pae.macqri	Paenibacillus macquariensis str. 673.
0.651	1430	Bb.thruber	Brevibacillus thermoruber str. BT2.
0.648	1357	Pae.larvae	Paenibacillus larvae subsp. larvae.
0.639	1360	B.acvorans	Bacillus acidovorans.
0.639	1398	Spo.ureae3	Sporosarcina ureae.
0.633	1419	B.infernus	Bacillus infernus str. TH-22 (=SMCC/W 478).
0.633	1310	Pae.valid2	Paenibacillus validus str. Q1.
0.621	1387	B.macroide	Bacillus macroides.
0.618	1360	Pae.macqr2	Paenibacillus macquariensis str. 673.
0.618	1442	B.amvorans	Bacillus aminovorans.

0.612 1422 B.smithii2 Bacillus smithii.
0.609 1413 B.pantoth2 Bacillus pantothenticus.
0.606 1408 B.infernu2 Bacillus infernus str. TH-23 (=SMCC/W 479).
0.606 1428 B.sp6 Bacillus sp.

---

458 **apr23** Unknown

S_ab Olis Short-ID Full name

0.710 1382 Rb.sphrrnA Rhodobacter sphaeroides str. ATH 2.4.1.
0.710 1382 Rb.sphrrnB Rhodobacter sphaeroides str. ATH 2.4.1.
0.710 1314 Rb.sphaer2 Rhodobacter sphaeroides.
0.710 1382 Rb.sphrrnC Rhodobacter sphaeroides str. ATH 2.4.1.
0.707 1314 Rb.sphaer3 Rhodobacter sphaeroides str. IL106.
0.653 1330 Rb.veldkam Rhodobacter veldkampii.
0.646 1313 Rb.capsul5 Rhodobacter capsulatus str. C5.
0.635 1319 str.SW2 str. SW2.
0.631 1318 Rhv.euryhl Rhodovulum euryhalinum str. KA-65.
0.624 1325 Rhv.MB-G2 Rhodovulum sp. str. MB-G2.
0.624 1306 Rhv.MB263 Rhodovulum sp. str. MB263.
0.620 1303 Rhv.MB2602 Rhodovulum sp. str. MB260.
0.609 1301 Par.thcyan Paracoccus thiocyanatus str. THI 011; 1-8A.
0.605 1312 Rb.capsul4 Rhodobacter capsulatus str. ATH 2.3.1.
0.603 1303 Rhv.sulfi5 Rhodovulum sulfidophilum str. W12.
0.603 1304 Rhv.sulfi4 Rhodovulum sulfidophilum str. TW13.
0.600 1296 Rb.capsul2 Rhodobacter capsulatus str. B10.
0.600 1293 Rb.capsula Rhodobacter capsulatus str. B10.
0.600 1318 Par.amphil Paracoccus aminophilus str. DM-15.
0.600 1366 Par.denitr Paracoccus denitrificans.

---

591 **apr24** Unknown

S_ab Olis Short-ID Full name

0.633 1343 Rps.virid3 Rhodopseudomonas viridis.
0.633 1341 Rps.viridi Rhodopseudomonas viridis.
0.633 1368 Srh.fredi2 Sinorhizobium fredii.
0.631 1346 Thb.sp051 Thiobacillus sp. str. THI051.
0.629 1407 Srh.fredi5 Sinorhizobium fredii.
0.628 1367 Srh.xinjia Sinorhizobium xinjiangensis.
0.626 1340 Myp.bullat Mycoplana bullata.
0.624 1360 Rhb.sp7834 Rhizobium sp.

0.624 1360 Srh.terang Sinorhizobium teranga.
0.623 1362 Srh.saheli Sinorhizobium saheli.
0.623 1401 Srh.melil7 Sinorhizobium meliloti.
0.619 1364 Srh.fredi3 Sinorhizobium fredii.
0.618 1362 Srh.melil3 Sinorhizobium meliloti.
0.618 1340 Rhb.ciceri Rhizobium ciceri str. UPM-Ca7.
0.616 1349 Srh.melil4 Sinorhizobium meliloti.
0.614 1356 Rhb.loti2 Rhizobium loti.
0.613 1359 Myp.dmorph Mycoplana dimorpha.
0.613 1336 Rhp.roseus Rhodoplanes roseus str. 941.
0.613 1362 Rhb.loti3 Rhizobium loti str. 3F6g2.
0.611 1329 Rdb.orient Rhodobium orientis str. MB312.

---

542 **apr25** Unknown

S_ab Olis Short-ID Full name

0.708 1370 Mag.magne2 Magnetospirillum magnetotacticum.
0.705 1349 Mag.spAMB1 Magnetospirillum sp. str. AMB-1.
0.696 1355 Azs.lipof2 Azospirillum lipoferum str. VPI Sp 59b.
0.696 1322 R.fulvum2 Rhodospirillum fulvum str. 1360.
0.690 1376 Mag.gryphi Magnetospirillum gryphiswaldense str. MSR-1.
0.688 1366 R.molischi Rhodospirillum molischianum.
0.683 1319 Azs.sp4 Azospirillum sp. str. 4 AZ.
0.679 1353 Azs.iraken Azospirillum irakense str. KBC1.
0.675 1365 Rhb.sp7854 Rhizobium sp.
0.675 1397 Rhb.spOK50 Rhizobium sp. str. OK-50.
0.675 1365 Rhb.sp7836 Rhizobium sp.
0.673 1404 Zoo.ramig5 Zoogloea ramigera.
0.670 1336 Rhc.cente2 Rhodocista centenaria.
0.670 1364 Rhb.huaku2 Rhizobium huakuii.
0.670 1365 Azs.brasi2 Azospirillum brasilense str. Sp 7.
0.668 1330 Mag.magnet Magnetospirillum magnetotacticum.
0.666 1395 Rhb.loti5 Rhizobium loti.
0.666 1359 Rhb.huakui Rhizobium huakuii.
0.664 1337 Zoo.ramig2 Zoogloea ramigera.
0.662 1360 Rhb.loti4 Rhizobium loti.

---

553 **apr26** Unknown

S_ab Olis Short-ID Full name

0.624 1315 Hyp.vulgar Hyphomicrobium vulgare str. MC-750.
0.615 1343 Rps.virid3 Rhodopseudomonas viridis.
0.615 1341 Rps.viridi Rhodopseudomonas viridis.
0.599 1374 str.Hyp353 str. US-353.
0.597 1372 Ag.tumefa4 Agrobacterium tumefaciens.
0.597 1371 Ag.tumefa2 Agrobacterium tumefaciens.
0.597 1419 Ag.tumefac Agrobacterium tumefaciens.
0.597 1402 Ag.tumefa6 Agrobacterium tumefaciens.
0.595 1340 Myp.bullat Mycoplana bullata.
0.593 1346 Thb.sp051 Thiobacillus sp. str. THI051.
0.591 1372 Ag.rubi2 Agrobacterium rubi.
0.591 1336 Rhp.roseus Rhodoplanes roseus str. 941.
0.590 1400 Ag.rubi3 Agrobacterium rubi.
0.590 1301 Azs.sp2 Azospirillum sp. str. A1-3.
0.588 1402 Ag.tumef10 Agrobacterium tumefaciens.
0.588 1368 Ag.tumefa3 Agrobacterium tumefaciens str. ICPB TT111.
0.586 1325 Rhp.elegan Rhodoplanes elegans str. AS130.
0.584 1372 Ag.vitis2 Agrobacterium vitis.
0.584 1402 Ag.vitis3 Agrobacterium vitis.
0.584 1371 Ag.vitis Agrobacterium vitis.

---

562 **apr27** Unknown

S_ab Olis Short-ID Full name

0.541 1358 Flx.ruber Flexibacter ruber str. Lewin GEY.
0.525 200 L.homohioc Lactobacillus homohiochii.
0.523 1385 Msc.serice "Microscilla sericea" str. SIO-7.
0.520 1334 str.srb str. TD3.
0.511 1362 Msc.furves Microscilla furvescens str. TV-2.
0.505 1361 Flx.flexil Flexibacter flexilis str. Lewin CR-63.
0.504 1374 Flx.tractu Flexibacter tractuosus str. Lewis BA-3.
0.502 1386 Cy.hutchin Cytophaga hutchinsonii str. D465 (P.H.A. Sneath).
0.498 1406 Msc.arenar Microscilla arenaria str. HJ-1.
0.496 1396 Azr.cauli3 Azorhizobium caulinodans.
0.496 1431 Trp.pallid Treponema pallidum str. Nichols.
0.495 216 L.kefir2 Lactobacillus kefir.
0.495 1386 Rps.palus2 Rhodopseudomonas palustris str. ATH 2.1.6.
0.495 1364 Bdr.japoni Bradyrhizobium japonicum.
0.495 1385 Ntb.hambur Nitrobacter hamburgensis str. X14.
0.495 1366 Azr.cauli2 Azorhizobium caulinodans.
0.493 219 L.hilgard2 Lactobacillus hilgardii.
0.493 1406 Bdr.sp129 Bradyrhizobium sp. str. 129.

0.493 1381 Ntb.winogr Nitrobacter winogradskyi str. W.
0.493 1392 Bar.elizab Bartonella elizabethae str. F9251; B91-002005.

---

603 **apr29** Unknown

S_ab Olis Short-ID Full name

0.650 1343 Rps.virid3 Rhodopseudomonas viridis.
0.650 1341 Rps.viridi Rhodopseudomonas viridis.
0.650 1346 Thb.sp051 Thiobacillus sp. str. THI051.
0.638 1368 Srh.fredi2 Sinorhizobium fredii.
0.633 1407 Srh.fredi5 Sinorhizobium fredii.
0.632 1367 Srh.xinjia Sinorhizobium xinjiangensis.
0.630 1360 Srh.terang Sinorhizobium teranga.
0.630 1360 Rhb.sp7834 Rhizobium sp.
0.629 1362 Srh.saheli Sinorhizobium saheli.
0.627 1401 Srh.melil7 Sinorhizobium meliloti.
0.627 1340 Myp.bullat Mycoplana bullata.
0.625 1340 Rhb.ciceri Rhizobium ciceri str. UPM-Ca7.
0.624 1362 Srh.melil3 Sinorhizobium meliloti.
0.624 1364 Srh.fredi3 Sinorhizobium fredii.
0.622 1356 Rhb.loti2 Rhizobium loti.
0.622 1336 Rhp.roseus Rhodoplanes roseus str. 941.
0.620 1349 Srh.melil4 Sinorhizobium meliloti.
0.620 1325 Rhp.elegan Rhodoplanes elegans str. AS130.
0.619 1362 Rhb.loti3 Rhizobium loti str. 3F6g2.
0.619 1329 Rdb.orient Rhodobium orientis str. MB312.

---

518 **apr30** Unknown

S_ab Olis Short-ID Full name

0.716 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.712 1399 Lptx.disc3 Leptothrix discophora str. SS-1.
0.710 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.710 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.695 1430 Brd.holmes Bordetella holmesii.
0.695 1411 Id.dechlrt Ideonella dechloratans.
0.691 1360 Brd.pperts Bordetella parapertussis.
0.691 1379 Rcy.tenui2 Rhodocyclus tenuis str. 3760.
0.689 1407 Ral.eutrop Ralstonia eutropha str. 335 (R.Y. Stanier).
0.689 1391 Rcy.purpur Rhodocyclus purpureus str. 6770.

0.689 1398 Lptx.disc2 Leptothrix discophora str. SP-6.
0.687 1371 Vrv.pardox Variovorax paradoxus.
0.681 1364 Rub.gelat2 Rubrivivax gelatinosus str. ATH 2.2.1.
0.681 1352 Brd.bronc2 Bordetella bronchiseptica str. Dog 71.
0.680 1350 Brd.avium Bordetella avium.
0.680 1370 Rub.gelat3 Rubrivivax gelatinosus str. A3.
0.675 265 env.PAD37 str. PAD37.
0.674 1448 sym.Critsp symbiont of Crithidia sp.
0.670 1377 Brch.denit Brachymonas denitrificans str. AS-P1.
0.668 1363 Alc.spM913 Alcaligenes sp. str. M91-3.

---

597 **apr31** Unknown

S_ab Olis Short-ID Full name

0.495 200 L.homohioc Lactobacillus homohiochii.
0.477 216 L.kefir2 Lactobacillus kefir.
0.466 1435 She.algaBr Shewanella alga str. BrY.
0.459 1435 She.putre2 Shewanella putrefaciens.
0.457 1447 Frm.balear Ferrimonas balearica str. PAT.
0.456 1334 str.srb str. TD3.
0.447 1382 Sln.lactic Selenomonas lacticifex str. VB4b.
0.447 1457 Leg.lytica Legionella lytica.
0.444 1404 Dmn.tiedje Desulfomonile tiedjei str. DCB-1.
0.442 1365 Azs.brasi2 Azospirillum brasilense str. Sp 7.
0.442 1447 She.algaFe Shewanella alga str. FeRed.
0.441 1384 Leg.cincin Legionella cincinnatiensis.
0.441 1406 Bdr.sp129 Bradyrhizobium sp. str. 129.
0.439 1367 Leg.stheln Legionella sainthelensi.
0.439 1396 Azr.cauli3 Azorhizobium caulinodans.
0.439 1394 Bdr.japon4 Bradyrhizobium japonicum.
0.439 1340 Myp.bullat Mycoplana bullata.
0.437 1355 Azs.lipof2 Azospirillum lipoferum str. VPI Sp 59b.
0.432 1385 Ntb.hambur Nitrobacter hamburgensis str. X14.
0.432 271 env.FIE17 str. FIE17.

---

375 **nov2** Unknown

S_ab Olis Short-ID Full name

0.547 1376 Mag.gryphi Magnetospirillum gryphiswaldense str. MSR-1.
0.541 1366 R.molischi Rhodospirillum molischianum.

0.539 1370 Mag.magne2 Magnetospirillum magnetotacticum.
0.539 1322 R.fulvum2 Rhodospirillum fulvum str. 1360.
0.528 1349 Mag.spAMB1 Magnetospirillum sp. str. AMB-1.
0.523 1269 Ppb.neusto Porphyrobacter neustonensis.
0.517 1297 Azs.amazo2 Azospirillum amazonense str. Y-1.
0.515 1330 Mag.magnet Magnetospirillum magnetotacticum.
0.515 1336 Rhc.cente2 Rhodocista centenaria.
0.515 1374 str.Hyp353 str. US-353.
0.509 1315 Hyp.vulgar Hyphomicrobium vulgare str. MC-750.
0.507 1319 Azs.sp4 Azospirillum sp. str. 4 AZ.
0.504 1365 Rhb.sp7854 Rhizobium sp.
0.504 1365 Rhb.sp7836 Rhizobium sp.
0.504 1339 Br.diminu2 Brevundimonas diminuta.
0.499 1340 Rhb.ciceri Rhizobium ciceri str. UPM-Ca7.
0.499 1356 Rhb.loti2 Rhizobium loti.
0.499 1306 Rhv.MB253 Rhodovulum sp. str. MB253.
0.499 1337 Zoo.ramig2 Zoogloea ramigera.
0.499 1340 Myp.bullat Mycoplana bullata.

---

475 **nov3** Unknown

S_ab Olis Short-ID Full name

0.781 1365 Rhb.sp7836 Rhizobium sp.
0.781 1365 Rhb.sp7854 Rhizobium sp.
0.766 1364 Rhb.huaku2 Rhizobium huakuii.
0.766 1395 Rhb.loti5 Rhizobium loti.
0.766 1351 Bar.vinso2 Bartonella vinsonii subsp. vinsonii str. Baker.
0.762 1387 Bar.vinso3 Bartonella vinsonii subsp. vinsonii.
0.762 1342 Bar.vinson Bartonella vinsonii.
0.762 1356 Rhb.loti2 Rhizobium loti.
0.762 1360 Bar.vinso4 Bartonella vinsonii subsp. berkhoffii str. 93-C01.
0.760 1338 Bar.quint2 Bartonella quintana str. Fuller.
0.758 1412 Bar.quinta Bartonella quintana str. Fuller.
0.756 1344 Bru.canis Brucella canis.
0.756 1392 Bar.elizab Bartonella elizabethae str. F9251; B91-002005.
0.747 1330 Bru.melten Brucella melitensis.
0.747 1338 Bru.neotom Brucella neotomae.
0.747 1340 Bru.ovis Brucella ovis.
0.745 1340 Rhb.ciceri Rhizobium ciceri str. UPM-Ca7.
0.745 1362 Rhb.loti3 Rhizobium loti str. 3F6g2.
0.739 1359 Rhb.huakui Rhizobium huakuii.
0.737 1360 Rhb.loti4 Rhizobium loti.

644 **nov4** Unknown

S_ab	Olis	Short-ID	Full name
0.812	1371	Vrv.pardox	Variovorax paradoxus.
0.772	1398	Lptx.disc2	Leptothrix discophora str. SP-6.
0.761	1371	Rhf.ferme3	Rhodoferax fermentans str. FR3.
0.761	1371	Rhf.ferme2	Rhodoferax fermentans str. FR2.
0.753	1377	Brch.denit	Brachymonas denitrificans str. AS-P1.
0.750	1411	Id.dechlrt	Ideonella dechloratans.
0.747	1399	Lptx.disc3	Leptothrix discophora str. SS-1.
0.745	263	env.PAD44	str. PAD44.
0.733	1364	Rub.gelat2	Rubrivivax gelatinosus str. ATH 2.2.1.
0.731	1444	Com.testos	Comamonas testosteroni str. RH 1104.
0.728	1370	Rub.gelat3	Rubrivivax gelatinosus str. A3.
0.693	844	str.Stripa	Stripa-derived bacterium
0.688	1387	Rcy.tenuis	Rhodocyclus tenuis str. 2761.
0.683	1395	Rub.gelati	Rubrivivax gelatinosus str. ATH 2.2.1.
0.680	1385	Rcy.tenui3	Rhodocyclus tenuis str. SW18.
0.674	1430	Brd.holmes	Bordetella holmesii.
0.671	1274	Lptx.disco	Leptothrix discophora str. SS-1.
0.669	1427	Brd.bronch	Bordetella bronchiseptica str. S-1.
0.668	1275	Spt.natans	Sphaerotilus natans str. 565.
0.652	1360	Brd.pperts	Bordetella parapertussis.

653 **nov5** Unknown

S_ab	Olis	Short-ID	Full name
0.640	1402	F.ferrugin	Flavobacterium ferrugineum.
0.615	273	env.PAD4	str. PAD4.
0.526	272	env.PAD10	str. PAD10.
0.504	1378	Flx.sancti	Flexibacter sancti.
0.455	1174	Cy.arvensi	Cytophaga arvensicola.
0.453	1351	Flx.filfor	Flexibacter filiformis str. FX e 1.
0.431	269	env.PAD33	str. PAD33.
0.392	1377	Flc.major	Flectobacillus major.
0.378	1386	Cy.hutchin	Cytophaga hutchinsonii str. D465 (P.H.A. Sneath).
0.375	1330	env.agg32	str. agg32.
0.375	1354	Hal.hydros	Haliscomenobacter hydrossis.
0.375	1362	Msc.furves	Microscilla furvescens str. TV-2.

0.372 1351 Flx.ele.NZ Flexibacter elegans str. NZ-1.
0.371 1386 Cy.difflue Cytophaga diffluens str. Lewin LIM-1.
0.368 1394 Emb.brevi2 Empedobacter brevis.
0.368 1373 Cyb.marinu Cyclobacterium marinus str. WH-A; Raj.
0.368 1410 Lps.sp183 Leptospira sp. str. A-183.
0.368 1383 Flx.litora Flexibacter litoralis str. Lewin SIO-4.
0.363 1385 Msc.serice "Microscilla sericea" str. SIO-7.
0.363 1366 Lps.biflAa Leptospira biflexa str. Porto.

---

654 **nov6** Unknown

S_ab Olis Short-ID Full name

0.788 273 env.PAD4 str. PAD4.
0.766 1402 F.ferrugin Flavobacterium ferrugineum.
0.699 272 env.PAD10 str. PAD10.
0.624 1378 Flx.sancti Flexibacter sancti.
0.580 1351 Flx.filfor Flexibacter filiformis str. FX e 1.
0.572 1174 Cy.arvensi Cytophaga arvensicola.
0.561 269 env.PAD33 str. PAD33.
0.443 1330 env.agg32 str. agg32.
0.440 1386 Cy.hutchin Cytophaga hutchinsonii str. D465 (P.H.A. Sneath).
0.437 1358 Flx.ruber Flexibacter ruber str. Lewin GEY.
0.434 1386 Cy.difflue Cytophaga diffluens str. Lewin LIM-1.
0.433 1354 Hal.hydros Haliscomenobacter hydrossis.
0.430 1406 Msc.arenar Microscilla arenaria str. HJ-1.
0.424 1434 Mgb.bavarc Magnetobacterium bavaricum.
0.424 1362 Msc.furves Microscilla furvescens str. TV-2.
0.422 1390 Tnm.lapsum Thermonema lapsum.
0.422 460 E.coli3 Escherichia coli.
0.416 1347 Msc.marina Microscilla marina str. SIO-8.
0.416 1377 Flc.major Flectobacillus major.
0.414 1373 Cyb.marinu Cyclobacterium marinus str. WH-A; Raj.

---

471 **nov7** Unknown

S_ab Olis Short-ID Full name

0.705 1377 Brch.denit Brachymonas denitrificans str. AS-P1.
0.700 263 env.PAD44 str. PAD44.
0.679 1371 Vrv.pardox Variovorax paradoxus.
0.675 1411 Id.dechlrt Ideonella dechloratans.

0.665 1398 Lptx.disc2 Leptothrix discophora str. SP-6.
0.648 1371 Rhf.ferme3 Rhodoferax fermentans str. FR3.
0.648 1371 Rhf.ferme2 Rhodoferax fermentans str. FR2.
0.643 1364 Rub.gelat2 Rubrivivax gelatinosus str. ATH 2.2.1.
0.639 1370 Rub.gelat3 Rubrivivax gelatinosus str. A3.
0.635 1399 Lptx.disc3 Leptothrix discophora str. SS-1.
0.631 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.626 1430 Brd.holmes Bordetella holmesii.
0.622 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.622 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.611 1352 Brd.bronc2 Bordetella bronchiseptica str. Dog 71.
0.607 1360 Brd.pperts Bordetella parapertussis.
0.605 1444 Com.testos Comamonas testosteroni str. RH 1104.
0.605 1395 Rub.gelati Rubrivivax gelatinosus str. ATH 2.2.1.
0.603 267 env.PAD58 str. PAD58.
0.599 1359 Brd.pertus Bordetella pertussis.

---

420 **nov8** Unknown

S_ab Olis Short-ID Full name

0.545 1343 Rps.virid3 Rhodopseudomonas viridis.
0.545 1341 Rps.viridi Rhodopseudomonas viridis.
0.524 267 env.PAD53 str. PAD53.
0.524 1336 Rhp.roseus Rhodoplanes roseus str. 941.
0.523 266 env.PAD54 str. PAD54.
0.521 1368 Srh.fredi2 Sinorhizobium fredii.
0.521 1419 Ag.tumefac Agrobacterium tumefaciens.
0.521 1372 Ag.tumefa4 Agrobacterium tumefaciens.
0.521 1402 Ag.tumefa6 Agrobacterium tumefaciens.
0.521 1407 Srh.fredi5 Sinorhizobium fredii.
0.519 1367 Srh.xinjia Sinorhizobium xinjiangensis.
0.519 1368 Ag.tumefa3 Agrobacterium tumefaciens str. ICPB TT111.
0.519 1364 Srh.fredi3 Sinorhizobium fredii.
0.519 1340 Myp.bullat Mycoplana bullata.
0.517 1371 Ag.tumefa2 Agrobacterium tumefaciens.
0.514 1346 Thb.sp051 Thiobacillus sp. str. THI051.
0.514 1325 Rhp.elegan Rhodoplanes elegans str. AS130.
0.514 1349 Srh.melil4 Sinorhizobium meliloti.
0.514 1360 Rhb.sp7834 Rhizobium sp.
0.514 1360 Srh.terang Sinorhizobium teranga.

---

652 **nov9** Unknown

S_ab	Olis	Short-ID	Full name
0.788	273	env.PAD4	str. PAD4.
0.741	1402	F.ferrugin	Flavobacterium ferrugineum.
0.699	272	env.PAD10	str. PAD10.
0.586	1378	Flx.sancti	Flexibacter sancti.
0.558	269	env.PAD33	str. PAD33.
0.548	1174	Cy.arvensi	Cytophaga arvensicola.
0.538	1351	Flx.filfor	Flexibacter filiformis str. FX e 1.
0.433	1330	env.agg32	str. agg32.
0.431	1354	Hal.hydros	Haliscomenobacter hydrossis.
0.426	1386	Cy.difflue	Cytophaga diffluens str. Lewin LIM-1.
0.425	1362	Msc.furves	Microscilla furvescens str. TV-2.
0.423	1406	Msc.arenar	Microscilla arenaria str. HJ-1.
0.422	1386	Cy.hutchin	Cytophaga hutchinsonii str. D465 (P.H.A. Sneath).
0.419	1358	Flx.ruber	Flexibacter ruber str. Lewin GEY.
0.419	1434	Mgb.bavarc	Magnetobacterium bavaricum.
0.417	460	E.coli3	Escherichia coli.
0.416	1394	Emb.brevi2	Empedobacter brevis.
0.414	1373	Cyb.marinu	Cyclobacterium marinus str. WH-A; Raj.
0.414	1367	F.johnsoni	Flavobacterium johnsoniae str. MYX.1.1.1.
0.414	1347	Msc.marina	Microscilla marina str. SIO-8.

569 **nov12** Unknown

S_ab	Olis	Short-ID	Full name
0.498	253	env.PAD21	str. PAD21.
0.488	258	env.PAD20	str. PAD20.
0.474	228	env.PAD63	str. PAD63.
0.466	1370	Tmc.roseum	Thermomicrobium roseum.
0.457	1334	Mlsc.plut2	Melissococcus pluton str. M40P.
0.452	1440	Rum.amylop	Ruminobacter amylophilus.
0.448	1370	Stc.miller	Streptococcus milleri str. SJMC.
0.443	1359	Thb.caldus	Thiobacillus caldus str. KU.
0.443	1328	Mlsc.plutn	Melissococcus pluton str. M43P.
0.441	1418	Stm.galbus	Streptomyces galbus.
0.439	1335	C.esterth2	Clostridium estertheticum.
0.439	1309	Glb.violac	Gloeobacter violaceus.
0.439	1413	Stm.subrut	Streptomyces subrutilus.
0.439	1335	C.esterthe	Clostridium estertheticum.

0.436 1396 Th.agreste Thermocrispum agreste str. CHB77.
0.436 1431 B.sp2 Bacillus sp.
0.434 251 env.PAD66 str. PAD66.
0.434 1415 Arm.salmo8 Aeromonas salmonicida subsp. achromogenes.
0.434 1389 Nc.mobilis Nitrococcus mobilis.
0.434 1410 Arm.salmo3 Aeromonas salmonicida subsp. salmonicida.

---

569 **nov12re** Unknown

S_ab Olis Short-ID Full name

0.498 253 env.PAD21 str. PAD21.
0.488 258 env.PAD20 str. PAD20.
0.474 228 env.PAD63 str. PAD63.
0.466 1370 Tmc.roseum Thermomicrobium roseum.
0.457 1334 Mlsc.plut2 Melissococcus pluton str. M40P.
0.452 1440 Rum.amylop Ruminobacter amylophilus.
0.448 1370 Stc.miller Streptococcus milleri str. SJMC.
0.443 1359 Thb.caldus Thiobacillus caldus str. KU.
0.443 1328 Mlsc.plutn Melissococcus pluton str. M43P.
0.441 1418 Stm.galbus Streptomyces galbus.
0.439 1335 C.esterth2 Clostridium estertheticum.
0.439 1309 Glb.violac Gloeobacter violaceus.
0.439 1413 Stm.subrut Streptomyces subrutilus.
0.439 1335 C.esterthe Clostridium estertheticum.
0.436 1396 Th.agreste Thermocrispum agreste str. CHB77.
0.436 1431 B.sp2 Bacillus sp.
0.434 251 env.PAD66 str. PAD66.
0.434 1415 Arm.salmo8 Aeromonas salmonicida subsp. achromogenes.
0.434 1389 Nc.mobilis Nitrococcus mobilis.
0.434 1410 Arm.salmo3 Aeromonas salmonicida subsp. salmonicida.

---

620 **nov14** Unknown

S_ab Olis Short-ID Full name

0.525 255 env.PAD28 str. PAD28.
0.345 200 L.homohioc Lactobacillus homohiochii.
0.340 1416 Arm.sobria Aeromonas sobria.
0.339 1374 Arm.sobri2 Aeromonas sobria.
0.337 1410 Arm.salmo3 Aeromonas salmonicida subsp. salmonicida.
0.337 1415 Arm.salmo8 Aeromonas salmonicida subsp. achromogenes.

0.337 1410 Arm.salmo4 Aeromonas salmonicida subsp. achromogenes.
0.337 1413 Arm.sp2 Aeromonas sp.
0.335 1371 Arm.salmo6 Aeromonas salmonicida subsp. salmonicida.
0.335 1376 Arm.salmo5 Aeromonas salmonicida subsp. masoucida str. 1-1-1.
0.334 1411 Arm.eucren Aeromonas eucrenophila.
0.334 1419 Arm.cavia3 Aeromonas caviae.
0.334 1414 Arm.cavia2 Aeromonas caviae.
0.333 216 L.kefir2 Lactobacillus kefir.
0.332 1420 Arm.trota Aeromonas trota.
0.332 1372 Arm.eucre2 Aeromonas eucrenophila.
0.331 1416 Arm.allsac Aeromonas allosaccharophila.
0.331 1374 Arm.cavia4 Aeromonas caviae.
0.331 1418 Arm.hphila Aeromonas hydrophila.
0.331 1418 Arm.media Aeromonas media.

---

629 **nov16** Unknown

S_ab Olis Short-ID Full name

0.814 1382 Rb.sphrrnA Rhodobacter sphaeroides str. ATH 2.4.1.
0.814 1382 Rb.sphrrnB Rhodobacter sphaeroides str. ATH 2.4.1.
0.814 1382 Rb.sphrrnC Rhodobacter sphaeroides str. ATH 2.4.1.
0.812 1314 Rb.sphaer2 Rhodobacter sphaeroides.
0.808 1314 Rb.sphaer3 Rhodobacter sphaeroides str. IL106.
0.792 1330 Rb.veldkam Rhodobacter veldkampii.
0.777 1313 Rb.capsul5 Rhodobacter capsulatus str. C5.
0.758 1318 Par.amphil Paracoccus aminophilus str. DM-15.
0.757 1301 Par.thcyan Paracoccus thiocyanatus str. THI 011; 1-8A.
0.755 1366 Par.denitr Paracoccus denitrificans.
0.749 1306 Rhv.MB263 Rhodovulum sp. str. MB263.
0.746 1303 Rhv.MB2602 Rhodovulum sp. str. MB260.
0.742 1302 Par.versu3 Paracoccus versutus.
0.742 1318 Rhv.euryhl Rhodovulum euryhalinum str. KA-65.
0.741 1319 str.SW2 str. SW2.
0.738 1317 Par.amvora Paracoccus aminovorans str. DM-82.
0.734 1303 Rhv.sulfi5 Rhodovulum sulfidophilum str. W12.
0.734 1325 Rhv.MB-G2 Rhodovulum sp. str. MB-G2.
0.734 1304 Rhv.sulfi4 Rhodovulum sulfidophilum str. TW13.
0.733 1304 Par.versu2 Paracoccus versutus.

---

520 **nov17** Unknown

S_ab	Olis	Short-ID	Full name
0.504	1343	env.agg58	str. agg58.
0.502	1420	Sph.heparn	Sphingobacterium heparinum str. HIM 762-3.
0.487	1406	Msc.arenar	Microscilla arenaria str. HJ-1.
0.471	1376	Sph.sprvo2	Sphingobacterium spiritivorum.
0.460	1384	F.columna2	Flavobacterium columnare str. RR-1a.
0.458	1362	Msc.furves	Microscilla furvescens str. TV-2.
0.456	1367	Sph.thalpo	Sphingobacterium thalpophilum.
0.456	1380	Rik.mifusu	Rikenella microfusus.
0.454	271	env.PAD36	str. PAD36.
0.450	1379	Cy.ferment	Cytophaga fermentans.
0.448	1371	Flx.canada	Flexibacter canadensis.
0.446	1392	Flx.aggre1	Flexibacter aggregans str. Lewin NN-3.
0.446	1368	F.hydatis	Flavobacterium hydatis.
0.444	1403	Prv.disien	Prevotella disiens.
0.439	271	env.PAD30	str. PAD30.
0.439	269	env.PAD48	str. PAD48.
0.438	1362	env.agg13	str. agg13.
0.438	1369	Run.slithy	Runella slithyformis.
0.437	1328	Gbc.slfred	Geobacter sulfurreducens str. PCA.
0.437	1412	Rht.marin2	Rhodothermus marinus.

337 **nov18** Unknown

S_ab	Olis	Short-ID	Full name
0.602	1344	Pln.spS664	Planctomyces sp. str. Schlesner 664.
0.549	1366	Pln.spS668	Planctomyces sp. str. Schlesner 668.
0.528	1365	Pln.spS269	Planctomyces sp. str. Schlesner 269.
0.472	1365	Pln.spS658	Planctomyces sp. str. Schlesner 658.
0.472	1089	Pln.brasil	Planctomyces brasiliensis.
0.433	1379	Pln.spS642	Planctomyces sp. str. Schlesner 642.
0.430	1368	Pln.spS638	Planctomyces sp. str. Schlesner 638.
0.409	1367	Iso.spS640	Isosphaera sp. str. Schlesner 640.
0.407	1419	Pln.spS130	Planctomyces sp. str. Schlesner 130.
0.404	1403	Pir.spS678	Pirellula sp. str. Schlesner 678.
0.401	1346	Pir.stale2	Pirellula staleyi.
0.398	1381	Pir.spS302	Pirellula sp. str. Schlesner 302.
0.395	1397	Pln.limnop	Planctomyces limnophilus.
0.392	1419	Pir.spS139	Pirellula sp. str. Schlesner 139.
0.392	1414	Spl.inulin	Sporolactobacillus inulinis.
0.389	1383	Iso.spS666	Isosphaera sp. str. Schlesner 666.

0.386 1391 Spl.racmi2 Sporolactobacillus racemicus str. M-86.
0.386 1390 Spl.inuli2 Sporolactobacillus inulinus.
0.383 1464 B.methanol Bacillus methanolicus str. C1.
0.383 1465 B.methano2 Bacillus methanolicus str. C1.

---

225 **nov19** Unknown

S_ab Olis Short-ID Full name

0.782 1377 Brch.denit Brachymonas denitrificans str. AS-P1.
0.724 844 str.Stripa Stripa-derived bacterium
0.724 1275 Spt.natans Sphaerotilus natans str. 565.
0.707 1444 Com.testos Comamonas testosteroni str. RH 1104.
0.702 1411 Id.dechlrt Ideonella dechloratans.
0.698 1398 Lptx.disc2 Leptothrix discophora str. SP-6.
0.693 1364 Rub.gelat2 Rubrivivax gelatinosus str. ATH 2.2.1.
0.689 1370 Rub.gelat3 Rubrivivax gelatinosus str. A3.
0.684 1399 Lptx.disc3 Leptothrix discophora str. SS-1.
0.676 1371 Vrv.pardox Variovorax paradoxus.
0.667 1395 Rub.gelati Rubrivivax gelatinosus str. ATH 2.2.1.
0.662 1274 Lptx.disco Leptothrix discophora str. SS-1.
0.649 1427 Brd.bronch Bordetella bronchiseptica str. S-1.
0.640 1371 Rhf.ferme2 Rhodoferax fermentans str. FR2.
0.640 1371 Rhf.ferme3 Rhodoferax fermentans str. FR3.
0.627 1430 Brd.holmes Bordetella holmesii.
0.609 1387 Rcy.tenuis Rhodocyclus tenuis str. 2761.
0.609 1385 Rcy.tenui3 Rhodocyclus tenuis str. SW18.
0.600 1360 Brd.pperts Bordetella parapertussis.
0.600 1352 Brd.bronc2 Bordetella bronchiseptica str. Dog 71.

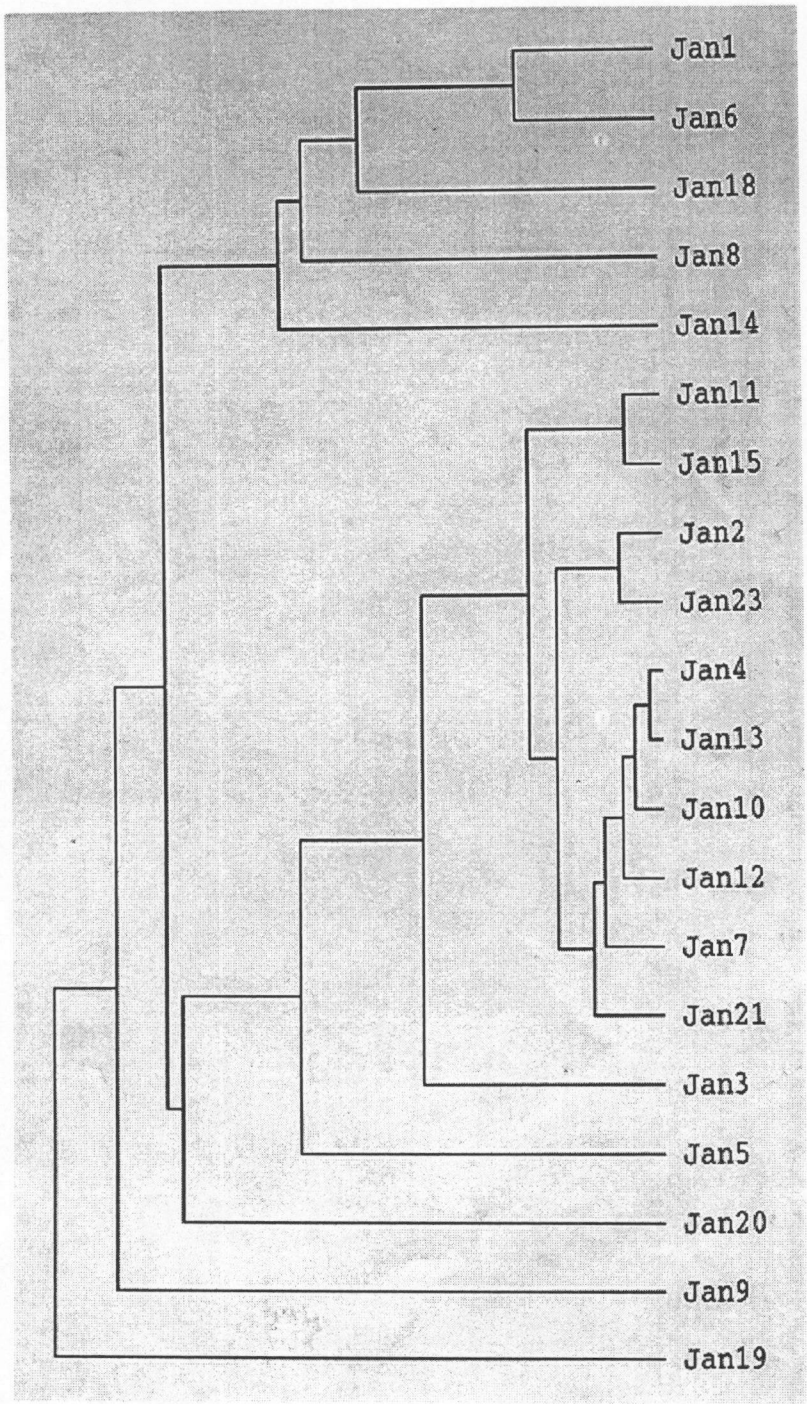
---

253 **nov22** Unknown

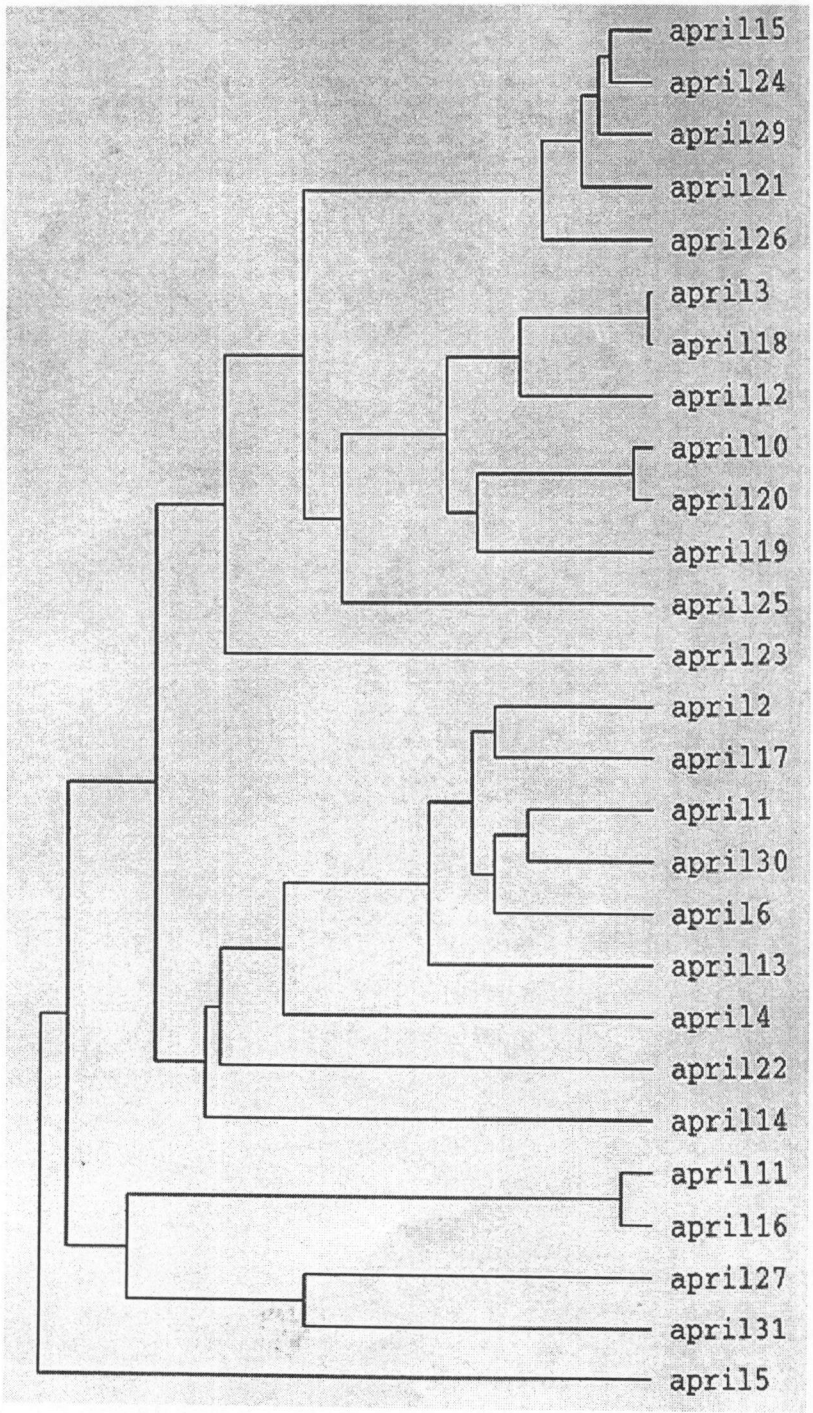
S_ab Olis Short-ID Full name

0.791 1374 str.Hyp353 str. US-353.
0.787 1315 Hyp.vulgar Hyphomicrobium vulgare str. MC-750.
0.684 1342 Rps.palus7 Rhodopseudomonas palustris.
0.680 1373 Rps.palust Rhodopseudomonas palustris str. GH.
0.676 1414 Bdr.japon5 Bradyrhizobium japonicum.
0.676 1394 Bdr.japon4 Bradyrhizobium japonicum.
0.652 1364 Bdr.japoni Bradyrhizobium japonicum.
0.652 1370 Bdr.sp9966 Bradyrhizobium sp.

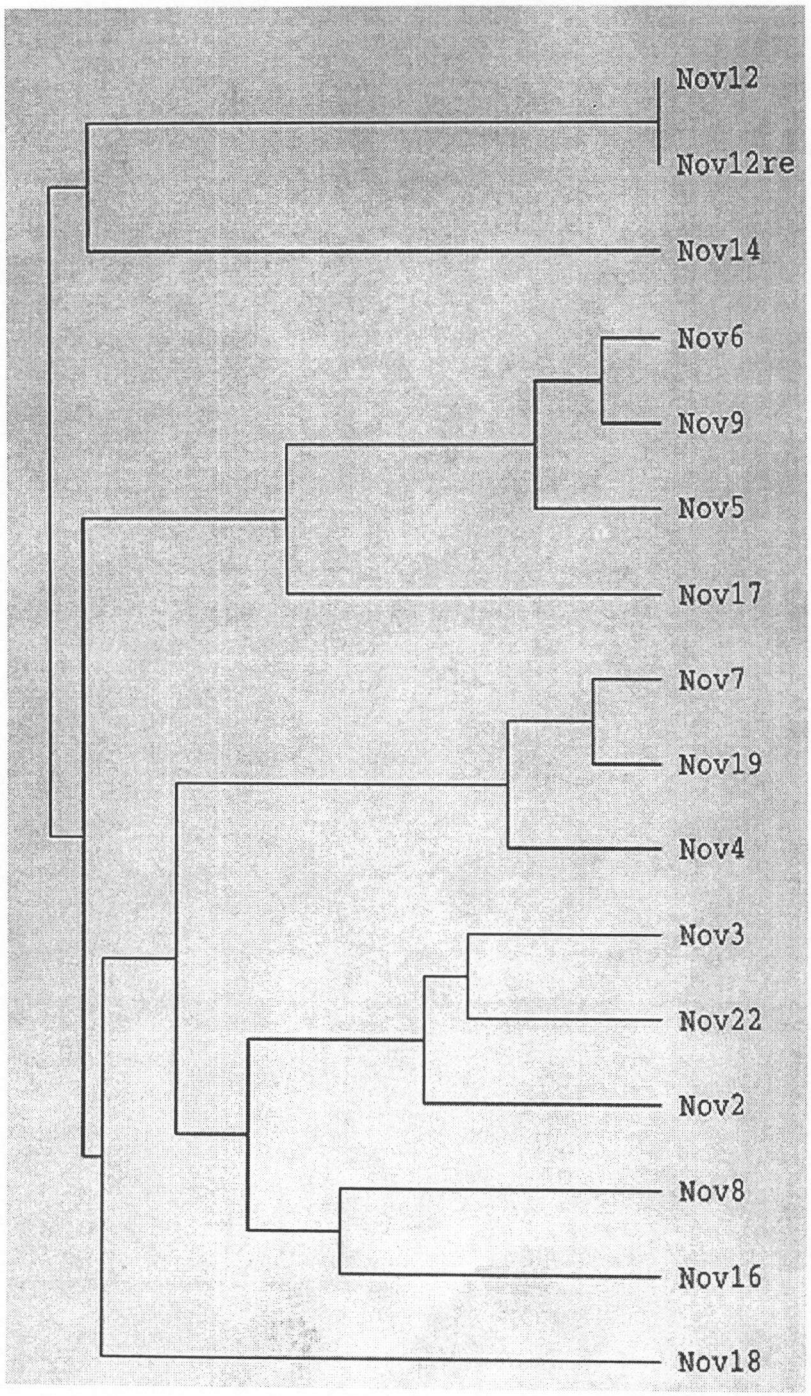
0.652 1369 Bdr.sp9520 Bradyrhizobium sp.
0.652 1372 Bdr.sp0689 Bradyrhizobium sp.
0.652 1368 Bdr.sp9514 Bradyrhizobium sp.
0.652 1370 Bdr.sp9980 Bradyrhizobium sp.
0.648 1359 Bdr.japon2 Bradyrhizobium japonicum.
0.644 1344 Afp.cleve Afipia clevelandensis.
0.644 1406 Bdr.sp129 Bradyrhizobium sp. str. 129.
0.628 1325 Rhp.elegan Rhodoplanes elegans str. AS130.
0.625 1357 Blb.denitr Blastobacter denitrificans.
0.605 1385 Ntb.hambur Nitrobacter hamburgensis str. X14.
0.601 1355 Azs.lipof2 Azospirillum lipoferum str. VPI Sp 59b.
0.589 1396 Azr.cauli3 Azorhizobium caulinodans.



Appendix C: Phylogenetic tree showing relationships of the nucleotide sequences of the 16S rDNA clones obtained from the January activated sludge sample.



Appendix D: Phylogenetic tree showing relationships of the nucleotide sequences of the 16S rDNA clones obtained from the April activated sludge sample.



Appendix E: Phylogenetic tree showing relationships of the nucleotide sequences of the16S rDNA clones obtained from the November activated sludge sample.

Appendix F: Multiple sequence alignments of the partial 16S rDNA sequences obtained from January, April and November clones.

```
                1                                                   50
      Nov12     .......... .......... .......... .......... ..........
    Nov12re     .......... .......... .......... .......... ..........
    april23     .......... .......... .......... .......... ..........
      Nov16     .......... .......... .......... .......... ..........
    Sphingo     .......... .......... .......... .......... ..........
      A8AN3     .......... .......... .......... .......... ..........
       jan8     .......... .......... .......... .......... ..........
      jan14     .......... .......... .......... .......... ..........
      Brady     .......... .......... .......... .......... ..........
    april21     .......... .......... .......... .......... ..........
       Nov8     .......... .......... .......... .......... ..........
    april29     .......... .......... .......... .......... ..........
      jan18     .......... .......... .......... .......... ..........
    april24     .......... .......... .......... .......... ..........
    april15     .......... .......... .......... .......... ..........
    april26     .......... .......... .......... .......... ..........
        3M3     .......... .......... .......... .......... ..........
        6M3     .......... .......... .......... .......... ..........
    april19     .......... .......... .......... .......... ..........
      Nov22     .......... .......... .......... .......... ..........
     april3     .......... .......... .......... .......... ..........
    april18     .......... .......... .......... .......... ..........
       jan1     .......... .......... .......... .......... ..........
    april12     .......... .......... .......... .......... ..........
    april10     .......... .......... .......... .......... ..........
    april20     .......... .......... .......... .......... ..........
      Hypho     .......... .......... .......... .......... ..........
      US353     .......... .......... .......... .......... ..........
       jan6     .......... .......... .......... .......... ..........
       Nov3     .......... .......... .......... .......... ..........
  Zoogloea1     .......... .......... .......... .......... ..........
    april25     .......... .......... .......... .......... ..........
       Nov2     .......... .......... .......... .......... ..........
      jan20     .......... .......... .......... .......... ..........
 Polyangium     NTTAACTGGA GAGTTTGATC CTGGCTCAGA ACGAACGTTA GCGGCGCGCC
    april22     .......... .......... .......... .......... ..........
   Bacillus     .......... .......... .......... .......... ACGAACGCTG
      Lacto     .......... .......... .......... .......... ..........
 Leptothrix     .......... .......... .......... .......... ..........
 Rubrivivax     .......... .......... .......... .......... .....ATTGA
    Sphaero     .......... .......... .......... .......... ..........
    april17     .......... .......... .......... .......... ..........
       jan3     .......... .......... .......... .......... ..........
    april30     .......... .......... .......... .......... ..........
      jan15     .......... .......... .......... .......... ..........
      jan11     .......... .......... .......... .......... ..........
     april6     .......... .......... .......... .......... ..........
     april1     .......... .......... .......... .......... ..........
       jan2     .......... .......... .......... .......... ..........
       Nov7     .......... .......... .......... .......... ..........
      jan23     .......... .......... .......... .......... ..........
      Nov19     .......... .......... .......... .......... ..........
       jan4     .......... .......... .......... .......... ..........
```

```
      jan13 .......... .......... .......... .......... ..........
      jan10 .......... .......... .......... .......... ..........
       Nov4 .......... .......... .......... .......... ..........
      jan12 .......... .......... .......... .......... ..........
       jan7 .......... .......... .......... .......... ..........
      jan21 .......... .......... .......... .......... ..........
  Variovorax .......... .......... .......... .......... .....ATTGA
      april2 .......... .......... .......... .......... ..........
   Zoogloea2 .......... .......... .......AGA GTTTGATNNT GGCTCAGATT
     april13 .......... .......... .......... .......... ..........
     april4 .......... .......... .......... .......... ..........
       jan5 .......... .......... .......... .......... ..........
  Legionella .......... .......... .......... ........GA TTGAACGCTG
     april14 .......... .......... .......... .......... ..........
        Nov6 .......... .......... .......... .......... ..........
        Nov9 .......... .......... .......... .......... ..........
        Nov5 .......... .......... .......... .......... ..........
     april11 .......... .......... .......... .......... ..........
     april16 .......... .......... .......... .......... ..........
       jan19 .......... .......... .......... .......... ..........
       Nov17 .......... .......... .......... .......... ..........
   Cytophaga .......... ......TTTA CAATGGAGAG TTTGATCCTG GCTCAGGATG
Flavobacter .......... ......NTTA CAATGGAGAG TTTGATCCTG GCTCAGGATN
     april31 .......... .......... .......... .......... ..........
        jan9 .......... .......... .......... .......... ..........
     april27 .......... .......... .......... .......... ..........
       Nov18 .......... .......... .......... .......... ..........
      april5 .......... .......... .......... .......... ..........
       Nov14 .......... .......... .......... .......... ..........

             51                                                  100
       Nov12 .......... .......... .......... .......... ..........
     Nov12re .......... .......... .......... .......... ..........
     april23 .......... .......... .......... .......... ..........
       Nov16 .......... .......... .......... .......... ..........
     Sphingo .......... .......... .......... .........G AACGAACGCT
       A8AN3 .......... .......... .......... .......... ..........
        jan8 .......... .......... .......... .......... ..........
       jan14 .......... .......... .......... .......... ..........
       Brady .......... AACTTGAAGA GTTTGATCCT GGCTCAGAGC GAACGCTGGC
     april21 .......... .......... .......... .......... ..........
        Nov8 .......... .......... .......... .......... ..........
     april29 .......... .......... .......... .......... ..........
       jan18 .......... .......... .......... .......... ..........
     april24 .......... .......... .......... .......... ..........
     april15 .......... .......... .......... .......... ..........
     april26 .......... .......... .......... .......... ..........
         3M3 .......... ..........CTTTTTGA TCATGGCTCA GAACGAACGC
         6M3 .......... ..........AGAGTTTGA TCATGGCTCA GAACGAACGC
     april19 .......... .......... .......... .......... ..........
       Nov22 .......... .......... .......... .......... ..........
      april3 .......... .......... .......... .......... ..........
     april18 .......... .......... .......... .......... ..........
        jan1 .......... .......... .......... .......... ..........
     april12 .......... .......... .......... .......... ..........
     april10 .......... .......... .......... .......... ..........
     april20 .......... .......... .......... .......... ..........
       Hypho .......... .......... .......... .......... ..........
       US353 .......... .......... .......... ....CTCAGA ACGAACGCTG
        jan6 .......... .......... .......... .......... ..........
```

```
      Nov3  .......... .......... .......... .......... ..........
 Zoogloea1  .......... .......... .AGAGTTTGA TNNTGGCTCA GAACGAACGC
   april25  .......... .......... .......... .......... ..........
      Nov2  .......... .......... .......... .......... ..........
     jan20  .......... .......... .......... .......... ..........
Polyangium  TAACACATGC AAGTCGTGCG AGAAAGGGCT TCGGCCCCGG TAAAGCGGCG
   april22  .......... .......... .......... .......... ..........
  Bacillus  GCGGCGTGCC TAATACATGC AAGTCGAGCA GGCTCTGCCT TCGGGTGGAG
     Lacto  .......... .......... .......... .......... ..........
Leptothrix  .......... .......... .......... .......... .........C
Rubrivivax  ACGCTGGCGG CATGCCTTAC ACATGCAAGT CGAACGGTAA CAGGCCGCAA
   Sphaero  .......... .......... .......... .......... .........C
   april17  .......... .......... .......... .......... ..........
      jan3  .......... .......... .......... .......... ..........
   april30  .......... .......... .......... .......... ..........
     jan15  .......... .......... .......... .......... ..........
     jan11  .......... .......... .......... .......... ..........
    april6  .......... .......... .......... .......... ..........
    april1  .......... .......... .......... .......... ..........
      jan2  .......... .......... .......... .......... ..........
      Nov7  .......... .......... .......... .......... ..........
     jan23  .......... .......... .......... .......... ..........
     Nov19  .......... .......... .......... .......... ..........
      jan4  .......... .......... .......... .......... ..........
     jan13  .......... .......... .......... .......... ..........
     jan10  .......... .......... .......... .......... ..........
      Nov4  .......... .......... .......... .......... ..........
     jan12  .......... .......... .......... .......... ..........
      jan7  .......... .......... .......... .......... ..........
     jan21  .......... .......... .......... .......... ..........
Variovorax  ACGCTGGCGG CATGCCTTAC ACATGCAAGT CGAACGGCAG CGCGGGAGCA
    april2  .......... .......... .......... .......... ..........
 Zoogloea2  GAACGCTGGC GGCATGCCTT ACACATGCAA GTCGAACGGC AGCGCGGGGC
   april13  .......... .......... .......... .......... ..........
    april4  .......... .......... .......... .......... ..........
      jan5  .......... .......... .......... .......... ..........
Legionella  GCGGCATGCT TAACACATGC AAGTCGAACG GCAGCATTAT CTAGCTTGCT
   april14  .......... .......... .......... .......... ..........
      Nov6  .......... .......... .......... .......... ..........
      Nov9  .......... .......... .......... .......... ..........
      Nov5  .......... .......... .......... .......... ..........
   april11  .......... .......... .......... .......... ..........
   april16  .......... .......... .......... .......... ..........
     jan19  .......... .......... .......... .......... ..........
     Nov17  .......... .......... .......... .......... ..........
 Cytophaga  AACGCTAGCG GCAGGCCTAA TACATGCAAG TCGAACGAGA TTAAGGGGCT
Flavobacter AACGCTAGCG GCAGGCCTAA TACATGCAAG TCGAACGAGA TTATCCAGCT
   april31  .......... .......... .......... .......... ..........
      jan9  .......... .......... .......... .......... ..........
   april27  .......... .......... .......... .......... ..........
     Nov18  .......... .......... .......... .......... ..........
    april5  .......... .......... .......... .......... ..........
     Nov14  .......... .......... .......... .......... ..........

            101                                                 150
     Nov12  .......... .......... .......... .......... ..........
   Nov12re  .......... .......... .......... .......... ..........
   april23  .......... .......... .......... .......... ..........
     Nov16  .......... .......... .......... .......... ..........
   Sphingo  GGCGGCATGC CTAACACATG CAAGTCGAAC GAACCCTTCG GGTTAGTGGC
```

```
      A8AN3  .......... .......... .......... .......... ..........
       jan8  .......... .......... .......... .......... ..........
       jan14 .......... .......... .......... .......... ..........
       Brady GGCAGGCCTT ACACATGCAA GTCGAGCGGG CGTAGCAATA CGTCAGCGGC
     april21 .......... .......... .......... .......... ..........
       Nov8  .......... .......... .......... .......... ..........
     april29 .......... .......... .......... .......... ..........
       jan18 .......... .......... .......... .......... ..........
     april24 .......... .......... .......... .......... ..........
     april15 .......... .......... .......... .......... ..........
     april26 .......... .......... .......... .......... ..........
         3M3 TGGCGGCAGG CCTAACACAT GCAAGTCGAA CGCCCCGCAA GGGGAGTGGC
         6M3 TGGCGGCAGG CCTAACACAT GCAAGTCGAA CGCCCCGCAA GGGGAGTGGC
     april19 .......... .......... .......... .......... ..........
       Nov22 .......... .......... .......... .......... ..........
      april3 .......... .......... .......... .......... ..........
     april18 .......... .......... .......... .......... ..........
       jan1  .......... .......... .......... .......... ..........
     april12 .......... .......... .......... .......... ..........
     april10 .......... .......... .......... .......... ..........
     april20 .......... .......... .......... .......... ..........
       Hypho .......GCC TAACACATGC AAGTCGAACG C..GCAGCAC AGGGAGTGGC
       US353 GCGGCAGGCC TAACACATGC AAGTCGAACG CTGTAGCAAT ACAGAGTGGC
        jan6 .......... .......... .......... .......... ..........
        Nov3 .......... .......... .......... .......... ..........
   Zoogloea1 TGGCGGCAGG CTTAACACAT GCAAGTCGAA CGCCCCGCAA GGGGAGTGGC
     april25 .......... .......... .......... .......... ..........
        Nov2 .......... .......... .......... .......... ..........
       jan20 .......... .......... .......... .......... ..........
  Polyangium CACGNGTGAG TAACACGTAG GTAACCTACC CTCTGGTGGT GGATAACCTT
     april22 .......... .......... .......... .......... ..........
    Bacillus CTAGCGGCGG ACGGGTGAGT AACACGTAGG CAACCTGCCT GTAAGACTGG
       Lacto .......... .......... .......... .......... ..........
  Leptothrix AATCCTCGAG AGTGGCGAAC GGGTGAGTAA TGCATCGGAA CGTGCCCAGT
  Rubrivivax GGTGCTGACG AGTGGCGAAC GTGTGAGTAA TGCATCGGAA CGTGCCCAGT
     Sphaero AATCCTCGAG AGTGGCGAAC GGGTGAGTAA TACATCGGAA CGTGCCCAGT
     april17 .......... .......... .......... .......... ..........
        jan3  .......... .......... .......... .......... ..........
     april30 .......... .......... .......... .......... ..........
       jan15 .......... .......... .......... .......... ..........
       jan11 .......... .......... .......... .......... ..........
      april6 .......... .......... .......... .......... ..........
      april1 .......... .......... .......... .......... ..........
        jan2  .......... .......... .......... .......... ..........
        Nov7  .......... .......... .......... .......... ..........
       jan23 .......... .......... .......... .......... ..........
       Nov19 .......... .......... .......... .......... ..........
        jan4  .......... .......... .......... .......... ..........
       jan13 .......... .......... .......... .......... ..........
       jan10 .......... .......... .......... .......... ..........
        Nov4  .......... .......... .......... .......... ..........
       jan12 .......... .......... .......... .......... ..........
        jan7  .......... .......... .......... .......... ..........
       jan21 .......... .......... .......... .......... ..........
  Variovorax ATCCTGGCGG CGAGTGGCGA ACGGGTGAGT AATACATCGG AACGTGCCCA
     april2  .......... .......... .......... .......... ..........
   Zoogloea2 AACCTGGCGG CGAGTGGCGA ACGGGTGAGT AATATATCGG AACGTACCCT
     april13 .......... .......... .......... .......... ..........
     april4  .......... .......... .......... .......... ..........
        jan5  .......... .......... .......... .......... ..........
```

```
Legionella   AGATTGATGG CGAGTGGCGA ACGGGTGAGT AACGCGTAGG AATATGCCTT
   april14   .......... .......... .......... .......... ..........
      Nov6   .......... .......... .......... .......... ..........
      Nov9   .......... .......... .......... .......... ..........
      Nov5   .......... .......... .......... .......... ..........
   april11   .......... .......... .......... .......... ..........
   april16   .......... .......... .......... .......... ..........
     jan19   .......... .......... .......... .......... ..........
     Nov17   .......... .......... .......... .......... ..........
 Cytophaga   TGCTCCTTAT GAAAGTGGCG CACGGGTGCG TAACGCGTAT GCAACCTACC
Flavobacter  TGCTGGATAT GAAAGTGGCG CACGGGTGCG TAACGCGTGA GCAACCTACC
   april31   .......... .......... .......... .......... ..........
      jan9   .......... .......... .......... .......... ..........
   april27   .......... .......... .......... .......... ..........
     Nov18   .......... .......... .......... .......... ..........
    april5   .......... .......... .......... .......... ..........
     Nov14   .......... .......... .......... .......... ..........

             151                                                 200
     Nov12   .......... .......... .......... .......... ..........
   Nov12re   .......... .......... .......... .......... ..........
   april23   .......... .......... .......... .......... ..........
     Nov16   .......... .......... .......... .......... ..........
   Sphingo   GCACGGGTGC GTAACGCGTG GGAATCTGCC CTTTGCTTCG GAATAACAGT
     A8AN3   .......... .......... .......... .......... ..........
      jan8   .......... .......... .......... .......... ..........
     jan14   .......... .......... .......... .......... ..........
     Brady   AGACGGGTGA GTAACGCGTG GGAACGTACC TTTTGGTTCG GAATAACACA
   april21   .......... .......... .......... .......... ..........
      Nov8   .......... .......... .......... .......... ..........
   april29   .......... .......... .......... .......... ..........
     jan18   .......... .......... .......... .......... ..........
   april24   .......... .......... .......... .......... ..........
   april15   .......... .......... .......... .......... ..........
   april26   .......... .......... .......... .......... ..........
       3M3   AGACGGGTGA GTAACACGTG GGAACCTTCC CTATAGTACG GAATAGCCCA
       6M3   AGACGGGTGA GTAACACGTG GGAACCTTCC CTATAGTACG GAATAGCCCA
   april19   .......... .......... .......... .......... ..........
     Nov22   .......... .......... .......... .......... ..........
    april3   .......... .......... .......... .......... ..........
   april18   .......... .......... .......... .......... ..........
      jan1   .......... .......... .......... .......... ..........
   april12   .......... .......... .......... .......... ..........
   april10   .......... .......... .......... .......... ..........
   april20   .......... .......... .......... .......... ..........
     Hypho   AGACGGGTGA GTCACCCGTG GGAATCTTCC TATCGGTACG GAATAGCTCA
     US353   AGACGGGTGA GTAACACGTG GGAATCTTCC TATCGGTACG GAATAGCTCA
      jan6   .......... .......... .......... .......... ..........
      Nov3   .......... .......... .......... .......... ..........
Zoogloea1    AGACGGGTGA GTAACGCGTG GGAATCTACC CAACTCTACG GAATAACTCA
   april25   .......... .......... .......... .......... ..........
      Nov2   .......... .......... .......... .......... ..........
     jan20   .......... .......... .......... .......... ..........
Polyangium   CCGAAAGGAG GGCTAATACA GCATAAGACC ACGGCCTCGC AAGAGGGAGG
   april22   .......... .......... .......... .......... ..........
  Bacillus   GATAACTACC GGAAACGGTA GCTAAGACCG GATAAGTGGT CTTCTCGCAT
     Lacto   .......... .......... .......... .......... ..........
Leptothrix   CGTGGGGGAT AACTACTCGA AAGAGTGGCT AATACCGCAT ACGACCTGAG
Rubrivivax   AGTGGGGGAT AGCCCGGCGA AAGCCGGATT AATACCGCAT ACGATCTACG
   Sphaero   CGTGGGGGAT AACGTAGCGA AA.CTACGCT AATACCGCAT ACGACCCGAG
```

```
     april17  .......... .......... .......... .......... ..........
        jan3  .......... .......... .......... .......... ..........
     april30  .......... .......... .......... .......... ..........
       jan15  .......... .......... .......... .......... ..........
       jan11  .......... .......... .......... .......... ..........
      april6  .......... .......... .......... .......... ..........
      april1  .......... .......... .......... .......... ..........
        jan2  .......... .......... .......... .......... ..........
        Nov7  .......... .......... .......... .......... ..........
       jan23  .......... .......... .......... .......... ..........
       Nov19  .......... .......... .......... .......... ..........
        jan4  .......... .......... .......... .......... ..........
       jan13  .......... .......... .......... .......... ..........
       jan10  .......... .......... .......... .......... ..........
        Nov4  .......... .......... .......... .......... ..........
       jan12  .......... .......... .......... .......... ..........
        jan7  .......... .......... .......... .......... ..........
       jan21  .......... .......... .......... .......... ..........
  Variovorax  ATCGTGGGGG ATAACGCAGC GAGNGCTGTG CTAATNCCGC ATACGATCTA
     april2   .......... .......... .......... .......... ..........
  Zoogloea2   GGAGTGGGGG ATAACGTAGC GAAAGTTACG CTAATACCGC ATACGATCTA
     april13  .......... .......... .......... .......... ..........
     april4   .......... .......... .......... .......... ..........
        jan5  .......... .......... .......... .......... ..........
  Legionella  GAAGTGGGGG ACAACTTGGG GAAACTCMAG CTAATACCGC ATAATGTCTA
     april14  .......... .......... .......... .......... ..........
        Nov6  .......... .......... .......... .......... ..........
        Nov9  .......... .......... .......... .......... ..........
        Nov5  .......... .......... .......... .......... ..........
     april11  .......... .......... .......... .......... ..........
     april16  .......... .......... .......... .......... ..........
       jan19  .......... .......... .......... .......... ..........
       Nov17  .......... .......... .......... .......... ..........
  Cytophaga   TTAATCAGGG GGATAGCCCG AAGAAATTCG GATTAACACC GCATAAAAAC
Flavobacter   TCTGTCAGGG GGATAGCCCG GCGAAAGTCG GATTAACACC GCATGACATT
     april31  .......... .......... .......... .......... ..........
        jan9  .......... .......... .......... .......... ..........
     april27  .......... .......... .......... .......... ..........
       Nov18  .......... .......... .......... .......... ..........
      april5  .......... .......... .......... .......... ..........
       Nov14  .......... .......... .......... .......... ..........

              201                                                  250
       Nov12  .......... .......... .......... .......... ..........
     Nov12re  .......... .......... .......... .......... ..........
     april23  .......... .......... .......... .......... ..........
       Nov16  .......... .......... .......... .......... ..........
     Sphingo  TAGAAATGAC TGCTAATACC GGATGATGAC TTCGGTCCAA AGATTTATCG
       A8AN3  .......... .......... .......... .......... ..........
        jan8  .......... .......... .......... .......... ..........
       jan14  .......... .......... .......... .......... ..........
       Brady  GGGAAACTTG TGCTAATACC GGATAAGCCC TTACGGGGAA AGATTTATCG
     april21  .......... .......... .......... .......... ..........
        Nov8  .......... .......... .......... .......... ..........
     april29  .......... .......... .......... .......... ..........
       jan18  .......... .......... .......... .......... ..........
     april24  .......... .......... .......... .......... ..........
     april15  .......... .......... .......... .......... ..........
     april26  .......... .......... .......... .......... ..........
         3M3  GGGNAAACTT GGAGTAATAC CGTATACGCC CGAAAGGGGA AAGAATTTCG
```

```
       6M3  GGGGAAACTT GGAGTAATAC CGTATACGCC CGAAAGGGGA AAGAATTTCG
    april19 .......... .......... .......... .......... ..........
      Nov22 .......... .......... .......... .......... ..........
     april3 .......... .......... .......... .......... ..........
    april18 .......... .......... .......... .......... ..........
       jan1 .......... .......... .......... .......... ..........
    april12 .......... .......... .......... .......... ..........
    april10 .......... .......... .......... .......... ..........
    april20 .......... .......... .......... .......... ..........
      Hypho  GGGAAACTTG GGGTAATACC GCCTACGCCC TTCGGGGGAA AGATTTATCG
      US353  GGGAAACTTG GGGTAATACC GCATACGCCC TTCGGGGNAA AGATTTATCG
       jan6 .......... .......... .......... .......... ..........
       Nov3 .......... .......... .......... .......... ..........
  Zoogloea1  GGGAAACTTG TGCTAATACC GTATACGCCC TTCGGGGGAA AGATTTATCG
    april25 .......... .......... .......... .......... ..........
       Nov2 .......... .......... .......... .......... ..........
      jan20 .......... .......... .......... .......... ..........
 Polyangium  GGTAAAAGCG GGCCTCTGCA TGCAAGCTCG CACCAGGGNN TGGGCCTGCG
    april22 .......... .......... .......... .......... ..........
   Bacillus  GAGAGGATCA AGAAACACGG GGCAACCTGT GGCTTACAGA TGCGCGTGCG
      Lacto .......... .......... .......... .......... ..........
 Leptothrix  GGTGAAAGCG GGGGACTCGC AAGAGCCTCG CGCGATTGGA GCGGCCGATG
 Rubrivivax  GGTGAAAGCG GGGGA..CCG TAAGGCCTCG CGCTATTGGA GCGGCCGATG
    Sphaero  GGTGAAAGCG GGGGACTCGC AAGAGCCTCG CGCGATTGGA GCGGCCGATG
    april17 .......... .......... .......... .......... ..........
       jan3 .......... .......... .......... .......... ..........
    april30 .......... .......... .......... .......... ..........
      jan15 .......... .......... .......... .......... ..........
      jan11 .......... .......... .......... .......... ..........
     april6 .......... .......... .......... .......... ..........
     april1 .......... .......... .......... .......... ..........
       jan2 .......... .......... .......... .......... ..........
       Nov7 .......... .......... .......... .......... ..........
      jan23 .......... .......... .......... .......... ..........
      Nov19 .......... .......... .......... .......... ..........
       jan4 .......... .......... .......... .......... ..........
      jan13 .......... .......... .......... .......... ..........
      jan10 .......... .......... .......... .......... ..........
       Nov4 .......... .......... .......... .......... ..........
      jan12 .......... .......... .......... .......... ..........
       jan7 .......... .......... .......... .......... ..........
      jan21 .......... .......... .......... .......... ..........
 Variovorax  CGGATGAAAG CAGGGGATCG CAAGACCTTG CGCGAATGGA GCGGCCGATG
     april2 .......... .......... .......... .......... ..........
  Zoogloea2  AGGATGAAAG TGGGGGATCG CAAGACCTCA TGCTCCTGGA GCGGCCGATA
    april13 .......... .......... .......... .......... ..........
     april4 .......... .......... .......... .......... ..........
       jan5 .......... .......... .......... .......... ..........
 Legionella  AGGACSAAAG CTGGGGACCT TCGGGCCTGG CGCTTTAAGA TTAGCCTGCG
    april14 .......... .......... .......... .......... ..........
       Nov6 .......... .......... .......... .......... ..........
       Nov9 .......... .......... .......... .......... ..........
       Nov5 .......... .......... .......... .......... ..........
    april11 .......... .......... .......... .......... ..........
    april16 .......... .......... .......... .......... ..........
      jan19 .......... .......... .......... .......... ..........
      Nov17 .......... .......... .......... .......... ..........
 Cytophaga  ACAG.GATAG CATTATCCAA TGTTCAAATA TTTATAGGAT TAAGATGGGC
Flavobacter ATTGATGTGG CATCACATTA TAATCAAATA TTTATAGGAC AGAGATGGGC
    april31 .......... .......... .......... .......... ..........
```

```
      jan9  .......... .......... .......... .......... ..........
    april27  .......... .......... .......... .......... ..........
     Nov18  .......... .......... .......... .......... ..........
     april5  .......... .......... .......... .......... ..........
     Nov14  .......... .......... .......... .......... ..........

            251                                                   300
     Nov12  .......... .......... .......... .......... ..........
   Nov12re  .......... .......... .......... .......... ..........
    april23  .......... .......... .......... .......... ..........
     Nov16  .......... .......... .......... .......... ..........
   Sphingo  GCAAAGGATG AGCCCGCGTA GGATTAGGTA GTTGGTGGGG TAAAGGCCTA
     A8AN3  .......... .......... .......... .......... ..........
      jan8  .......... .......... .......... .......... ..........
     jan14  .......... .......... .......... .......... ..........
     Brady  CCGAAAGATC GGCCCGCGTC TGATTAGCTA GTTGGTGAGG TAATGGCTCA
    april21  .......... .......... .......... .......... ..........
      Nov8  .......... .......... .......... .......... ..........
    april29  .......... .......... .......... .......... ..........
     jan18  .......... .......... .......... .......... ..........
    april24  .......... .......... .......... .......... ..........
    april15  .......... .......... .......... .......... ..........
    april26  .......... .......... .......... .......... ..........
       3M3  CTATAGGATG GGCCCGCGTA GGATTAGCTA GTTGGTGAGG TAATGGCTCA
       6M3  CTATAGGATG GGCCCGCGTA GGATTAGCTA GTTGGTGAGG TAATGGCTCA
    april19  .......... .......... .......... .......... ..........
     Nov22  .......... .......... .......... .......... ..........
     april3  .......... .......... .......... .......... ..........
    april18  .......... .......... .......... .......... ..........
      jan1  .......... .......... .......... .......... ..........
    april12  .......... .......... .......... .......... ..........
    april10  .......... .......... .......... .......... ..........
    april20  .......... .......... .......... .......... ..........
      Hypho  CCGATAGATG AGCCCGCGTG TGATTAGCTG GTTGGTGAGG TAATGGCTCA
      US353  CCGATAGATG AGCCCGCGTC TGATTAGCTA GTTGGTGAGG TAATGGCTCA
       jan6  .......... .......... .......... .......... ..........
       Nov3  .......... .......... .......... .......... ..........
  Zoogloea1  GAGTTGGATG AGCCCGCGTT GGATTAGCTA GTTGGTGGGG TAAAGGCCTA
    april25  .......... .......... .......... .......... ..........
       Nov2  .......... .......... .......... .......... ..........
      jan20  .......... .......... .......... .......... ..........
 Polyangium  GCCCATCAGC TAGTTGGTGA GGTAATGGCT CACCAAGGCA AAGACGGGTA
    april22  .......... .......... .......... .......... ..........
   Bacillus  GCGCATTAGC TAGTTGGTGG GGTAACGGCT CACCAAGGCG ACGATGCGTA
      Lacto  .......... .......... .......... .......... ..........
 Leptothrix  TCAGATTAGG TAGTTGGTGG GGTAAAAGCT CACCAAGCCG ACGATCTGTA
Rubrivivax  TCAGATTAGG TAGTTGGTGG GGTAAAGGCC TACCAAGCCT GCGATCTGTA
    Sphaero  GCAGATTAGG TAGTTGGTGG GGTAAAGGCC CACCAAGCCT GCGATCTGTA
    april17  .......... .......... .......... .......... ..........
      jan3  .......... .......... .......... .......... ..........
    april30  .......... .......... .......... .......... ..........
     jan15  .......... .......... .......... .......... ..........
     jan11  .......... .......... .......... .......... ..........
     april6  .......... .......... .......... .......... ..........
     april1  .......... .......... .......... .......... ..........
      jan2  .......... .......... .......... .......... ..........
      Nov7  .......... .......... .......... .......... ..........
     jan23  .......... .......... .......... .......... ..........
     Nov19  .......... .......... .......... .......... ..........
      jan4  .......... .......... .......... .......... ..........
```

```
      jan13  .......... .......... .......... .......... ..........
      jan10  .......... .......... .......... .......... ..........
       Nov4  .......... .......... .......... .......... ..........
      jan12  .......... .......... .......... .......... ..........
       jan7  .......... .......... .......... .......... ..........
      jan21  .......... .......... .......... .......... ..........
  Variovorax GCAGATTAGG TAGTTGGTGA GGTAAAGGCT CACCAAGCCT TCGATCTGTA
      april2 .......... .......... .......... .......... ..........
  Zoogloea2  TCTGATTAGC TAGTTGGTAG GGTAAAAGCC TACCAAGGCA TCGATCAGTA
     april13 .......... .......... .......... .......... ..........
      april4 .......... .......... .......... .......... ..........
       jan5  .......... .......... .......... .......... ..........
  Legionella TCCGATTAGC TAGTTGGTGG GGTAAGGGCC TACCAAGGCG ACGATCGGTA
     april14 .......... .......... .......... .......... ..........
        Nov6  .......... .......... .......... .......... ..........
        Nov9  .......... .......... .......... .......... ..........
        Nov5  .......... .......... .......... .......... ..........
     april11 .......... .......... .......... .......... ..........
     april16 .......... .......... .......... .......... ..........
      jan19  .......... .......... .......... .......... ..........
       Nov17 .......... .......... .......... .......... ..........
   Cytophaga ATGCGTGTCA TTAGCTAGTT GGCGGGGTAA CGGCCCACCA AGGCGACGAT
Flavobacter  TCGCGTGACA TTAGCTAGTT GGAGAGGTAA CGGCTCACCA AGGCAACGAT
     april31 .......... .......... .......... .......... ..........
       jan9  .......... .......... .......... .......... ..........
     april27 .......... .......... .......... .......... ..........
       Nov18 .......... .......... .......... .......... ..........
      april5 .......... .......... .......... .......... ..........
       Nov14 .......... .......... .......... .......... ..........

             301                                                  350
       Nov12 .......... .......... .......... .......... ..........
     Nov12re .......... .......... .......... .......... ..........
     april23 .......... .......... .......... .......... ..........
       Nov16 .......... .......... .......... .......... ..........
     Sphingo CCAAGCCGAC GATCCTTAGC TGGTCTGAGA GGATGATCAG CCACACTGGG
       A8AN3 .......... .......... .......... .......... ..........
        jan8 .......... .......... .......... .......... ..........
       jan14 .......... .......... .......... .......... ..........
       Brady CCAAGGCGAC GATCAGTAGC TGGTCTGAGA GGATGATCAG CCACATTGGG
     april21 .......... .......... .......... .......... ..........
        Nov8 .......... .......... .......... .......... ..........
     april29 .......... .......... .......... .......... ..........
       jan18 .......... .......... .......... .......... ..........
     april24 .......... .......... .......... .......... ..........
     april15 .......... .......... .......... .......... ..........
     april26 .......... .......... .......... .......... ..........
         3M3 CCAAGGCGAC GATCCTTAGC TGGTTTGAGA GAACGACCAG CCACACTGGG
         6M3 CCAAGGCGAC GATCCTTAGC TGGTTTGAGA GAACGACCAG CCACACTGGG
     april19 .......... .......... .......... .......... ..........
       Nov22 .......... .......... .......... .......... ..........
      april3 .......... .......... .......... .......... ..........
     april18 .......... .......... .......... .......... ..........
        jan1 .......... .......... .......... .......... ..........
     april12 .......... .......... .......... .......... ..........
     april10 .......... .......... .......... .......... ..........
     april20 .......... .......... .......... .......... ..........
       Hypho CCAAGGCGAC GATCCGTAGC TGGTCTCAGA GGATGACCAG CCACACTGGG
       US353 CCAAGGCGAC GATCAGTAGC TGGTCTGAGA GGATGACCAG CCACACTGGG
        jan6 .......... .......... .......... .......... ..........
```

```
      Nov3  .......... .......... .......... .......... ..........
 Zoogloea1  CCAAGGCGAC GATCCATAGC TGGTCTGAGA GGATGATCAG CCACATTGGG
    april25 .......... .......... .......... .......... ..........
      Nov2  .......... .......... .......... .......... ..........
     jan20  .......... .......... .......... .......... ..........
 Polyangium GCTGGTCTGA GAGGATGATC AGCCACACTG GAACTGAGAC ACGGTCCAGA
    april22 .......... .......... .......... .......... ..........
  Bacillus  GCCGACCTGA GAGGGTGATC GGCCACACTG GGACTGAGAC ACGGCCCAGA
     Lacto  .......... .......... .......... .......... ..........
 Leptothrix GCTGGTCTGA GAGGACGACC AGCCACACTG GGACTGAGAC ACGGCCCAGA
 Rubrivivax GCTGGTCTGA GAGGACGACC AGCCACACTG GGACTGAGAC ACGGCCCAGA
    Sphaero GCTGGTCTGA GAGG.CGACC AGCCACACTG GGACTGAGAC ACGGCCCAGA
    april17 .......... .......... .......... .......... ..........
       jan3  .......... .......... .......... .......... ..........
    april30 .......... .......... .......... .......... ..........
      jan15  .......... .......... .......... .......... ..........
      jan11  .......... .......... .......... .......... ..........
     april6  .......... .......... .......... .......... ..........
     april1  .......... .......... .......... .......... ..........
       jan2  .......... .......... .......... .......... ..........
       Nov7  .......... .......... .......... .......... ..........
      jan23  .......... .......... .......... .......... ..........
      Nov19  .......... .......... .......... .......... ..........
       jan4  .......... .......... .......... .......... ..........
      jan13  .......... .......... .......... .......... ..........
      jan10  .......... .......... .......... .......... ..........
       Nov4  .......... .......... .......... .......... ..........
      jan12  .......... .......... .......... .......... ..........
       jan7  .......... .......... .......... .......... ..........
      jan21  .......... .......... .......... .......... ..........
 Variovorax GCTGGTCTGA GAGGACGACC AGCCACACTG GGACTGAGAC ACGGCCCAGA
    april2  .......... .......... .......... .......... ..........
 Zoogloea2  GCTGGTCTGA GAGGACGACC AGCCACACTG GAACTGAGAC ACGGTCCAGA
    april13 .......... .......... .......... .......... ..........
     april4 .......... .......... .......... .......... ..........
       jan5  .......... .......... .......... .......... ..........
 Legionella GCTGGTCTGA GAGGATGGCC AGCCACACTG GAACTGAGAC ACGGTCCAGA
    april14 .......... .......... .......... .......... ..........
       Nov6  .......... .......... .......... .......... ..........
       Nov9  .......... .......... .......... .......... ..........
       Nov5  .......... .......... .......... .......... ..........
    april11 .......... .......... .......... .......... ..........
    april16 .......... .......... .......... .......... ..........
      jan19  .......... .......... .......... .......... ..........
      Nov17  .......... .......... .......... .......... ..........
 Cytophaga  GACTAGGGGA TCTGAGAGGA TGACCCCCCA CACTGGTACT GAGACACGGA
Flavobacter GTCTAGGGGC TCTGAGAGGA GAATCCCCCA CACTGGTACT GAGACACGGA
    april31 .......... .......... .......... .......... ..........
       jan9  .......... .......... .......... .......... ..........
    april27 .......... .......... .......... .......... ..........
      Nov18  .......... .......... .......... .......... ..........
     april5  .......... .......... .......... .......... ..........
      Nov14  .......... .......... .......... .......... ..........

             351                                                 400
      Nov12  .......... .......... .......... .......... ..........
    Nov12re  .......... .......... .......... .......... ..........
    april23  .......... .......... .......... .......... ..........
      Nov16  .......... .......... .......... .......... ..........
    Sphingo  ACTGAGACAC GGCCCAGACT CCTACGGGAG GCAGCAGTGG GGAATATTGG
```

```
      A8AN3  .......... .......... .......... .......... ..........
       jan8  .......... .......... .......... .......... ..........
      jan14  .......... .......... .......... .......... ..........
      Brady  ACTGAGACAC GGCCCAAACT CCTACGGGAG GCAGCAGTGG GGAATATTGG
     april21 .......... .......... .......... .......... ..........
       Nov8  .......... .......... .......... .......... ..........
     april29 .......... .......... .......... .......... ..........
      jan18  .......... .......... .......... .......... ..........
     april24 .......... .......... .......... .......... ..........
     april15 .......... .......... .......... .......... ..........
     april26 .......... .......... .......... .......... ..........
        3M3  ACTGAGACAC GGCCCCA.AC TCCTACGGGA GGCAGCNAGT GGGGAATATT
        6M3  ACTGAGACAC GGCCCCAGAC TCCTACGGGG AGGCAGCAGT GGGGAATATT
     april19 .......... .......... .......... .......... ..........
      Nov22  .......... .......... .......... .......... ..........
      april3 .......... .......... .......... .......... ..........
     april18 .......... .......... .......... .......... ..........
       jan1  .......... .......... .......... .......... ..........
     april12 .......... .......... .......... .......... ..........
     april10 .......... .......... .......... .......... ..........
     april20 .......... .......... .......... .......... ..........
      Hypho  ACTGAGACAC GGCCCAGACT CCTACGGG.. ...AGGCAGC AGTGGGGAAT
      US353  ACTGAGACAC GGCCCAGACT CCTACGGG.. ...AGGCAGC AGTGGGGAAT
       jan6  .......... .......... .......... .......... ..........
       Nov3  .......... .......... .......... .......... ..........
  Zoogloea1  ACTGAGACAC GGCCCAAACT CCTACGGGAG GCAGCAGTGG GGAATATTGG
     april25 .......... .......... .......... .......... ..........
       Nov2  .......... .......... .......... .......... ..........
      jan20  .......... .......... .......... .......... ..........
 Polyangium  CTCCTACGGG AGGCAGCAGT GGGGAATCTT GCGCAATGGG CGAAAGCCTG
     april22 .......... .......... .......... .......... ..........
    Bacillus CTCCTACGGG AGGCAGCAGT AGGGAATCTT CCGCAATGGA CGCAAGTCTG
       Lacto .......... .......... .......... .......... ..........
 Leptothrix  CTCCTACGGG AGGCAGCAGT GGGGAATTTT GGACAAT.GG CGAAAGCCAG
 Rubrivivax  CTCCTACGGG AGGCAGCAGT GGGGAATTTT GGACAATGGG CGCAAGCCTG
     Sphaero CT.CTACGGG AGGCAGCAGT GGGGAATTTT GGACAATGGG CGAAAGCCTG
     april17 .......... .......... .......... .......... ..........
        jan3  .......... .......... .......... .......... ..........
     april30 .......... .......... .......... .......... ..........
      jan15  .......... .......... .......... .......... ..........
      jan11  .......... .......... .......... .......... ..........
      april6 .......... .......... .......... .......... ..........
      april1 .......... .......... .......... .......... ..........
       jan2  .......... .......... .......... .......... ..........
       Nov7  .......... .......... .......... .......... ..........
      jan23  .......... .......... .......... .......... ..........
      Nov19  .......... .......... .......... .......... ..........
       jan4  .......... .......... .......... .......... ..........
      jan13  .......... .......... .......... .......... ..........
      jan10  .......... .......... .......... .......... ..........
       Nov4  .......... .......... .......... .......... ..........
      jan12  .......... .......... .......... .......... ..........
       jan7  .......... .......... .......... .......... ..........
      jan21  .......... .......... .......... .......... ..........
 Variovorax  CTCCTACGGG AGGCAGCAGT GGGGAATTTT GGACAATGGG CGAAAGCCTG
     april2  .......... .......... .......... .......... ..........
  Zoogloea2  CTCCTACGGG AGGCAGCAGT GGGGAATTTT GGACAATGGG CGCAAGCCTG
     april13 .......... .......... .......... .......... ..........
     april4  .......... .......... .......... .......... ..........
       jan5  .......... .......... .......... .......... ..........
```

```
Legionella   CTCCTACGGG AGTCAGCAGT GGGGAATATT GGACAATGGG GGGAACCCTG
   april14   .......... .......... .......... .......... ..........
      Nov6   .......... .......... .......... .......... ..........
      Nov9   .......... .......... .......... .......... ..........
      Nov5   .......... .......... .......... .......... ..........
   april11   .......... .......... .......... .......... ..........
   april16   .......... .......... .......... .......... ..........
     jan19   .......... .......... .......... .......... ..........
     Nov17   .......... .......... .......... .......... ..........
 Cytophaga   CCAGACTCCT ACGGGAGGCA GCAGTAAGGA ATATTGGTCA ATGGAGGGAA
Flavobacter  CCAGACTCCT ACGGGAGGCA GCAGTAAGGA ATATTGGTCA ATGGAGGGAA
   april31   .......... .......... .......... .......... ..........
      jan9   .......... .......... .......... .......... ..........
   april27   .......... .......... .......... .......... ..........
     Nov18   .......... .......... .......... .......... ..........
    april5   .......... .......... .......... .......... ..........
     Nov14   .......... .......... .......... .......... ..........

             401                                                 450
     Nov12   .......... .......... .......... .......... ..........
   Nov12re   .......... .......... .......... .......... ..........
   april23   .......... .......... .......... .......... ..........
     Nov16   .......... .......... .......... .......... ..........
   Sphingo   ACAATGGGCG CAAGCCTGAT CCAGCAATGC .......CGC GTGAGTGATG
     A8AN3   .......... .......... .......... .......... ..........
      jan8   .......... .......... .......... .......... ..........
     jan14   .......... .......... .......... .......... ..........
     Brady   ACAATGGGGG CAACCCTGAT CCAGCCATGC .......CGC GTGAGTGATG
   april21   .......... .......... .......... .......... ..........
      Nov8   .......... .......... .......... .......... ..........
   april29   .......... .......... .......... .......... ..........
     jan18   .......... .......... .......... .......... ..........
   april24   .......... .......... .......... .......... ..........
   april15   .......... .......... .......... .......... ..........
   april26   .......... .......... .......... .......... ..........
       3M3   GGAACAATGG GGCGCAAAGC CTGAATCCAC CATGCGGCGT GAGTGATGAA
       6M3   GGACAATGGG CGC....AAG CCTGATCAGC CATGCCGCGT GAGTGATGAA
   april19   .......... .......... .......... .......... ..........
     Nov22   .......... .......... .......... .......... ..........
    april3   .......... .......... .......... .......... ..........
   april18   .......... .......... .......... .......... ..........
      jan1   .......... .......... .......... .......... ..........
   april12   .......... .......... .......... .......... ..........
   april10   .......... .......... .......... .......... ..........
   april20   .......... .......... .......... .......... ..........
     Hypho   ATTGGACAAT GGGCGCAAGC CTGATCCAGC CATGCCGCGT GAGTGACGAA
     US353   ATTGGACAAT GGGCGCAAGC CTGATCCAGC CATGCCGCGT GAGTGACGAA
      jan6   .......... .......... .......... .......... ..........
      Nov3   .......... .......... .......... .......... ..........
 Zoogloea1   ACAATGGGCG CAAGCCTGAT CCAGCCATGC .......CGC GTGAGTGATG
   april25   .......... .......... .......... .......... ..........
      Nov2   .......... .......... .......... .......... ..........
     jan20   .......... .......... .......... .......... ..........
Polyangium   ACGCAGCGAC GCCGCGTGAG TGATGAAGGC CTTCGGGTTG TAAAGCTCTG
   april22   .......... .......... .......... .......... ..........
  Bacillus   ACGGAGCAAC GCCGCGTGAG TGATGAAGGT TTTCGGATCG TAAAGCTCTG
     Lacto   .......... .......... .......... .......... ..........
Leptothrix   ATCCAGCCAT G.CGCGTGCG GGAAGAAGGC CTTCGGGTTG TAAACCGCTT
Rubrivivax   ATCCAGCCAT GCCGCGTGCG GGAAGAAGGC CTTCGGGTTG TAAACCGCTT
   Sphaero   ATCCAGCCAT .ACGCGTGCG GGAAGAAGG. CTTCGGGTTG TAAACCGCTT
```

```
april17  .......... .......... .......... .......... ..........
   jan3  .......... .......... .......... .......... ..........
april30  .......... .......... .......... .......... ..........
  jan15  .......... .......... .......... .......... ..........
  jan11  .......... .......... .......... .......... ..........
  april6 .......... .......... .......... .......... ..........
  april1 .......... .......... .......... .......... ..........
   jan2  .......... .......... .......... .......... ..........
   Nov7  .......... .......... .......... .......... ..........
  jan23  .......... .......... .......... .......... ..........
  Nov19  .......... .......... .......... .......... ..........
   jan4  .......... .......... .......... .......... ..........
  jan13  .......... .......... .......... .......... ..........
  jan10  .......... .......... .......... .......... ..........
   Nov4  .......... .......... .......... .......... ..........
  jan12  .......... .......... .......... .......... ..........
   jan7  .......... .......... .......... .......... ..........
  jan21  .......... .......... .......... .......... ..........
Variovorax ATCCAGCCAT GCCGCGTGCA GGATGAAGGC CTTCGGGTTG TAAACTGCTT
  april2 .......... .......... .......... .......... ..........
Zoogloea2 ATCCAGCAAT GCCGCGTGAG TGAAGAAGGC CTTCGGGTTG TAAAGCTCTT
 april13 .......... .......... .......... .......... ..........
 april4  .......... .......... .......... .......... ..........
   jan5  .......... .......... .......... .......... ..........
Legionella ATCCAGCAAT GCCGCGTGTG TGAAGAAGGC CTGAGGGTTG TAAAGCACTT
 april14 .......... .......... .......... .......... ..........
   Nov6  .......... .......... .......... .......... ..........
   Nov9  .......... .......... .......... .......... ..........
   Nov5  .......... .......... .......... .......... ..........
 april11 .......... .......... .......... .......... ..........
 april16 .......... .......... .......... .......... ..........
  jan19  .......... .......... .......... .......... ..........
  Nov17  .......... .......... .......... .......... ..........
Cytophaga CTCTGAACCA GCCATGCCGC GTGCAGGAAG ACAGCCCTCT GGGTCGTAAA
Flavobacter CTCTNAACCA GCCATGCCGC GTGCAGGATG ACTGCCCTAT GGGTTGTAAA
 april31 .......... .......... .......... .......... ..........
   jan9  .......... .......... .......... .......... ..........
 april27 .......... .......... .......... .......... ..........
  Nov18  .......... .......... .......... .......... ..........
  april5 .......... .......... .......... .......... ..........
  Nov14  .......... .......... .......... .......... ..........

         451                                                 500
  Nov12  .......... .......... .......... .......... ..........
Nov12re  .......... .......... .......... .......... ..........
 april23 .......... .......... .......... .......... ..........
  Nov16  .......... .......... .......... .......... ..........
 Sphingo AAGGCCTTCG GGTCGTAAAG CTCTTTTACC AGGGATGATA ATGACAGTAC
   A8AN3 .......... .......... .......... .......... ..........
    jan8 .......... .......... .......... .......... ..........
   jan14 .......... .......... .......... .......... ..........
   Brady AAGGCCCTAG GGTTGTAAAG CTCTTTTGTG CGGGAAGATA ATGACGGTAC
 april21 .......... .......... .......... .......... ..........
    Nov8 .......... .......... .......... .......... ..........
 april29 .......... .......... .......... .......... ..........
   jan18 .......... .......... .......... .......... ..........
 april24 .......... .......... .......... .......... ..........
 april15 .......... .......... .......... .......... ..........
 april26 .......... .......... .......... .......... ..........
     3M3 GGCTTAGGGG TTGTAAAGNT TTTTGGGCGG GGACGATAAT GACGGTAA.C
```

```
       6M3  GGCTTAAGGG TTGTAAAGNT TTTTGGCGGG GGACGATAAT GACGGTAACC
   april19  .......... .......... .......... .......... ..........
     Nov22  .......... .......... .......... .......... ..........
    april3  .......... .......... .......... .......... ..........
   april18  .......... .......... .......... .......... ..........
      jan1  .......... .......... .......... .......... ..........
   april12  .......... .......... .......... .......... ..........
   april10  .......... .......... .......... .......... ..........
   april20  .......... .......... .......... .......... ..........
     Hypho  GGTCTTCGGA TTGTAAAGCT CTTTTGCCAG GGACGATAAT GACGGTA..C
     US353  GGTCTTCGGA TTGTAAAGCT CTTTTGGCGG GGACGATAAT GACGGTA..C
      jan6  .......... .......... .......... .......... ..........
      Nov3  .......... .......... .......... .......... ..........
 Zoogloea1  AAGGCCCTAG GGTTGTAAAG CTCTTTCACC GGTGAAGATA ATGACGGTAA
   april25  .......... .......... .......... .......... ..........
      Nov2  .......... .......... .......... .......... ..........
     jan20  .......... .......... .......... .......... ..........
Polyangium  TGGAGGGGGA CGAATAAGTG TCGTCGAATA GA.CGGCATG ATGACGGTAC
   april22  .......... .......... .......... .......... ..........
  Bacillus  TTGCCAGGGA AGAACGTCGC GGAGAGTAAC TGCTCTGCGA ATGACGGTAC
     Lacto  .......... .......... .......... .......... ..........
Leptothrix  TTGTCAGGGA AGAAATCTTC TGGGTTAATA CCTCGGGAGG ATGACGGTAC
Rubrivivax  TTGTCAGGGA AGAAATCTTC TGGGCTAATA CCTCGGGAGG ATGACGGTAC
   Sphaero  TTGTCAGGGA AGAAATACTC CGGGCTAATA CCCTGGGGTG ATGACGGTAC
   april17  .......... .......... .......... .......... ..........
      jan3  .......... .......... .......... .......... ..........
   april30  .......... .......... .......... .......... ..........
     jan15  .......... .......... .......... .......... ..........
     jan11  .......... .......... .......... .......... ..........
    april6  .......... .......... .......... .......... ..........
    april1  .......... .......... .......... .......... ..........
      jan2  .......... .......... .......... .......... ..........
      Nov7  .......... .......... .......... .......... ..........
     jan23  .......... .......... .......... .......... ..........
     Nov19  .......... .......... .......... .......... ..........
      jan4  .......... .......... .......... .......... ..........
     jan13  .......... .......... .......... .......... ..........
     jan10  .......... .......... .......... .......... ..........
      Nov4  .......... .......... .......... .......... ..........
     jan12  .......... .......... .......... .......... ..........
      jan7  .......... .......... .......... .......... ..........
     jan21  .......... .......... .......... .......... ..........
Variovorax  TTGTACGGAA CGAAACGGCC TTTTCTAATA AAGAGGGCTA ATGACGGTAC
    april2  .......... .......... .......... .......... ..........
 Zoogloea2  TTGTCAGGGA AGAAACGGGT TTTTCTAATA CAGAGATCTA ATGACGGTAC
   april13  .......... .......... .......... .......... ..........
    april4  .......... .......... .......... .......... ..........
      jan5  .......... .......... .......... .......... ..........
Legionella  TCAGTGGGGA GGAGGGTTGA TTGGTTAAGA GCTGATTAAC TGGACGTTAC
   april14  .......... .......... .......... .......... ..........
      Nov6  .......... .......... .......... .......... ..........
      Nov9  .......... .......... .......... .......... ..........
      Nov5  .......... .......... .......... .......... ..........
   april11  .......... .......... .......... .......... ..........
   april16  .......... .......... .......... .......... ..........
     jan19  .......... .......... .......... .......... ..........
     Nov17  .......... .......... .......... .......... ..........
 Cytophaga  CTGCTTTTAT TCGGGAATAA ACCTACTTAC GTGTAAGTAG CTGAATGTAC
Flavobacter CTGCTTTTGT CGGGGAATAA ACCTACGTTT GTAAACGTAG CTGAATGTAC
   april31  .......... .......... .......... .......... ..........
```

```
      jan9  .......... .......... .......... .......... ..........
    april27  .......... .......... .......... .......... ..........
     Nov18  .......... .......... .......... .......... ..........
     april5  .......... .......... .......... .......... ..........
     Nov14  .......... .......... .......... .......... ..........

            501                                                  550
     Nov12  .......... .......... .......... .......... ..........
   Nov12re  .......... .......... .......... .......... ..........
    april23  .......... .......... .......... .......... ..........
     Nov16  .......... .......... .......... .......... ..........
   Sphingo  CTGGAGAATA AGCTCCGGCT AACTCCGTGC CAGCAGCCGC GGTAATACGG
     A8AN3  .......... .......... .......... .......... ..........
      jan8  .......... .......... .......... .......... ..........
     jan14  .......... .......... .......... .......... ..........
     Brady  CGCAAGAATA AGCCCCGGCT AAGTTCGTGC CAGCAGCCGC GGTAATACGA
    april21  .......... .......... .......... .......... ..........
      Nov8  .......... .......... .......... .......... ..........
    april29  .......... .......... .......... .......... ..........
     jan18  .......... .......... .......... .......... ..........
    april24  .......... .......... .......... .......... ..........
    april15  .......... .......... .......... .......... ..........
    april26  .......... .......... .......... .......... ..........
       3M3  CGCAGAATAA GTCCCGGCTA AATTTCGTGC CAGCAGCCGC GGTAATACGG
       6M3  CGCAGAATAA GTCCCGGCTA AA.TTCGTGC CAGCAGCCGC GGTAATACGA
    april19  .......... .......... .......... .......... ..........
     Nov22  .......... .......... .......... .......... ..........
     april3  .......... .......... .......... .......... ..........
    april18  .......... .......... .......... .......... ..........
      jan1  .......... .......... .......... .......... ..........
    april12  .......... .......... .......... .......... ..........
    april10  .......... .......... .......... .......... ..........
    april20  .......... .......... .......... .......... ..........
      Hypho  CTCGAGAATA AGCCCCGGCT AACTTCGTGC CAGCGGCCGC GGTAATACGA
      US353  CCGCAGAATA AGCCCCGGCT AACTTCGTGC CAGCAGCCGC GGTAATACGA
       jan6  .......... .......... .......... .......... ..........
       Nov3  .......... .......... .......... .......... ..........
 Zoogloea1  CCGGAGAAGA AGCCCCGGCT AACTTCGTGC CAGCAGCCGC GGTAATACGA
    april25  .......... .......... .......... .......... ..........
       Nov2  .......... .......... .......... .......... ..........
      jan20  .......... .......... .......... .......... ..........
Polyangium  CCNTTTAGCA AGCACCGGCT AACTCTGTGC CAGCAGCCGC GGTAAGACAG
    april22  .......... .......... .......... .......... ..........
   Bacillus  CTGAGAAGAA AGCCCCGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT
      Lacto  .......... .......... .......... .......... ..........
Leptothrix  CTGAAGAATA AGCACCGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT
Rubrivivax  CTGAAGAATA AGCACCGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT
    Sphaero  CTGAAGAATA AGCACCGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT
    april17  .......... .......... .......... .......... ..........
       jan3  .......... .......... .......... .......... ..........
    april30  .......... .......... .......... .......... ..........
     jan15  .......... .......... .......... .......... ..........
     jan11  .......... .......... .......... .......... ..........
     april6  .......... .......... .......... .......... ..........
     april1  .......... .......... .......... .......... ..........
      jan2  .......... .......... .......... .......... ..........
      Nov7  .......... .......... .......... .......... ..........
     jan23  .......... .......... .......... .......... ..........
     Nov19  .......... .......... .......... .......... ..........
      jan4  .......... .......... .......... .......... ..........
```

```
      jan13  .......... .......... .......... .......... ..........
      jan10  .......... .......... .......... .......... ..........
       Nov4  .......... .......... .......... .......... ..........
      jan12  .......... .......... .......... .......... ..........
       jan7  .......... .......... .......... .......... ..........
      jan21  .......... .......... .......... .......... ..........
 Variovorax  CGTAAGAATA AGCACCGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT
      april2  .......... .......... .......... .......... ..........
  Zoogloea2  CTGAAGAATA AGCACCGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT
     april13  .......... .......... .......... .......... ..........
      april4  .......... .......... .......... .......... ..........
       jan5  .......... .......... .......... .......... ..........
 Legionella  CCACAGAAGA AGCACCGGCT AACTCCGTGC CAGCAGCCGC GGTAATACGG
     april14  .......... .......... .......... .......... ..........
       Nov6  .......... .......... .......... .......... ..........
       Nov9  .......... .......... .......... .......... ..........
       Nov5  .......... .......... .......... .......... ..........
     april11  .......... .......... .......... .......... ..........
     april16  .......... .......... .......... .......... ..........
      jan19  .......... .......... .......... .......... ..........
      Nov17  .......... .......... .......... .......... ..........
 Cytophaga  CGAAGGAATA AGGATCGGCT AACTCCGTGC CAGCAGCCGC GGTAATACGG
Flavobacter  CCNAAGAATA AGGATCGGCT AACTCCGTGC CAGCAGCCGC GNNNATACGG
     april31  .......... .......... .......... .......... ..........
       jan9  .......... .......... .......... .......... ..........
     april27  .......... .......... .......... .......... ..........
      Nov18  .......... .......... .......... .......... ..........
      april5  .......... .......... .......... .......... ..........
      Nov14  .......... .......... .......... .......... ..........

             551                                                  600
       Nov12  .......... .......... .......... .......... ..........
     Nov12re  .......... .......... .......... .......... ..........
     april23  .......... .......... .......... .......... ..........
       Nov16  .......... .......... .......... .......... ..........
     Sphingo  AGGGAGCTAG CGTTGTTCGG AATTACTGGG CGTAAAGCGC ACGTAGGCGG
       A8AN3  .......... .......... .......... .......... ..........
        jan8  .......... .......... .......... .......... ..........
       jan14  .......... .......... .......... .......... ..........
       Brady  AGGGGGCTAG CGTTGCTCGG AATCACTGGG CGTAAAGGGT GCGTAGGCCG
     april21  .......... .......... .......... .......... ..........
        Nov8  .......... .......... .......... .......... ..........
     april29  .......... .......... .......... .......... ..........
       jan18  .......... .......... .......... .......... ..........
     april24  .......... .......... .......... .......... ..........
     april15  .......... .......... .......... .......... ..........
     april26  .......... .......... .......... .......... ..........
         3M3  AAGGGA.TAG CGTTGTTCGG AATCACTGGG CGTAAAGCGC ACGTAGGCGG
         6M3  AGGGGACTAG CGTTGTTCGG AATCACTGGG CGTAAAGCGC ACGTAGGCGG
     april19  .......... .......... .......... .......... ..........
      Nov22  .......... .......... .......... .......... ..........
      april3  .......... .......... .......... .......... ..........
     april18  .......... .......... .......... .......... ..........
        jan1  .......... .......... .......... .......... ..........
     april12  .......... .......... .......... .......... ..........
     april10  .......... .......... .......... .......... ..........
     april20  .......... .......... .......... .......... ..........
       Hypho  AGGGGGCTAG CGTTGTTCGG AATTACTGGG CGTAAAGCGC ACGTAGGCGG
       US353  AGGGGGCTAG CGTTGTTCGG AATCACTGGG CGTAAAGCGC ACGTAGGCGG
        jan6  .......... .......... .......... .......... ..........
```

```
      Nov3  .......... .......... .......... .......... ..........
 Zoogloea1  AGGGGGCTAG CGTTGTTCGG AATTACTGGG CGTAAAGCGC ACGTAGGCGG
    april25 .......... .......... .......... .......... ..........
      Nov2  .......... .......... .......... .......... ..........
     jan20  .......... .......... .......... .......... ..........
Polyangium  AGGGTGCAAA CGTTGTTCGG AATTACTGGG CGTAAAGCGC GTGTAGGCTG
    april22 .......... .......... .......... .......... ..........
  Bacillus  AGGGGGCAAG CGTTGTCCGG AATTATTGGG CGTAAAGCGC GCGCAGGCGG
     Lacto  .......... .......... .......... .......... ..........
Leptothrix  AGGGTGCAAG CGTTAATCGG AATTACTGGG CGTAAAGCGT GCGCAGGCGG
Rubrivivax  AGGGTGCAAG CGTTAATCGG AATTACTGGG CGTAAAGCGT GCGCAGGCGG
   Sphaero  AGGGTGCAAG CGTTAATCGG AATTACTGGG CGTAAAGCGT GCGCAGGCGG
    april17 .......... .......... .......... .......... ..........
       jan3 .......... .......... .......... .......... ..........
    april30 .......... .......... .......... .......... ..........
      jan15 .......... .......... .......... .......... ..........
      jan11 .......... .......... .......... .......... ..........
     april6 .......... .......... .......... .......... ..........
     april1 .......... .......... .......... .......... ..........
       jan2 .......... .......... .......... .......... ..........
      Nov7  .......... .......... .......... .......... ..........
      jan23 .......... .......... .......... .......... ..........
      Nov19 .......... .......... .......... .......... ..........
       jan4 .......... .......... .......... .......... ..........
      jan13 .......... .......... .......... .......... ..........
      jan10 .......... .......... .......... .......... ..........
      Nov4  .......... .......... .......... .......... ..........
      jan12 .......... .......... .......... .......... ..........
       jan7 .......... .......... .......... .......... ..........
      jan21 .......... .......... .......... .......... ..........
Variovorax  AGGGTGCAAG CGTTAATCGG AATTACTGGG CGTAAAGCGT GCGCAGGCGG
    april2  .......... .......... .......... .......... ..........
 Zoogloea2  AGGGTGCAAG CGTTAATCGG AATTACTGGG CGTAAAGCGT GCGCAGGCGG
    april13 .......... .......... .......... .......... ..........
     april4 .......... .......... .......... .......... ..........
       jan5 .......... .......... .......... .......... ..........
Legionella  AGGGTGCAAG CGTTAATCGG AATTACTGGG CGTAAAGCGT GCGTAGGTGG
    april14 .......... .......... .......... .......... ..........
      Nov6  .......... .......... .......... .......... ..........
      Nov9  .......... .......... .......... .......... ..........
      Nov5  .......... .......... .......... .......... ..........
    april11 .......... .......... .......... .......... ..........
    april16 .......... .......... .......... .......... ..........
      jan19 .......... .......... .......... .......... ..........
      Nov17 .......... .......... .......... .......... ..........
 Cytophaga  AGGATCCAAG CGTTATCCGG ATTTATTGGG TTTAAAGGGT GCGTAGGCGG
Flavobacter AGNATCCNAG CGTTATCCGG ATTTATTGGG TTTAAAGGGT GCGTAGGCGG
    april31 .......... .......... .......... .......... ..........
       jan9 .......... .......... .......... .......... ..........
    april27 .......... .......... .......... .......... ..........
      Nov18 .......... .......... .......... .......... ..........
     april5 .......... .......... .......... .......... ..........
      Nov14 .......... .......... .......... .......... ..........

            601                                                 650
      Nov12 .......... .......... .......... .......... ..........
    Nov12re .......... .......... .......... .......... ..........
    april23 .......... .......... .......... .......... ..........
      Nov16 .......... .......... .......... .......... ..........
    Sphingo TTACTCAAGT CAGAGGTGAA AGCCCGGGGC TCAACCCCGG AACTGCCTTT
```

```
      A8AN3  .......... .......... .......... .......... ..........
       jan8  .......... .......... .......... .......... ..........
      jan14  .......... .......... .......... .......... ..........
      Brady  GTCTTTAAGT CAGGGGTGAA ATCCTGGAGC TCAACTCCAG AACTGCCTTT
    april21  .......... .......... .......... .......... ..........
       Nov8  .......... .......... .......... .......... ..........
    april29  .......... .......... .......... .......... ..........
      jan18  .......... .......... .......... .......... ..........
    april24  .......... .......... .......... .......... ..........
    april15  .......... .......... .......... .......... ..........
    april26  .......... .......... .......... .......... ..........
        3M3  ATATGCCAGT CAGGGGTGAA ATCCCGGGGC TCAACCTCGG AACTGCCCTT
        6M3  ATATGCCAGT CAGGGGTGAA ATCCCGGGGC TCAACCTCGG AACTGCCCTT
    april19  .......... .......... .......... .......... ..........
      Nov22  .......... .......... .......... .......... ..........
     april3  .......... .......... .......... .......... ..........
    april18  .......... .......... .......... .......... ..........
       jan1  .......... .......... .......... .......... ..........
    april12  .......... .......... .......... .......... ..........
    april10  .......... .......... .......... .......... ..........
    april20  .......... .......... .......... .......... ..........
      Hypho  ATTTGTAAGT CAGGGGTGAA ATCCCGGGGC TCAACCTCGG AACTGCCTTT
      US353  ATTCGTAAGT CAGGGGTGAA ATCCCGGGGC TCAACCTCGG AACTGCCTTT
       jan6  .......... .......... .......... .......... ..........
       Nov3  .......... .......... .......... .......... ..........
 Zoogloea1  GTATTTAAGT CAGGGGTGAA ATCCCGGAGC TCAACTCCGG AACTGCCTTT
    april25  .......... .......... .......... .......... ..........
       Nov2  .......... .......... .......... .......... ..........
      jan20  .......... .......... .......... .......... ..........
Polyangium  CTCGGAAAGT CGGATGTGAA AGCCCTGGGC TCAACCTAGG AAGAGCATTC
    april22  .......... .......... .......... .......... ..........
   Bacillus  CCGCTTAAGT CTGGTGTTTA AGCC...GGG CTCAACCCCG GTTCGCACTG
      Lacto  .......... .......... .......... .......... ..........
Leptothrix  TTATATAAGA CAGATGTGAA ATCCCCGGGC TCAACCTGGG AACTGCATTT
Rubrivivax  TTATGTAAGA CAGATGTGAA ATCCCCGGGC TTAACCTGGG AACTGCATTT
    Sphaero  TTCTATAAGA CAGATGTGAA ATCCCCGGGC TCAACCTGGG AACTGCATTT
    april17  .......... .......... .......... .......... ..........
       jan3  .......... .......... .......... .......... ..........
    april30  .......... .......... .......... .......... ..........
      jan15  .......... .......... .......... .......... ..........
      jan11  .......... .......... .......... .......... ..........
     april6  .......... .......... .......... .......... ..........
     april1  .......... .......... .......... .......... ..........
       jan2  .......... .......... .......... .......... ..........
       Nov7  .......... .......... .......... .......... ..........
      jan23  .......... .......... .......... .......... ..........
      Nov19  .......... .......... .......... .......... ..........
       jan4  .......... .......... .......... .......... ..........
      jan13  .......... .......... .......... .......... ..........
      jan10  .......... .......... .......... .......... ..........
       Nov4  .......... .......... .......... .......... ..........
      jan12  .......... .......... .......... .......... ..........
       jan7  .......... .......... .......... .......... ..........
      jan21  .......... .......... .......... .......... ..........
Variovorax  TAATGTAAGA CAGTTGTGAA ATCCCCGGGC TCAACCTGGG AACTGCATCT
     april2  .......... .......... .......... .......... ..........
 Zoogloea2  TTTTGTAAGA CTGTCGTGAA ATCCCCGGGC TCAACCTGGG AATGGCGATG
    april13  .......... .......... .......... .......... ..........
     april4  .......... .......... .......... .......... ..........
       jan5  .......... .......... .......... .......... ..........
```

```
Legionella   TTGATTAAGT TATCTGTGAA ATCCCTGGGC TTAACCTGGG CAGGTCAGAT
   april14   .......... .......... .......... .......... ..........
      Nov6   .......... .......... .......... .......... ..........
      Nov9   .......... .......... .......... .......... ..........
      Nov5   .......... .......... .......... .......... ..........
   april11   .......... .......... .......... .......... ..........
   april16   .......... .......... .......... .......... ..........
     jan19   .......... .......... .......... .......... ..........
     Nov17   .......... .......... .......... .......... ..........
 Cytophaga   CCTGTTAAGT CAGGGGTGAA AGACGGTAGC TCAACTATCG CAGTGCCCTT
Flavobacter  TTCTTTAAGT CAGAGGTGAA AGACGGCAGC TTAACTGTCG CAGTGCCTTT
   april31   .......... .......... .......... .......... ..........
      jan9   .......... .......... .......... .......... ..........
   april27   .......... .......... .......... .......... ..........
     Nov18   .......... .......... .......... .......... ..........
    april5   .......... .......... .......... .......... ..........
     Nov14   .......... .......... .......... .......... ..........

             651                                                 700
     Nov12   .......... .......... .......... .......... ..........
   Nov12re   .......... .......... .......... .......... ..........
   april23   .......... .......... .......... .......... ..........
     Nov16   .......... .......... .......... .......... ..........
   Sphingo   GAAACTAGGT GACTGGAATC TTGGAGAGGC GAGTGGAATT CCGAGTGTAG
     A8AN3   .......... .......... .......... .......... ..........
      jan8   .......... .......... .......... .......... ..........
     jan14   .......... .......... .......... .......... ..........
     Brady   GATACTGAAG ATCTTGAGTT CGGGAGAGGT GAGTGGAACT GCGAGTGTAG
   april21   .......... .......... .......... .......... ..........
      Nov8   .......... .......... .......... .......... ..........
   april29   .......... .......... .......... .......... ..........
     jan18   .......... .......... .......... .......... ..........
   april24   .......... .......... .......... .......... ..........
   april15   .......... .......... .......... .......... ..........
   april26   .......... .......... .......... .......... ..........
       3M3   GATACAGCAT GTCTTGAGTC CGATAGAGGT GGGTGGAATT CCTAGTGTAG
       6M3   GATACAGCAT GTCTTGAGTC CGATAGAGGT GGGTGGAATT CCTAGTGTAG
   april19   .......... .......... .......... .......... ..........
     Nov22   .......... .......... .......... .......... ..........
    april3   .......... .......... .......... .......... ..........
   april18   .......... .......... .......... .......... ..........
      jan1   .......... .......... .......... .......... ..........
   april12   .......... .......... .......... .......... ..........
   april10   .......... .......... .......... .......... ..........
   april20   .......... .......... .......... .......... ..........
     Hypho   GATACTGCCA GTCTCGAGTC CGGAAGAGGT GAGTGGAATT CCTAGTGTAG
     US353   GATACTGACA GTCTTGAGTC CGGAAGAGGT GAGTGGAATT CCTTGTGTAT
      jan6   .......... .......... .......... .......... ..........
      Nov3   .......... .......... .......... .......... ..........
 Zoogloea1   GATACTGGGT ACCTAGAGTA TGGAAGAGGT AAGTGGAATT CCGAGTGTAG
   april25   .......... .......... .......... .......... ..........
      Nov2   .......... .......... .......... .......... ..........
     jan20   .......... .......... .......... .......... ..........
Polyangium   GAAACTTCCG AGCTCGAGTT TCGGAGAGGC AGGCGGAATT CTCGGTGTAG
   april22   .......... .......... .......... .......... ..........
  Bacillus   GAAACTGGGC GGCTTGAGTG CAGGAGAGGA AAGCGGAATT CCACGTGTAG
     Lacto   .......... .......... .......... .......... ..........
Leptothrix   GTGACTGTAT AGCTGGAGTG CGGCAGAGGG GGATGGAATT CCGCGTGTAG
Rubrivivax   GTGACTGCAT AGCTTGAGTG CGGCAGAGGG GGATGGAATT CCGCGTGTAG
   Sphaero   GTGACTGTAG AGCTAGAGTA CGGTAGAGGG GGATGGAATT CCGCGTGTAG
```

```
    april17 .......... .......... .......... .......... ..........
       jan3 .......... .......... .......... .......... ..........
    april30 .......... .......... .......... .......... ..........
      jan15 .......... .......... .......... .......... ..........
      jan11 .......... .......... .......... .......... ..........
     april6 .......... .......... .......... .......... ..........
     april1 .......... .......... .......... .......... ..........
       jan2 .......... .......... .......... .......... ..........
       Nov7 .......... .......... .......... .......... ..........
      jan23 .......... .......... .......... .......... ..........
      Nov19 .......... .......... .......... .......... ..........
       jan4 .......... .......... .......... .......... ..........
      jan13 .......... .......... .......... .......... ..........
      jan10 .......... .......... .......... .......... ..........
       Nov4 .......... .......... .......... .......... ..........
      jan12 .......... .......... .......... .......... ..........
       jan7 .......... .......... .......... .......... ..........
      jan21 .......... .......... .......... .......... ..........
 Variovorax GTGACTGCAT TGCTGGAGTA CGGCAGAGGG GGATGGAATT CCGCGTGTAG
    april2 .......... .......... .......... .......... ..........
  Zoogloea2 GTGACTGCAA GGCTAGAGTT TGGCAGAGGG GGGTAGAATT CCACGTGTAG
    april13 .......... .......... .......... .......... ..........
     april4 .......... .......... .......... .......... ..........
       jan5 .......... .......... .......... .......... ..........
 Legionella GATACTGGTT GACTCGAGTA TGGGAGAGGG TAGTGGAATT TCCGGTNNNN
    april14 .......... .......... .......... .......... ..........
       Nov6 .......... .......... .......... .......... ..........
       Nov9 .......... .......... .......... .......... ..........
       Nov5 .......... .......... .......... .......... ..........
april11  .......... .......... .......... .......... ..........
    april16 .......... .......... .......... .......... ..........
      jan19 .......... .......... .......... .......... ..........
       Nov17 .......... .......... .......... .......... ..........
  Cytophaga GATACTGACG GGCTTGAATA CACTAGAGGT AGGCGGAATG TGACAAGTAG
Flavobacter GATACTGAAG AACTTGAATT AGGTTGAGGA ATGCGGAATG AGACAAGTAG
    april31 .......... .......... .......... .......... ..........
       jan9 .......... .......... .......... .......... ..........
    april27 .......... .......... .......... .......... ..........
      Nov18 .......... .......... .......... .......... ..........
     april5 .......... .......... .......... .......... ..........
      Nov14 .......... .......... .......... .......... ..........

            701                                                  750
      Nov12 .......... .......... .......... .......... ..........
    Nov12re .......... .......... .......... .......... ..........
    april23 .......... .......... .......... .......... ..........
      Nov16 .......... .......... .......... .......... ..........
    Sphingo AGGTGAAATT CGTAGATATT CGGAAGAACA CCAGTGGCGA AGGGCACTCG
      A8AN3 .......... .......... .......... .......... ..........
       jan8 .......... .......... .......... .......... ..........
      jan14 .......... .......... .......... .......... ..........
      Brady AGGTGAAATT CGTAGATATT CGCAAGAACA CCAGTGGCGA AGGCGGCTCA
    april21 .......... .......... .......... .......... ..........
       Nov8 .......... .......... .......... .......... ..........
    april29 .......... .......... .......... .......... ..........
      jan18 .......... .......... .......... .......... ..........
    april24 .......... .......... .......... .......... ..........
    april15 .......... .......... .......... .......... ..........
    april26 .......... .......... .......... .......... ..........
        3M3 AGGTGAAATT CGTAGATATT AGGAAGAACA CCGGTGGCGA AGGCGGCCCA
```

```
          6M3  AGGTGAAATT CGTAGATATT AGGAAGAACA CCGGTGGCGA AGGCGGCCCA
      april19  .......... .......... .......... .......... ..........
        Nov22  .......... .......... .......... .......... ..........
       april3  .......... .......... .......... .......... ..........
      april18  .......... .......... .......... .......... ..........
         jan1  .......... .......... .......... .......... ..........
      april12  .......... .......... .......... .......... ..........
      april10  .......... .......... .......... .......... ..........
      april20  .......... .......... .......... .......... ..........
        Hypho  AGGTGAAATT CGTAGATATT CGGAAGAACA CCAGTGGCGA AGGCGGCTCA
        US353  AGGTGAAATT CGTAGATATT AGGAAGAACA CCAGTGGCGA AGGCGGCTCA
         jan6  .......... .......... .......... .......... ..........
         Nov3  .......... .......... .......... .......... ..........
   Zoogloea1   AGGTGAAATT CGTAGATATT CGGAGGAACA CCAGTGGCGA AGGCGGCTTA
      april25  .......... .......... .......... .......... ..........
         Nov2  .......... .......... .......... .......... ..........
        jan20  .......... .......... .......... .......... ..........
  Polyangium   AGGTGAAATT CGTAGATATC GAGAGGAACA CCGGTGGCGA AGGCGGCCTG
      april22  .......... .......... .......... .......... ..........
    Bacillus   CGGTGAAATG CGTAGAGATG TGGAGGAACA CCAGTGGCGA AGGCGGCTTT
       Lacto   .......... .......... .......... .......... ..........
  Leptothrix   CAGTGAAATG CGTAGATATG CGGAGGAACA CCGATGGCGA AGGCAATCCC
  Rubrivivax   CAGTGAAATG CGTAGATATG CGGAGGAACA CCGATGGCGA AGGCAATCCC
     Sphaero   CAGTGAAATG CGTAGATATG CGGAGGAACA CCGATGGCGA AGGCAATCCC
      april17  .......... .......... .......... .......... ..........
         jan3  .......... .......... .......... .......... ..........
      april30  .......... .......... .......... .......... ..........
        jan15  .......... .......... .......... .......... ..........
        jan11  .......... .......... .......... .......... ..........
       april6  .......... .......... .......... .......... ..........
       april1  .......... .......... .......... .......... ..........
         jan2  .......... .......... .......... .......... ..........
         Nov7  .......... .......... .......... .......... ..........
        jan23  .......... .......... .......... .......... ..........
        Nov19  .......... .......... .......... .......... ..........
         jan4  .......... .......... .......... .......... ..........
        jan13  .......... .......... .......... .......... ..........
        jan10  .......... .......... .......... .......... ..........
         Nov4  .......... .......... .......... .......... ..........
        jan12  .......... .......... .......... .......... ..........
         jan7  .......... .......... .......... .......... ..........
        jan21  .......... .......... .......... .......... ..........
  Variovorax   CAGTGAAATG CGTAGATATG CGGAGGAACA CCGATGGCGA AGGCAATCCC
      april2   .......... .......... .......... .......... ..........
   Zoogloea2   CAGTGAAATG CGTAGATATG TGGAGGAACA CCGATGGCGA AGGCAGCCCC
      april13  .......... .......... .......... .......... ..........
       april4  .......... .......... .......... .......... ..........
         jan5  .......... .......... .......... .......... ..........
  Legionella   NNNNNNNNNG CGTAGAGATC GGAAGGAACA CCAGTGGCGA AGGCGGCTAC
      april14  .......... .......... .......... .......... ..........
         Nov6  .......... .......... .......... .......... ..........
         Nov9  .......... .......... .......... .......... ..........
         Nov5  .......... .......... .......... .......... ..........
      april11  .......... .......... .......... .......... ..........
      april16  .......... .......... .......... .......... ..........
        jan19  .......... .......... .......... .......... ..........
        Nov17  .......... .......... .......... .......... ..........
   Cytophaga   CGGTGAAATG CATAGATATG TCACAGAACA CCGATTGCGA AGGCAGCTTA
Flavobacter   CGGTGAAATG CATAGATATG TCTCAGAACC CCGATTGCGA AGGCAGCATT
      april31  .......... .......... .......... .......... ..........
```

```
      jan9  .......... .......... .......... .......... ..........
    april27  .......... .......... .......... .......... ..........
     Nov18  .......... .......... .......... .......... ..........
     april5  .......... .......... .......... .......... ..........
     Nov14  .......... .......... .......... .......... ..........

            751                                                   800
     Nov12  .......... .......... .......... .......... ..........
   Nov12re  .......... .......... .......... .......... ..........
   april23  .......... .......... .......... .......... ..........
     Nov16  .......... .......... .......... .......... ..........
   Sphingo  CTGGACAAGT ATTGACGCTG AGGTGCGAAA GCGTGGGGAG CAAACAGGAT
     A8AN3  .......... .......... .......... .......... ..........
      jan8  .......... .......... .......... .......... ..........
     jan14  .......... .......... .......... .......... ..........
     Brady  CTGGCCCGAT ACTGACGCTG AGGCACGAAA GCGTGGGGAG CAAACAGGAT
   april21  .......... .......... .......... .......... ..........
      Nov8  .......... .......... .......... .......... ..........
   april29  .......... .......... .......... .......... ..........
     jan18  .......... .......... .......... .......... ..........
   april24  .......... .......... .......... .......... ..........
   april15  .......... .......... .......... .......... ..........
   april26  .......... .......... .......... .......... ..........
       3M3  CTGGATCGGT ACTGACGCTG AGGTGCGAAA GCGTGGGGAG CAAACAGGAT
       6M3  CTGGATCGGT ACTGACGCTG AGGTGCGAAA GCGTGGGGAG CAAACAGGAT
   april19  .......... .......... .......... .......... ..........
     Nov22  .......... .......... .......... .......... ..........
     april3  .......... .......... .......... .......... ..........
  april18  .......... .......... .......... .......... ..........
      jan1  .......... .......... .......... .......... ..........
   april12  .......... .......... .......... .......... ....CCCGTT
   april10  .......... .......... .......... .......... ..........
   april20  .......... .......... .......... .......... ..........
     Hypho  CTGGNCCGGT ACTGACGCTG AGGTGCGAAA GCGTGGGGAG CAAACAGGAT
     US353  CTGGTCCGGT ACTGACTCTG AGGTGCGAAA GCGTGGGGAT TAAACAGGAT
      jan6  .......... .......... .......... .......... ..........
      Nov3  .......... .......... .......... .......... ..........
 Zoogloea1  CTGGTCCATT ACTGACGCTG AGGTGCGAAA GCGTGGGGAG CAAACAGGAT
   april25  .......... .......... .......... .......... ..........
      Nov2  .......... .......... .......... .......... ..........
     jan20  .......... .......... .......... .......... ..........
Polyangium  CTGGACGATG ACTGACGCTG AGACGCNNAA GCGTGGGGAG CAAACAGGAT
   april22  .......... .......... .......... .......... ..........
  Bacillus  CTGGCCTGTA ACTGACGCTG AGGCGCGAAA GCGTGGGGAG CAAACAGGAT
     Lacto  .......... .......... .......... .......... ..........
Leptothrix  CTGGGCCTGC ACTGACGCTC ATGCACGAAA GCGTGGGGAG CAAACAGGAT
Rubrivivax  CTGGGCCTGC ACTGACGCTC ATGCACGAAA GCGTGGGGAG CAAACAGGAT
   Sphaero  CTGGACCTGT ACTGACGCTC ATGCACGAAA GCGTGGGGAG CAAACAGGAT
   april17  .......... .......... .......... .......... ..........
      jan3  .......... .......... .......... .......... ..........
   april30  .......... .......... .......... .......... ..........
     jan15  .......... .......... .......... .......... ..........
     jan11  .......... .......... .......... .......... ..........
    april6  .......... .......... .......... .......... ..........
    april1  .......... .......... .......... .......... ..........
      jan2  .......... .......... .......... .......... ..........
      Nov7  .......... .......... .......... .......... ..........
     jan23  .......... .......... .......... .......... ..........
     Nov19  .......... .......... .......... .......... ..........
      jan4  .......... .......... .......... .......... ..........
```

```
      jan13  .......... .......... .......... .......... ..........
      jan10  .......... .......... .......... .......... ..........
       Nov4  .......... .......... .......... .......... ..........
      jan12  .......... .......... .......... .......... ..........
       jan7  .......... .......... .......... .......... ..........
      jan21  .......... .......... .......... .......... ..........
 Variovorax  CTGGGCCTGT ACTGACGCTC ATGCACGAAA GCGTGGGGAG CAAACAGGAT
      april2  .......... .......... .......... .......... ..........
  Zoogloea2  CTGGGTCAAA ACTGACGCTC ATGCACGAAA GCGTGGGGAG CAAACAGGAT
     april13  .......... .......... .......... .......... ..........
      april4  .......... .......... .......... .......... ..........
       jan5  .......... .......... .......... .......... ..........
 Legionella  CTGGCCTAAT ACTGACACTG AGGCACGAAA GCGTGGGGAG CAAACAGGAT
     april14  .......... .......... .......... .......... ..........
       Nov6  .......... .......... .......... .......... ..........
       Nov9  .......... .......... .......... .......... ..........
       Nov5  .......... .......... .......... .......... ..........
     april11  .......... .......... .......... .......... ..........
     april16  .......... .......... .......... .......... ..........
      jan19  .......... .......... .......... .......... ..........
      Nov17  .......... .......... .......... .......... ..........
  Cytophaga  CTATGGTGTG ATTGACGCTG AGGCACGAAA GCGTGGGGAT CAAACAGGAT
Flavobacter  CCAAGCCTNT ATTGACGCTG ATGCACGAAA GCGTGGGGAT CGAACAGGAT
     april31  .......... .......... .......... .......... ..........
       jan9  .......... .......... .......... .......... ..........
     april27  .......... .......... .......... .......... ..........
      Nov18  .......... .......... .......... .......... ..........
      april5  .......... .......... .......... .......... ..........
      Nov14  .......... .......... .......... .......... ..........

             801                                                  850
      Nov12  .......... .......... .......... .......... ..........
    Nov12re  .......... .......... .......... .......... ..........
     april23  .......... .......... .......... .......... ..........
      Nov16  .......... .......... .......... .NANGATGAA AGCCASTTTT
    Sphingo  TAGATACCCT GGTAGTCCAC GCCGTAAACG ATGATAACTA GCTGTCCGGG
      A8AN3  .......... .......... .......... .......... ..........
       jan8  .......... .......... .......... .......... ..........
      jan14  .......... .......... GCNGTAAACG ATGAATGCCA GCCGTCGGGA
      Brady  TAGATACCCT GGTAGTCCAC GCCGTAAACG ATGAATGCCA GCCGTTAGTG
     april21  .......... .......... .......... .......... ..........
       Nov8  .......... .......... .......... .......... ..........
     april29  .......... .......... .......... .......... ..........
      jan18  .......... .......... .......... .......... ..........
     april24  .......... .......... .......... .......... ..........
     april15  .......... .......... .......... .......... ..........
     april26  .......... .......... .......... .......... ..........
        3M3  TAGATACCCT GGTAGTCCAC GCCGTAAACG ATGGATGCTA GCCGTCGGAT
        6M3  TAGATACCCT GGTAGTCCAC GCCGTAAACG ATGGATGCTA GCCGTCGGAT
     april19  .......... .......... .......... .......... ..........
      Nov22  .......... .......... .......... .......... ..........
      april3  .......... .......... .......... .......... ....AGCTTG
     april18  .......... .......... .......... .......... ..........
       jan1  .......... .......... .......... .......... ..........
     april12  AAAAACCGGA TTGGGATGGT TAAGCCNGTT CGGGAATAAG CTTGGTTAAT
     april10  .......... .......... .......... .......... ..........
     april20  .......... .......... .......... .......... ..........
      Hypho  TAGATACCCT GGTAGTCCAC GCCGTAAACT ATGGATGCTA GCAGTCGGCA
      US353  TAGATACCCT .GTAGTCCAC GCCGTAAACT ATGGATGCTA GCCGTCGGNA
       jan6  .......... .......... .......... .......... ..........
```

```
      Nov3  .......... .......... .......... .......... ..........
 Zoogloea1  TAGATACCCT GGTAGTCCAC GCCGTAAACG ATGAATGTTA GCCGTCGGCA
    april25 .......... .......... .......... .......... ..........
      Nov2  .......... .......... .......... .......... ..........
     jan20  .......... .......... .......... .......... ..........
Polyangium  TAGATACCCT GGTAGTCCAC GCCGTAAACG ATGGGTGCTA GGTGTCGCGG
    april22 .......... .......... .......... .......... ..........
  Bacillus  TAGATACCCT GGTAGTCCAC GCCGTAAACG ATGAGTGCTA GGTGTTAGGG
     Lacto  .......... .......... .......... .......... ..........
Leptothrix  TAGATACCCT GGTAGTCCAC GCCCTAAACG ATGTCAACTG GTTGTTGGGA
Rubrivivax  TAGATACCCT GGTAGTCCAC GCCCTAAACG ATGTCAACTG GTTGTTGGGA
   Sphaero  TAGATACCCT GGTAGTCCAC GCCCTAAACG ATGTCAACTG GTTGTTGGGA
    april17 .......... .......... .......... .......... ..........
      jan3  .......... .......... .......... .......... NTKNTSGGTC
    april30 .......... .......... .......... .......... ..........
     jan15  .......... .......... .......... .......... ..........
     jan11  .......... .......... .......... .......... ..........
     april6 .......... .......... .......... ....GGGGTT STTAACTGAA
     april1 .......... .......... .......... .........G GACGGGCTNT
      jan2  .......... .......... .......... .......... ..........
      Nov7  .......... .......... .......... .......... ..........
     jan23  .......... .......... .......... .......... ..........
     Nov19  .......... .......... .......... .......... ..........
      jan4  .......... .......... .......... .......... ..........
     jan13  .......... .......... .......... .......... ..........
     jan10  .......... .......... .......... .....AACKG GTTGTNGGGT
      Nov4  .......... .......... ....TAAACG ATGTCAACTG GTTGTTGGTT
     jan12  .......... .......... .......... .........N TWTGTTGGGT
      jan7  .......... .......... .......... .......... ..........
     jan21  .......... .......... .......... .......... ..........
Variovorax  TAGATACCCT GGTAGTCCAC GCCCTAAACG ATGTCAACTG GTTGTTGGGT
    april2  .......... .......... .......... .......... ....GGGTCT
 Zoogloea2  TAGATACCCT GGTAGTCCAC GCCCTAAACG ATGTCTACTA GTTGTCGGGT
    april13 .......... .......... .......... .......... ..........
     april4 .......... .......... .......... .......... ......ATTW
      jan5  .......... .......... .......... .......... ..........
Legionella  TAGATACCCT GGTAGTCCAC GCTGTAAACG ATGTCAACTA GCTGTTGGTT
    april14 .......... .......... ...TKTAAAM GATGAGWANT TAAAGGTTGG
      Nov6  .......... .....NNGCC CTTAAACTAT GGATAC.TCG ACATTTGCGA
      Nov9  .......... .....CACGC CNTAAACTAT GGATACTTNG ACATTTGNGA
      Nov5  ACCCTAGGTA GTCNAACGGC CGTAAAATGN NGGTAATTGG ACATTTGNGA
    april11 .......... .......... .........G GATAANTTCG CTGTTGGCGA
    april16 .......... .......... .......... .......... ..........
     jan19  .......NTT CCCACGCCCT TAAAACGGAK GRTAANTTSG MTGKTGGGMG
     Nov17  .......... .......... .......... .......... ..........
 Cytophaga  TAGATACCCT GGTAGTCCAC GCCCTAAACG ATGAATACTC GCTGTTAGCG
Flavobacter TAGATACCCT GGTAGTCCAC GCCCTAAACG ATGATAACTC GATGTTGGCG
    april31 .......... .......... .......... .......... ..........
      jan9  .......... .......... .......... .......... ..........
    april27 .......... .......... .......... .......... ..........
     Nov18  .......... .......... .......... .......... ..........
     april5 .......SCC CCTTAAAASG GATGTATGGS CTTTGGTGTT GGTTCCCCGC
     Nov14  .......... .......... .......... .......... .....GGAGG

            851                                                 900
     Nov12  .......... .......... .......... .......... ..........
   Nov12re  .......... .......... .......... .......... ..........
    april23 .......... .......... .......... .......... ..........
     Nov16  CGGCAAGCAN GGNTTTTCGG TGACACACCT AACGGATTAA GCATTCCGCC
    Sphingo CACTTGGTGT TTGGGTGGCG CACGTAACGC ATTAAGTTAT CCGCCTGGGG
```

```
      A8AN3  .......... .......... .......... .......... ..........
       jan8  .......... .......... .......... .......... ..........
      jan14  AGMTTGCTTT TCSGGTGGMG CRGMTAACGC TTTAAGCATT CCGCCTGGGG
      Brady  GGTTTAC.TC ACTAGTGGCG CACGTAACGC TTTAAGCATT CCGCCTGGGG
     april21 .......... .......... .......... .......... ..........
        Nov8 .......... .......... .......... .......... ..........
     april29 .......... .NCSGATCGG TGACGCAGCT AACGCAgTAA GCCNTCCCGC
      jan18  .......... .......... .......... .......... ..........
     april24 .......... .......... ...NGCAGCT AACGCAGTAA GCCTTCCGCC
     april15 .......... .......... .......... .......... ..........
     april26 .......... .......... .......... .......... ..........
         3M3 AGCTTGCTAT TCGGTGGCGC AGCT.AACGC ATTAAGCATC CCGCCTGGGG
         6M3 AGCTTGCTAT TCGGTGGCGC AGCT.AACGC ATTAAGCATC CCGCCTGGGG
     april19 .......... .......... .......... .......... ..........
       Nov22 .......... .......... .......... .......... ..........
      april3 CTAATTTCGG GTGGCGCAGC TAACGCATTA AGCATCCCGC CTGGGGAGTA
     april18 .......... .......... .......... .......... ..........
        jan1 .......... .......... .......... .......... ..........
     april12 TTCGGGTGGC CGCAGGCTTA AACGCATTAA GCATCCCCGC CTGGGGAGTA
     april10 .......... .......... .......... .......... ..........
     april20 .......... .......... .......... .......... ..........
       Hypho AGCTAACTAG TCGGTGGAGC AGCT.AACGC ATTAAGCAT. CCCGCCTGGG
       US353 AGCTTGCTTG TCGGTGGCGC AGCT.AACGC TTTAAGCAT. CCCGCCTGGG
        jan6 .......... .......NGC AGCWAAACGC RTTAAGCATC CCCGCNTGGG
        Nov3 .......... .......... .......... .......... ..........
  Zoogloea1  TGCATGCATG TCGGTGGCGC AGCTAACGCA TTAAACATTC CGCCTGGGGA
     april25 .......... .......... .......... .......... ..........
        Nov2 .......... .......... .......... .......... ..........
       jan20 .......... .......... .......... .......... ..........
  Polyangium GCTTTGACCC NTGCGGTGCC GTAGCTAACG CATTAAGCAC CCNGCCTGGG
     april22 .......... .......... .......... .......... ..........
    Bacillus GTTTCGATAC CCTTGGTGCC GAAGTAAACA CAATAAGCAC TCCGCCTGGG
       Lacto .......... .......... .......... .......... ..........
 Leptothrix GG.GTTTCTT CTCAGT.AAC GTAGCTAACG CGTGAAGTTG ACCGCCTGGG
 Rubrivivax GG.GTTTCTT CTCAGT.AAC GTAGCTAACG CGTGAAGTTG ACCGCCTGGG
     Sphaero GG.GTTTCTT CTCAGT.AAC GAAG..AACG CGTGAAGTTG ACCGCCTGGG
     april17 .......... .......... .......... .......... ..........
        jan3 GG.MTTGCCG ATCAGWGACG CAGCTAAACG CAGTAAGCCC TCCCGCNTGG
     april30 .......... .......... .......... .......... ..........
       jan15 .......... .......... .......... .......... ..........
       jan11 .......... .......... .......... .......... ..........
      april6 CT.TCCAGTA AACGAAAGCT TAACGCGGTG AAAGTTGACC SGCCTGGGGA
      april1 GC.KGGTTTC AGTAACGGAA GCTAACGGCG TGAAGTTGAC CGCCTGGGGA
        jan2 .......... .......... .......... .......... ..........
        Nov7 .......... .......... .......... .......... ..........
       jan23 .......... .......... .......... .......... ..........
       Nov19 .......... .......... .......... .......... ..........
        jan4 .......... .......... .......... .......... ..........
       jan13 .......... .......... .......... .......... ..........
       jan10 CT.TTACTGA CTCAGT.AAC GAAGCTAACG CGTGAAGTTG ACCGCCTGGG
        Nov4 CT.TAGCTGA TTCAGT.AAC GAAGCTAACG CGTGAAGTTG ACCGCCTGGG
       jan12 CT.TAGCTGA NTCAGTAAAC GAAGMTAACG CGTGAAGTTG ACCGCCTGGG
        jan7 .......... .......... .......... .......... ..........
       jan21 .......... .......... .......... .......... ..........
 Variovorax CT.TNACTGA CTCAGT.AAC GAAGCTAACG CGTGAAGTTG ACCGCCTGGG
     april2 TT.AACTGAC TTCCAGTAAC GAAGCTAACG CGTGAAGTTG ACCGCCTGGG
  Zoogloea2 TT.TAATTAA CTTGGT.AAC GCAGCTAACG CGTGAAGTAG ACCGCCTGGG
     april13 .......... .......... .......... .......... ..........
     april4  GAAGGGGCTT AGTGGSGCAG GSTAAACGSG ATTAAAGTTG ACCGCCTGGG
        jan5 .......... .......... .......... .......... ..........
```

```
Legionella  ATATGAAAAT AATTAGTGGC GCAGCAAACG CGATAAGTTG ACCGCCTGGG
   april14  TAGGGGAACC TATCAGTCTT GAaGCTAACA CGATAAGTAA TTCSGCCTGG
      Nov6  TAAACTGT.A AGTGTCT.GA GCGAAAGCAT TAAGTAT.CC CACCTGGGAA
      Nov9  TAAACTGT.A AGNGTCTGGA GCGAAAGCAT TAAGTAT.CC CACCTGGGAA
      Nov5  TAAACTNTAA AGNGTCAGAG CGGAAAGCAT TAAGTATCCC CAANTGGGAA
   april11  TACANCAGCC AGCGGCCAAG CGAAAGCATT AAGTTATCCC ACCTGGGGGA
   april16  .......... .......... .......... .......... ..........
     jan19  AAACACAGCC AGCGGCCAAG CGAAARGCAT TAAGTTATCC ACCTGGGGAG
      Nov17 .......... .......... .......... .......... ..........
 Cytophaga  ATATACAGTT AGCGGCTAAG CGAAAGCGTT AAGTATTCC. .ACCTGGGGA
Flavobacter ATAGACAGTC AGCGTCCCAG CGAAAGCGTT AAGTTATCC. .ACCTGGGGA
   april31  .......... .......... .......... .......... ...CTGGGGA
      jan9  .......... ..TGTGCCGA AGTTAACGCA TTAAGCATCC CGCMTGGGGA
   april27  .......... .......... .......... .......... ..........
     Nov18  .......... .......... .......... .......... ..........
    april5  AAGGGGATCA GKGCCCGTAG GAAATCTTGA TTAAGSCATA CCCACCCTGG
     Nov14  ARGGATAAGC ACTTTTTGAK TGTCGCAGCT AACGCGTTAA ACAGGGCGCM

            901                                                  950
     Nov12  .........N GACCGCAAGG TTAAAACTCA AAGGAATTGA CGGGGGCCCG
   Nov12re  .........N GACCGCAAGG TTAAAACTCA AAGGAATTGA CGGGGGCCCG
   april23  .......... .......... .......... .......... ..........
     Nov16  TGGGGAGTAC GGTCGCAAGA TTAAAACTCA AAGGAATTGA CGGGGGCCCG
   Sphingo  A.....GTAC GGTCGCAAGA TTAAAACTCA AAGGAATTGA CGGGGGCCTG
     A8AN3  .......... .......... .......... .......... ..........
      jan8  .......... .......... .......... .ANGAATTGA CGGGGGCNTG
     jan14  A.....GTAC GGTCGCAAGA TTAAAACTCA AAGGAATTGA CGGGGGCCCG
     Brady  A.....GTAC GGTCGCAAGA TTAAAACTCA AAGGAATTGA CGGGGGCCCG
   april21  .......... .......... .......... AAGGAATKGA SGGGGGCCCG
      Nov8  .......... .......... .......... .......... ..........
   april29  CtGGGaGTAC GGTCGCAAGA TTAAAACTCA AAGGAATTGA CGGGGGCCCG
     jan18  .......... .......... .......... .......... CGGGGGCCCG
   april24  TGGGGAGTAC GGTCGCAAGA TTAAAACTCA AAGGAATTGA CGGGGGCCCG
   april15  .......... .......... .......... .......... ..........
   april26  ...GGAGTAC GCCCSCAAGG TTAAAACTCA AAGGAATTGA CGGGGGCCCG
       3M3  AGTTACGGCC GCAAGGTTAA AACTCAAAGG AATTGACGGG GGCCCGGCNG
       6M3  AGTTACGGCC GCAAGGTTAA AACTCAAAGG AATTGACGGG GGC....CCG
   april19  .......... .......... .......... .......... ..........
     Nov22  .......... .......... .......... .......... ..........
    april3  ACGGCCGCAA GGTTAAAACT TCAAAGGAA. .TTGACGGGG GCC....CGC
   april18  .......... .......... .......... .......... ..........
      jan1  .......... .......... .......... .......... ..........
   april12  ACGGCCGCAA GGTTAAAAAC TTCAAAGGAA TTTGACGGGG GCC....CGC
   april10  .......... .......... .......... .......... ..........
   april20  .......... .......... .......... .......... ..........
     Hypho  GAGTACGGCC GCAAGGTTAA AACTCAAAGG AATTGACGGG GGC....CTG
     US353  GAGTACGGCC GCAAGNTTAA AACTCAAAGG AATTGACGGG GGC....CCG
      jan6  GAGWACGGCC GCAAGGCTAA AACTCAAAGG AATTGACGGG GGC....CCG
      Nov3  .......... .......... .......... .......... ..........
 Zoogloea1  GTACGGTCGC AAGATTAAAA CTCAAAGGAA TTGACGGGGG ......CCCG
   april25  .......... .......... .......GAA TTGACGGGGG ......CCCG
      Nov2  .......... .......... .......... .......... ..........
     jan20  .......... .......... .......... .......... ..........
Polyangium  GAGTACGGCC GCAAGGCTAA AAC...T.CA AAGGAATTGA NNGGGNCNNN
   april22  .......... .......... .......... .......... ..........
  Bacillus  GAGTACGCTC GCAAGAGTGA AAC...T.CA AAGGAATTGA CGGGGACCCG
     Lacto  .......... .......... .......... .......... ..........
Leptothrix  GAGTACGGCC GCAAGGTTGA AAC..TC.AA AGGAATTGAC GGGGAGC..C
Rubrivivax  GAGTACGGCC GCAAGGTTGA AAC..TC.AA AGGAATTGAC GGGGACCCGC
   Sphaero  GAGTACGGCC GCAAG..TGA AAC..TC.AA AGGAATTGAC GGGGACCCGC
```

```
     april17  .......... .......... .......... .......... ..........
        jan3  GGAGTACGGT CGCAAGATTN AAA..CT.CA AAGGAATTGA CGGGGGCCCG
     april30  .......... .......... .......... .......... ..........
       jan15  .......... .......... .......... .......... ..........
       jan11  .......... .......... .......... .......... ..........
       april6 GTACGGCCGC CAAGGTTGAA ACT..NC.AA AGGAATTGAA CGGGGACCCG
       april1 GTACSGCCCG CAAGGTTGAA ACT..CC.AA AGGGAATTGA CGGGGACCCG
        jan2  .......... .......... .......... .......... ..........
        Nov7  .......... .......... .......... .......... ..........
       jan23  ......SGGC CGCAAGGTTG AAA..CT.CA AAGGAATWGA CGGGGACCCG
       Nov19  .......... .......... .......... .......... ..........
        jan4  .......... .......... .AA..TT.CA AAGGAATTGA CGGGACCCGC
       jan13  .......... .......... .......... .......... ..........
       jan10  GAGTACGGCC GCAAGGTTGA AAC..TC.AA AGGAATTGAC KGGGACCCGC
        Nov4  GAGTACGGCC GCAAGGTTGA AAC..TC.AA AGGAATTGAC GGGGACCCGC
       jan12  GAGTACGGCC GCAAGGTTGA AAC..TC.AA AGGAATTGAC GGGGACCCGC
        jan7  .......... ....NGTTGA AAC..TC.AA AGGAATTGAY GGG..ACCCG
       jan21  .......... .......... .......... .......... ..........
   Variovorax GAGTACGGCC GCAAGGTTGA AAC..TC.AA AGGAATTGAC GGGGACCCGC
      april2  GAGTACGGCC GCAAGGTTGA AAC..TC.AA AGGAATTGAC GGGGGCCCGC
   Zoogloea2  GAGTACGGTC GCAAGATTAA AAC..TC.AA AGGAATTGAC GGGGACCCGC
     april13  .......... .......... .......... .......... ..NGGACCCG
      april4  GRGTACCGGC CGCAAGGTTA AAACTTC.AA AGGAATTGAC GGGGGGCCCG
        jan5  .......... .......... .......... .......... ..........
   Legionella GAGTACGGTC GCAAGATTAA AA...CT.CA AAGGAATTGA CGGGGGCCCG
     april14  AAGTACGGCC GCAAGGTTGA AAC..TC.AA AtGAATTGAC GGGGGGCCCG
        Nov6  GTACGACCGC AAGGTTGAAA CTC.....AA AGGAATTGGC .GGGGGTCCG
        Nov9  GTACGACCGC AAGGTTGAAA CTC.....AA AGGAATTGGC GGGGGGTCCG
        Nov5  GTACGACCGC AANGTTGAAA CTC.....AA AGGAATTGGC .GGGGGTCCG
     april11  GTACGACCGC AAGGTTGAAA CTC.....AA AGGAATTGAC .GGGGGCCCG
     april16  .......... .......... .......... .......... ..........
       jan19  TACGACCGCA AGGTTGAAAC TTC.....AA AGGAATTGAC .GGGGGCCCG
       Nov17  .......... .......... .......... .......... ..........
    Cytophaga GTACGCCCGC AAGGGTGAAA CTC.....AA AGGAATTGAC .GGGGGCCCG
Flavobacter   GTACGCCCGC AAGGGTGAAA CTC.....AA AGGAATTGAC .GGGGGCCNG
     april31  GTAC.GTCGC AAGATTGAAA CTY.....AA AGGAATTGGC .GGGGGCCCG
        jan9  GTACGGTCGC AAGATTGAAA CTC.....AA AGGAATTGGC .GGGGGCCCG
     april27  .......... .......... MTC.....AA AGGAATTGAC .GGGGGTCCG
       Nov18  .......... .......... .......... .......... ..........
      april5  GGGAGKACGA TCGCCAAGGK TGAAACTTCA AAGGAATTGA CGGGGGCCSG
       Nov14  TGTGAAGTAC GACCGCAAGG TTAAAACACA AAGGAATTGA CGGGGACCCG

              951                                                1000
       Nov12  CACAAGCAGC GGAGCGTGTG GTTTAATTC. GATGCAACGC GAAGAACCTT
     Nov12re  CACAAGCAGC GGAGCGTGTG GTTTAATTC. GATGCAACGC GAAGAACCTT
     april23  .......... .......... .......... ........GG GCAGRACBTT
       Nov16  CACAAGCGGT GGAGCATGTG GTTTAATTCG AAGCAAC.GC GCAGAACCTT
     Sphingo  CACAAG.CGG TGGAGCATGT GGTTTAATTC GAAGCAACGC GCAGAACCTT
       A8AN3  .......... .......... .......... .......... ..........
        jan8  CACAAGCSGK WGGAGCATGT GGTTTAACTC GAAGCAACGC GCAGAACCTT
       jan14  CACAAGCGGT GGAGCATGTG GTTTAATTCG AAGCAAC.GC GCAGAACCTT
       Brady  CACAAGCGGT GGAGCATGTG GTTTAATTCG ACGCAAC.GC GCAGAACCTT
     april21  CACAAGCGGT GGAGCATGTG GKTaAATTTV GAaGCAACGc GAAGAACNgT
        Nov8  .......... .......... .......... .......... ..........
     april29  CACAAGCGGT GGAGCATGTG GTTTAA.TTC GAAGCAACGC GAAGAACCTT
       jan18  CACAAGCGGT GGAGCATGTG GTWTAA.TTC GAAGCAACGC GAAGAACCWW
     april24  CACAAGCGGT GGAGCATGTG GTTTAA.TTC GAAGCAACGC GAAGAACCTT
     april15  .......... .......TGK GGKTTAATTC GAAGCAACGC GAAGAACcTt
     april26  CACAAGCGGt GGAGCATGTG GTTTAA.TTc GAAGCAACGC GAAGAACCTT
          3M3 CACAAGCGGT GGAGCATGTG GTTTAATTCG ACGCAAC.GC GAAGAACCTT
```

```
      6M3  CACAAGCGGT GGAGCATGTG GTTTAATTCG ACGCAAC.GC GAAGAAACTT
   april19 .....GCGGT GGAGCATGWG GTTTAATTTG GACGCAACGC GAAGAACCTT
     Nov22 .......... .......... .......... .......... ..........
    april3 ACAAGCGGT. GGAGCATGTG GTTTAATTCG ACGCAAC.GC GAAGAACCTT
   april18 .......... GGAGCAKGWG GTTTAATTCG ACGCAAC.GC GAAGAACCTT
      jan1 .......... .......... .......... .......... ..........
   april12 ACAAGCGGTG GGAGCATGTG GTTTAATTNG ACGCAACGGC GAAGAACCTT
   april10 .......... .......... .......... .......... ..........
   april20 .......... .......... .......... ....ACG.CG NARGAACYTT
     Hypho CACAAGCGGT GGAGCATGTG GTTTAATTCG AAGCAAC.GC GAAGAACCTT
     US353 CACAAGCGGT GGAGCATGTG GTTTAATTCG ACGCAAC.GC NAAGAACCTT
      jan6 CACAAGCGGT GGAGCATGNG GTTTAATTNG AAGCAAC.GC GAAGAACCTT
      Nov3 .......... .......... .......... .......... ..........
 Zoogloea1 CACAAGCGGT GGAGCATGTG GTTTAATTCG AAGCAAC.GC GCAGAACCTT
   april25 CACAAGCGGT GGAGCATGTG GTTTAATTCG AAGCAAC.GC GCAGAACYTT
      Nov2 .......... .......... .......... .......... ..........
     jan20 .ACAAGCGGT GGGGCATGTG GTTWAATTS. GASGCAASGC GGAAGACCTT
Polyangium CACAAGCGGT GGAGCATGTG GTTCAATTC. GNNNNAACGC GCAGAACCTT
   april22 .......... .......... .......... .......... ..........
  Bacillus CACAAGCAGT GGAGTATGTG GTTTAATTCG AAGCAACGCG AAGAACCTTA
     Lacto .......... .......... .......... .......... ..........
Leptothrix .ACAAGCGGT GGATGATGTG GTTTAATTC. GATGCAACGC GAAAAACCTT
Rubrivivax .ACAAGCGGT GGATGATGTG GTTTAATTC. GACGCAACGC GAAAAACCTT
   Sphaero .ACAAGCGTG GATCGATGTG GTTTAATTC. GATGCAACGC GAAAAACCTT
   april17 .......... .......... .......... .......... ..........
      jan3 CACAAGCGGT GGAGCATGTG GTTTAATTC. GAAGCAACGC GAAGAACCWT
   april30 .......... .......... GTTCAATTC. GATGCAAcGC GAAAAACCTT
     jan15 .......... .......... .......... ....CAACGC GAAAAACVTT
     jan11 .......... .......... .......... .......... ..........
    april6 CACAAGCGGT GGATGATGTG GTTTAATTC. GATGCAACGC GAAAAACCTT
    april1 CACAAGCGGT GGATGATGTG GTTTAATTS. GATGCAACGC GAAAAACCTT
      jan2 .......... .......... .......... .......... ..........
      Nov7 .......... .......... .......... .......... ..........
     jan23 CACAAGCGGT GGATGATGTG GTTTAATTC. GATGCAACGC GAAAAACCTT
     Nov19 .......... .......... .......... .......... ..........
      jan4 .ACAAGCGGT GGATGATGTG GTTTAATTC. GATGCAACGC GAAAAACCTT
     jan13 .......... .......... .......... .......... ..........
     jan10 .ACAAGCGGT GGATGAWGTG GTTTAATTC. GATGCAACGC GAAAAACCTT
      Nov4 .ACAAGCGGT GGATGATGTG GTTTAATTN. GATGCAACGC GAAAAACCTT
     jan12 AACAAGCGGW GGATGATGTG GTTTAATTC. GATGCAACGC GAAAAACCTT
      jan7 CACAAGCGGT GGATGAKTGG TNKAAWTCG. ..KGCAACGC GAAAAACNTT
     jan21 .......... .......... .......... .......... ..........
Variovorax .ACAAGCGGT GGATGATGTG GTTTAATTC. GATGCAACGC GAAAAACCTT
    april2 .ACAAGCGGT GGATGATGTG GTTTAATTC. GATGCAACGC GAAAAACCTT
 Zoogloea2 .ACAAGCGGT GGATGATGTG GATTAATTC. GATGCAACGC GAAAAACCTT
   april13 CACAAGCGGT GGAtGATGTG GATTAATTC. GAtGCAACGC GAAAAACCTT
   april4  CACAAGCGGT GGAGCATGWG GTTTAATTCG GATGCAACGC GAAGAACCTT
      jan5 .......... .......... .......... .......... ..........
Legionella CACAAGCGGT GGAGCATGTG GTTTAATTC. GATGCAACGC GAAGAACCTT
   april14 CACAAGCGGT GGAGCATGWG GCTTAATTC. GATGCAACGC GAAGAACCTT
      Nov6 CACAAGCGGT GGAGCATGTG GTTTAATTC. GATGATACGC GAGGAACCTT
      Nov9 CACAAGCGGT GGAGCATGTG GTTTAATTC. GATGATACGC GAGGAACCTT
      Nov5 CACAAGCGGT GGAGCATGTG GTTTAATTNA GATGATACGC GAGGAACCNT
   april11 CACAAGCGGA GGAGCATGTG GTTTAATTC. GATGATACGC GAGGAACCTT
   april16 .......... .......... .......TC. GATGATACGc GAGGAACCTT
     jan19 CACAAGCGGA GGAGCATGTG GTTTAATTC. GATGATACGC GAGGAACCTT
     Nov17 .ACAAGCGGA GGAGCATGTG GTTTAATTS. GATGATACGC GAGGAACCTT
 Cytophaga CACAAGCGGA GGAGCATGTG GTTTAATTC. GATGATACGC GAGGAACCTT
Flavobacter CACAAGCGGA GGAGCATGTG GTTTAATTC. GATGNTACGC GAGGAACCTT
   april31 CACAAGNGGT GGAGCATGTG GTTTAATTC. GATGCAAAGC GAAGAACCTT
```

```
      jan9  CACAAGCGGT GGAGCATGTG GTWTAACTM. GATGCAAAGC GAAGAACCTT
    april27 CACAAGCGGT GGAGCATGTG GTTTAATTC. GATGATACGC GAGGAACCTT
      Nov18 .......... .......... .......... .......... ..........
     april5 CACAAGCGGT GGATCATGTG GTTTAATTC. GATGCAACGS GAAGAACCTT
      Nov14 CACAAGCAGT GGAGTGCCTG GTTTAATTC. GATGATCAAC GAAGAACCTC

            1001                                                1050
      Nov12 ACCTAGGTTT GACGTAGTGG TAGTAGTGAA GTGAAAGCGG AACGACCCTT
    Nov12re ACCTAGGTTT GACGTAGTGG TAGTAGTGAA GTGAAAGCGG AACGACCCTT
    april23 ACCAACCcTT GACATGGCGA TcGCGGTTCC AGAGAT...G GTTCcTTCaG
      Nov16 ACCAACCCTT GACATGGCGA TCGCGGTTCC AGAGAT...G GTTCCTTCAG
    Sphingo ACCAGCCTTT GACATCC... .CG.CGCTAT ATCGAGAGAT CGATAGTTCC
      A8AN3 .......... .......... .......... .......... ..........
       jan8 ACCRGCGTWW GACATCCTCA TCG.CGATTT CCAGAGADGG ATTTCTTCAG
      jan14 ACCGGCTSTT GACATGCC.A CGACGACTTC CAGAGATGGA TTTCTTCCCT
      Brady ACCAGCCCTT GACATGTC.C AGGACCGGTC GCAGAGATGT GACCTTCTCT
    april21 ACCAGCCCTT GACATCC..C GGGAcGGTTT CCAGAGATGG GATCCTCNTA
       Nov8 .......... .......... .......... .......... .....TCNTA
    april29 ACCAGCCCTT GACATCC..C GGGACGGTTT CCAGAGATGG GATCCTCCTA
      jan18 ACCAGCCCTW GACATCC..C GGGACGGTTT CCAGAGATGG GATCCTCCTA
    april24 ACCAGCCCTT GACATCC..C GGGACGGTTT CCAGAGATGG GATCCTCCTA
    april15 ACCRGCCCKT GACATCC..c GGGAcGGTTT CCAGAGATGG GATCMTCgTA
    april26 ACCAGCTCTT GACATTC..A CTGACTGTTC CCAGAGatGG GATCGTCCTA
        3M3 ACCAGCTCTT GACATTC..A CTGATTGCCG GTANAGATGC CGGAGTTCCA
        6M3 ACCAGCTCTT GACATTC..A CTGATTGCCG GTANAGATGC CGGAGTTCCA
    april19 ACCAGCTCTT GACATGT..C CGGACGGTTT CCAGAGATGG GATCCTCCCA
      Nov22 .......... .......... .......... .......... ..........
     april3 ACCAGCTCTT GACATTC..A CTGATCGCCT GGGGAGACCT GGGAATCCTA
    april18 ACCAGCTSTT GACATTC..A CTGATCGCCT GGGGAGACCT GGGAATCCTA
       jan1 .......... .......... .......... .......... .ATCSTCMTA
    april12 ACCAGCTNTT GACATTC..A CTGATCGCCT GGAGAGATCC GGGAGTCCTA
    april10 .......... .......... .......... .......... ..........
    april20 ACCAGCTCTT GACATGT..C CGGACGGTTT CCaGAGATGG GATCSTCCTA
      Hypho ACCAGCTCTT GACATTC..A CTGATCGTTT CCAGAGATGG ATTCATCCTA
      US353 ACCAGCTCTT GACATTT..A CGGACCGTTT CCAGAGATGG ATTCATCCTA
       jan6 ACCAGCTCTT GACATTC..A CTGACTGTTC CCAGAGATGG GTTCGTCCTA
       Nov3 ......NNTN GACATCCCGG TNGAAGGANT CGAGANATGG GTCCTTCAGT
  Zoogloea1 ACCAGCCCTT GACATGTCGG TCG.CGGATT ACAGAGATGT TTTCCTTCAG
    april25 ACCAACCCTT GACATG.GGA SGTATGGGCT TCAGAGATGA GGTCCTTCAG
       Nov2 .......... .......... .......... .......... ..........
      jan20 ACCKGGGCTA GAAAAKG..C AGGAACSTGG GAGAAGTCTB GGGGTGCTCT
Polyangium ACCTGGGCTA GAAAATG..C AGGAACCTGG TTGAAAGATC GGGGTGCTCT
    april22 .......... .......... .......... .......... ..........
   Bacillus CCAGGTCTTG ACATCCC..T CTGAATATCC TAGAGATAGG ATAGGCTTCG
      Lacto .......... .......... .......... .......... ..........
Leptothrix ACCTACCCTT GACATGC..C AGGAATCCTG AAGAGATTTG GGAGTGCTCG
Rubrivivax ACCTACCCTT GACATGC..C AGGAATCCTG CAGAGATGTG GGAGTGCTCG
    Sphaero ACCTACCCTT GACATGT..C TGAAATCCTG CAGAGATGTG GGAGTGCTCG
    april17 .......... .......... .......... .......... ..........
       jan3 ACCAGCTCTT GACATTC..A CTGACKGTTC CCAGAGATGG GATCGTCCTA
    april30 ACCTGCGCTT GACATGC..C AGGAACCCCG AAGAGATTTG GGGGTGCCCG
      jan15 ACHTGCGCTT GACATGM..C AGGAACCCYG AAGAGATTTG GGGGWGCCCG
      jan11 .......... .......... .......... .......... ..........
     april6 ACCCACCTTT GACATGG..C AGGAAGTTTC CAGAGATGGA TTCGTGCTCG
     april1 ACYTACCCTT GACATGC..C AGGAACTTGC CAGAGATGGC TTGGTGCTCG
       jan2 .......... .......... .......... .......... ..........
       Nov7 .........C TCAAGCT..A ATGAGAAAGC ATTTTGNGTT GTAAGGCTTG
      jan23 ACCCACCTTT GACATGG..C AGGAAGTTTC CAGAGATGGA TTCGTGCTCG
      Nov19 .......... .......... .......... .......... ..........
       jan4 ACCCACCTTT GACATGC..A CGGAACCCTT TGGAAACAGA GGGGTGCTCG
```

```
      jan13  .......... .......... .......... .......... ...NKGNTNG
      jan10  ACCCACCTKT GACATGT..A CGGAACCCTT TGGAGACAGA GGGGTGcTCG
       Nov4  ACCCACCTTT GACATGT..A CGGAAACCTT TGGAGACAGA GGGGTGCTCG
      jan12  ACCCACCTTT GACATGT..A CGGAACCCTT TGGAGACAGA GGGGTGCTCG
       jan7  ACCCACSTTT GAMATGT..A CGGAACCCTT TGGAGACAGA GGGGTGCTCG
      jan21  .......... .......... .......... .......... .....NCTSG
 Variovorax  ACCCACCTTT GACATGT..A CGGAATTCGC CAGAGATGGC TTAGTGCTCG
      april2  ACCCACCTTT GACATGG..C AGGAAGTTTC CAGAGATGGA TTCGTGCTCG
  Zoogloea2  ACCTACCCTT GACATGG..C AGAAATCCTC GAGAGATTGA GGAGTGCTCG
     april13  ACCTACCCTt GACATGC..C AGGAACCTTG CTGAGAGGTG AGGGTGCCCG
     april4  AACCAGGTBT TGACATC..C TCGGA.ACTT TCCAGAAATG GATTGGTGCC
       jan5  ........NT WGACATC..C CGGGACGGTT TCCAGAGRTG GGATCcTCCT
 Legionella  .ACCTACCCT TGACATA..C AGTGA.ATTT TGCAGAGATG CATTAGTGCC
     april14  ACCtACCCTT GACATCC..T GCGAATCCCG ATGAGAGTTG GGAGTGCCGA
       Nov6  ACCTGGGCTA GAATGCTGGG AGACCGTGGG TGAAAGCTCA CTTTGTAGCA
       Nov9  ACNTGGGCTA GAATGCTGGG AGACCGTGGG TGAAAGCTCA CTTTGTAGCA
       Nov5  ACNTGGGATA GAATGATGGG AGACCGTGGG TGAAAGCTCA CTTTGTAGCA
     april11  ACCTGGGCTT GAAAGTT... AGCGACCGAC TCCGAAAAGA GTCTTTCAGA
     april16  ACSKGGGMTT GAAAGTt... aGCGACCGAC TCCGAAAAG. GTctTTCAGA
      jan19  ACSTGGGNTT GAAAGTT... AGNGACCGAC TCCGAAAAGA GTCTTTCAGA
      Nov17  ACCTGGGCTT GAAAGTT... AGCGACCGAC TCCGAARAGA GTCTTTCAGA
  Cytophaga  ACCCGGGCTT GAAAGTT... AGTGAATTAG ATAGAGATAT TTAAGTGAGC
Flavobacter  ACCCGGGCTT GAAAGTT... AGTGAATGAT CCAGAGACGG ATCAGTCCTT
     april31  ACCAAGGCTT GACATACTGG CTCAAGCTAA TGTGAAAGCA TTTTGTGTTG
       jan9  ACCAAGGCTT GACATACTGG CTCAAGCTAA TGTGAAAGCA TTTTGTGTWN
     april27  ACCTGGGCTA GAATGCCCTT GACAGATTCA GGGATGGATT TTTTCGCAAG
      Nov18  .......... .......... .......... .......... ..........
      april5  ACCCGGGSTT GAAGTGCAGG AAGTACAGAG ATGAAAATCG ACGGACCCGT
      Nov14  ACCTGGGCTT GAACTGTGGC TGCTAATCCT ATGAAAGTAG GACTCTTTCG

             1051                                                1100
      Nov12  CGGGGAGCCA TT........ ....ACAGGT GCTGCATGGC TGTCGTCAGC
    Nov12re  CGGGGAGCCA TT........ ....ACAGGT GCTGCATGGC TGTCGTCAGC
     april23  TTCGGCtGGA TCGCAC.... ....ACAGGT GctGCATGGc TGTcGTCaGC
      Nov16  TTCGGCTGGA TCGCAC.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
     Sphingo  TTTCGGGGAC GCGGTG.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
       A8AN3  .......... .......... .......... .......... ..........
       jan8  TTCGGCTGGA TGAGWG.... ....ACAGGT GCTGCAtGGC TGTCGTCAGC
      jan14  TCGGG..GAC GTGGAC.... ....ACAGGW GCWGCATGGC WGTCGTCAGC
      Brady  TCGGA..GCC TGGAGC.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
     april21  GCA.ATAGGC CCGGAG.... ....ACAGGW GTTGCAWGGM tGTCGTCAGC
       Nov8  GCA.ATAGGC CCGGAG.... ....ACAGGT GTTGCATGGC TGTNGTCAGC
     april29  GCA.ATAGGC CCGGAG.... ....ACAGGT GTTGCATGGC TGTCGTCAGC
      jan18  GCA.ATAGGC CCGGAG.... ....ACAGGT GTTGCATGGC KGTMGTCAGC
     april24  GCA.ATAGGC CCGGAG.... ....ACAGGT GTTGCATGGC TGTCGTCAGC
     april15  GCA.ATAGGC CCGGAG.... ....ACAGGT GTtGCATGGC TgTCGTCAGC
     april26  GCA.ATAGGC AGTGGG.... ....ACAGGT GcTGCATGGC TGTCGTCAGc
        3M3  GCA.ATGGAC AGTGGG.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
        6M3  GCA.ATGGAC AGTGGG.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
     april19  GCA.ATGGGC CGGAAC.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
      Nov22  .......... .......... .......... .......... ..........
      april3  GCA.ATAGGC AGTGGG.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
     april18  GCA.ATAGGC AGTGGG.... ....ACAGGT GCTGCATGGC tGTcGTCAGC
       jan1  GCA.ATAGGC AGaGGG.... ....ACAGGT GTTGCATGGC WGTCGTCAGC
     april12  GCA.ATAGGC AGTGGG.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
     april10  .......... .......... .......... .......... ..........
     april20  GCA.ATAGGC CGGAAC.... ....ACAGGT GCTGCATGGC gGTCGTCAGC
       Hypho  GCACATAGGC AGTGGG.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
       US353  GCANATAGGC CGTAGG.... ....ACAGGT GCTGCGTGGC TGTCGTCAGC
       jan6  GCA.ATAGGC AGTGGG.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
```

```
      Nov3  TNGGAGTGGA CCGATG.... ....ACAGGT GNTGCANGGC TGTCGTCAGC
 Zoogloea1  TTAGGCTGGA CCGAAC.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
   april25  TTCGGCTGGC GTCCAC.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
      Nov2  .......... .......... .......... .......... ..........
     jan20  TCGGAGAGCC TGTAGT.... ....TAGGW. GCTGCATGGC TGTCGTCAGC
Polyangium  TCGGAGAACC TGTAGT.... ....TAGGT. GCTGCATGGC TGTCGTCAGC
   april22  .......... .......... .......... .......... ..........
  Bacillus  GGACAGAGGA GACA...... .......GGT GGTGCATGGT TGTCGTCAGC
     Lacto  .......... .......... .......... .......... ..........
Leptothrix  AAAGAGAGCC TGGACA.... ....CAGGT. GCTGCATGGC CGTCGTCAGC
Rubrivivax  AAAGAGAACC TGGACA.... ....CAGGT. GCTGCATGGC CGTCGTCAGC
   Sphaero  AAAGAGAATC AGAACA.... ....CAGGT. GCTGCATGGC CGTCGTCAGC
   april17  .......NCA GTGGGA.... ....CAGGT. GgtGCATGGc TGTcGTCAGC
      jan3  GCAATAGGCA GTGGGA.... ....CAGGT. GCTGCATGGC TGTCGTCAGC
   april30  AAAGGGAGCC TGGACA.... ....CAGGT. GCTGCATGGC TGTCGTCAGC
     jan15  AAAGGGAGCM TGGACA.... ....CAGGW. GBTGCATGGC TGTMGTCAGC
     jan11  AAAGGGAGCC TGGACA.... ....CAGGK. GMWGCATGGC KGTMGTCAGC
    april6  AAAGAGAACC TGCACA.... ....CAGGT. GCTGCATGGC TGTCGTCAGC
    april1  AAAGAGAACC TGGACA.... ....CAGGT. GCTGCATGGC CGTCGTCAGC
      jan2  .......... .......... .......... .......... NGTCGTCAGM
      Nov7  CCTTACATAG TCAGTA.... ....CAGGN. GGNGCATGGC TGTCGTCAGC
     jan23  AAAGAGAACC TGCACA.... ....CAGGT. GCTGCATGGC TGTCGTCAGC
     Nov19  .......... .......... .......... .......... ..........
      jan4  AAAGAGAGCC GTAACA.... ....CAGGT. GCTGCATGGC TGTCGTCAGC
     jan13  AAAGAGAGCC GTNACA.... ....CAGGT. GCWGMATGGC KGTCGTCAGC
     jan10  AAAGAGAGCC GTAACA.... ....CAGGT. GMTGCATGGC TGTcGTCAGC
      Nov4  AAAGAGAGCC GTAACA.... ....CAGGT. GCTGCATGGC TGTCGTCAGC
     jan12  AAAGAGAGCC GTAACA.... ....CAGGT. GCTGCATGGC TGTCGTCAAC
      jan7  AAAGAGAGCC GTAACA.... ....CAGGT. GCTGCATGGC WGTCGTCAGC
     jan21  AAAGAGAGCM GTAACA.... ....CAGGTG GSTGCATGGC TGTCGTCAGC
Variovorax  AAAGAGAACC GTAACA.... ....CAGGT. GCTGCATGGC TGTCGTCAGC
    april2  AAAGAGAACC TGCACA.... ....CAGGT. GCTGCATGGC TGTCGTCAGC
 Zoogloea2  AAAGAGAATC TGCACA.... ....CAGGT. GCTGCATGGC TGTCGTCAGC
   april13  AAAGGGAGCC TGGACA.... ....CAGGK. GCTGCATGGC WGTSGTCAGC
    april4  TTCGGGA.AC CGAGAG.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
      jan5  AGCAATAGGC CCGGAG.... ....ACAGGT GTTGCAWGGS NGTCGTCAGC
Legionella  TTCGGGA.AC ACTGAT.... ....ACAGGT GCTGCATGGC TGTCGTCAGC
   april14  AAGGAGCGCA GAGACA.... .......GGT GCTGCATGGC TGTSGTCAGC
      Nov6  ATACACTGCC AGTA...... .......AGGT GCTGCATGGC TGTCGTCAGC
      Nov9  NTACACTGCC AGTA...... .......AGGT GCTGCATGGC TGTCGTCAGC
      Nov5  ATACANTGCC AGTA...... .......AGGT GCTGCATGGC TGTCGTCAGC
   april11  AATGACGCGA AACT...... .......AGGT GCTGCATGGC TGTCGTCAGC
   april16  AATGAcGCGA AACT...... .......aGGT GCTGCATGGC tGTCGTCAGC
     jan19  AATGACGCGA AACT...... .......AGGT GCTGCATGGC TGTCGTCAGC
     Nov17  AATGACGCGA AANT...... .......AGGT GCTGCATGGC TRTCGTCAGC
Cytophaga  AATCACACGA AACT...... .......AGGT GCTGCATGGC TGTCGTCAGC
Flavobacter CGGGACACGA AACT...... .......AGGT GCTGCATGGC TGTCGTCAGC
   april31  TAAGGCTTGC CTTACATAGT CAGTACAGGT GCTGCATGGC TGTCGTCAGC
      jan9  TAAGGCTTGC CTWACATAGT CAGTACAGGT GCTGCATGGC WGTCGTCAGC
   april27  AACAAGGAGC .......... .....AAGGT GCTGCATGGC TGTCGTCAGC
     Nov18  .......... .......... .......... .......... ..........
    april5  AAAGTCGGAA TCCTGC.... ....AGAGGT GCTGCATGGC TGTCGTCAGC
     Nov14  AAGGTGCCAC AG........ ......AGGT GATGCATGGC TGTCGTAAGC

            1101                                                1150
     Nov12  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCNTNGT
   Nov12re  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCNTNGT
   april23  TCGtGTCGtG AGatGDTCGg .TAAGTCcGG C.AAcGAGcG CAACCCACAS
     Nov16  TCGTGTCGTG AGATGTTCGG TTAAGTCCGG C.AACGAGCG CAACCCACAC
   Sphingo  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGT
```

```
      A8AN3  .......... .......... .........G C.AACGAGCG CAACCCTCGC
       jan8  TCGTGTCGaG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCcTCGT
      jan14  TCGWGTCGTG AGATGWTGGG TWAAGTCCCG C.AACGAGCG CAACCCTCGC
      Brady  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCCCGT
     april21 TCGTGTCGTg AGAtGttGGG TTAAGTCCCG C.AAcGAGCG CAACCCTCGC
       Nov8  TCGTGTCNTG AGATGNTGGG NTAAGTCCCG C.NACGAGCG CAACCCTCGC
     april29 TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGC
      jan18  TCGWGTCGTG AGATGWTGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGC
     april24 TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGC
     april15 TcGtGTCGTG AGATGaTGGG TTAAGTCCCG C.AACGAGcG CAACCcTCGG
     april26 TCGTGTCGTG AGATGTTGGG KTAAGTCCCG C.AACGAGCG CAACCCTCGC
         3M3  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGC
         6M3  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGC
     april19 TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGC
      Nov22  .......... .......... .......... .......... ..........
      april3 TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGC
     april18 TcGTGTCGTG GGATGTTGGG WTAAGTCCCG C.AACGAGCG CAACCcTCGC
        jan1 TCRTGTCGTG AGATGTWGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGC
     april12 TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGC
     april10 .......RTG aGADGTKGGG TKAAGTCCCG C.AaCGAGSG CAACCCTcGC
     april20 TCGTGTcgTG AGATGTTGGG TTAAGTCCCG C.AACGaGcG CAACCSTCGC
       Hypho  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGC
       US353  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGC
        jan6  TCGTGCCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGC
        Nov3  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTNGC
  Zoogloea1   TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTCGC
     april25 TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCCTAT
        Nov2  .......... ........GG TTAAGTCCCG C.AACGAGCG CNACCCCCAT
       jan20  TCGTGTCGTG AGATGKTGGG TTARGTCCCG C.AACGAGCG CAACCCcTAT
  Polyangium  TCGTGTCGTG AGATGTTGGG TTAAGTCCCN C.AACGAGCG CAACCCCTAT
     april22  .......... .......... .......... .......... ..........
    Bacillus  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTGA
       Lacto  .......... .......... .......... .......... ..........
  Leptothrix  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTGT
  Rubrivivax  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTGT
     Sphaero  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTGT
     april17  TCGTGTcGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CNACCCTTGT
        jan3  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTGT
     april30  TcGTGTCGTG AGATGTKGGG TTAAGTCCCG C.AACGAGCG CAACCCTTAT
       jan15  TCGWGTCGTG AGATGTTGGG WTAAGTCCCG C.AACGRGCG CAACCMTTAT
       jan11  TMGWGTCGTG AGATGWTGGG TWAAGTCCCG CAARCGAGCG CAACCSTTAT
      april6  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTGC
      april1  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTAT
        jan2  TCGTGTCGTG AGATGKKGGG TTAAGTCCCG C.AACGAGCG CRACCCTTGT
        Nov7  TCGTGCCGTG AGGTGTCCGG TTAAGTCCCG C.AACGAGCG CAACCCTTGT
       jan23  TCGTGTCGTG AGATGTTGGG TTAAG.CCCG C.AACGAGCG CAACCCTTGT
       Nov19  .......... .......... .......... .......... ..........
        jan4  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTGT
       jan13  TCGTGTCVWG AGATGTTGGG ATAAGTCCCG C.AACGAGSG CAACCSTNGT
       jan10  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTGT
        Nov4  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTGT
       jan12  TCGTGTCGTG AGATGWTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTGT
        jan7  TMGWGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCMTTGT
       jan21  TCGTGTMGWG AGATGKKGGG NTAAGTCCCG C.AACGAGCG CAACCCTKGT
  Variovorax  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTGT
      april2  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAACG CAACCCTTGC
   Zoogloea2  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTGT
     april13  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCTTGC
      april4  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG .TAACGAGCG CAACCCTTGT
        jan5  TYGTGTYGCG AGAWGWTGGG TTAAGTCCCG .WAACGAGSG CAACCCTTGT
```

```
Legionella  TCGTGTCGTG AGATGTTGGG TTAAGTCCCG .TAACGAGCG CAACCCTTGT
   april14  TCGTGTTGTG AGATGTTGGG TTAAGTCCcG .TAACGAGCG CAACCCTTGT
      Nov6  TCGTGCCGTG AGGTGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCCCAT
      Nov9  TCGTGCCGTG AGGTGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCCCAT
      Nov5  TCGTGCCGTG AGGTGTTGGG TTAAGTCCCG C.NACGANCG CAACCCCCAT
   april11  TCGTGCCGTG AGGTGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCCTAT
   april16  TCGTgCCGtG AGGTGKTGGG TTAAGTCCCG C.AACGAGCG CAACCCCTAT
     jan19  TCGTGCCGTG AGGTGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCCTAT
      Nov17 TCGTGCCGTG AGGTGTTGGG TTAAGTCCCG C.AACGRGCG CAACCCMTAT
  Cytophaga TCGTGCCGTG AGGTGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCCTAT
Flavobacter TCGTGCCGTG AGGTGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCCTAT
   april31  TCGTGCCGTG AGGTGTCCGG TTAAGTCCGG T.AACGAGCG CAACCCCTAT
      jan9  CCGTGCCGTG AGGTGTCCGG TTAAGTCCGG T.AACGAGCG CAACCCCTAT
   april27  TCGTGCCGTG AGGTGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCCTAT
      Nov18 .......... .......... .......... ........CG AAACCCCWAT
    april5  TCGTGCCGTG AGGTGTTGGG TTAAGTCCCG C.AACGAGCG CAACCCCTAT
      Nov14 TCGTGTCGTG AGATGTCGGG TTAAGTCCCA C.AACGAGCG CAACCCCTTT

            1151                                                  1200
      Nov12 CGCTAGTTAT ACGTGTCT.. .......... ....AGCGAG ACCGCCGGT.
    Nov12re CGCTAGTTAT ACGTGTCT.. .......... ....AGCGAG ACCGCCGGT.
   april23  TTgTAGTTGC CAGCA..TTC AGTTGGGCAc TgTAGAAG.A ACTGCCGATG
      Nov16 TTCTAGTTGC CAGCA..TTC AGTTGGGCAC TCTAGAAG.A ACTGCCGATG
    Sphingo CCTTAGTTGC CAGCA..TTA AGTTGGGCAC TCTAAAGGAA ACTGCCGGTG
      A8AN3 CTTTAGTTGC CAGCA..TTT GGTTGGGTAC TCTAAAGG.A ACCGCCGGTG
       jan8 CCTTAGTTGC CATCA..TTT AGTTGGGCAC TCT.AAGGAA ACCGCCGGTG
      jan14 CTMTAGTTGC CAGCA..TTC AGTTGGGCAC TCTAGAGG.G ACTGCCGGTG
      Brady CCTTAGTTGC TACCA..TTT AGTTGAGCAC TCTAAGGA.G ACTGCCGGTG
   april21  tGTTAGTtGC CaTCa..TTT AGTTGGGCAc TcTAACGG.G ACCGCCGGTG
       Nov8 TGTTAGTNGC CATCA..TTT AGTTGGGCAC TCTAACGG.G ACCNCCGGTG
   april29  TGTTAGTTGC CATCA..TTT AGTTGGGCAC TCTAACGG.G ACCGCCGGTG
      jan18 TGTTAGWTGC CATCA..TTT AGTTGGGCAC TMTAACGG.G ACCGCCGGTG
   april24  TGTTAGTTGC CATCA..TTT AGTTGGGCAC TMTAACGG.G ACCGCCGGTG
   april15  WGWKAGTTGC CATCA..TTt AGTtGGGCAC TSTAACGG.G ACCGCcGGTG
   april26  CATTAGTtGC CATCA..TTC AGTTGGGCAC THTAGTGG.G ASTGCCGGTG
        3M3 CATTAGTTGC CATCA..TTC AGTTGGGCAC TCTAGTGG.G ACTGCCGGTG
        6M3 CATTAGTTGC CATCA..TTC AGTTGGGCAC TCTAGTGG.G ACTGCCGGTG
   april19  CATCAGTTGC CATCA..WTA AGTTGGGCAC TCTGGTGG.G ACTGCCGGTG
      Nov22 .......... .......... .......... .......... ..........
    april3  CATTAGTTGC CATCA..TTC AGTTGGGCAC TMTAGTGG.G ACTGCCGGTG
   april18  CATTAGTTGC CATCA..TTC AGTtGGGCAC TMTAGTGG.G ACTGCCGGTG
       jan1 CATTAGTWGC CATCA..TTC AGTTGGGCAC TCTAGTGG.G ACTGCCGGTG
   april12  CATTAGTTGC CATCA..TTT AGTTGGGCAC TCTAGTGG.G ACTGCCGGTG
   april10  CAKKAGTtGC CATCA..tTA AGtKGGGCAC TWTAGTGG.G AMTGCCGGWG
   april20  CATTAGTtGC CATCA..TTA AGTTGGGCAC TCTAGTGG.G ACTGCCGGTG
      Hypho CATTAGTTGC CATCA..TTC AGTTGGGCAC TCTAGTGG.G ACTGCCGGTG
      US353 CATTAGTTGC CATCA..TTT AGTTGGGCAC TCTAGTGG.G ACTGCCGGTG
       jan6 CATTAGTTGC CATCA..TTA AGTTGGGCAC TCTAGTGG.G ACTGCCGGTG
       Nov3 CCTTAGTTGC CATCA..TTC AGATGGGCAC TCTAAGGG.G ACTGCCGGTG
 Zoogloea1  CCTTAGTTGC CAGCA..TTC AGTTGGGCAC TCTAAGGG.G ACTGCCGGTG
   april25  BTTCAGTTGC CATCA..TTC AGTTGGGCAC TCTGAAGA.A ACTGCCGGTG
       Nov2 CCTTAGTTGC CATCA..TTA AGTTGGGCAC TNTAAGGA.A ACTGCCGGTG
      jan20 CACTAGTTAC CAGCGGGTCA TGCCGGGGAC TGTAGTGA.G ACCGCCGATA
Polyangium  CGTTAGTTGC CAGC.GGTCC GGCCGGGNAC TCTAACGA.G ACTGCCGATA
   april22  .......... .GGCA..TTA AGTKGGGCAN TTTAGSGK.G ACTGCCGGt.
   Bacillus ACTTAGTTGC CAGCA..TTC AGTTGGGCAC TCTAAGTT.G ACTGCCGGT.
      Lacto .......... .......... .......... .......... ..........
Leptothrix  CATTAGTTGC TA.......C GAAAGGGCAC TCTAATGA.G ACT.CCGGT.
Rubrivivax  CATTAGTTGC TA.......C GTAAGGGCAC TCTAATGA.G ACTGCCGGT.
   Sphaero  CATTAGTTGC TA.......C GAAAGGGCAC TCTAATGA.G ACTGCCGGT.
```

```
    april17  CATTAGTtGg TA.C..... GAaAGGGCAC TgTAATGA.G ASTGCCGGT.
       jan3  CATTAGTTGC TA.C..... GAAAGGGCAC TMTAATGA.G ACTGCCGGT.
    april30  CCTTAGTTGc TA.C..... GCAAGGGCAC TCTAAGGA.G ACTGCCGGT.
      jan15  CMTTAGTTGC TA.C..... GCAAGGGCAH TCTAAGGA.G ACTGCCGGT.
      jan11  CCTTAGTTGM TA.C..... GCAAGGGCAS TMTAAGGA.G ACTGCCGGT.
      april6 CATTAGTTGC TA.C..... GAAAGGGCAC TCTAATGG.G ACTGCCGGT.
      april1 CATTAGTTGC TA.C..... GCAAGGGCAC TCTAATGA.G ACTGCCGGT.
       jan2  CATTAGTKGN TA.C..... GAAAGGGCAC TcTAATGA.G ACTGCCGGT.
       Nov7  CATTAGTTGN TA.C..... GAAAGGGCAC TNTAATGA.G ACTGCCGGT.
      jan23  CATTAGTTGC TA.C..... GAAAGGGCAC TCTAATGA.G ACTGCCGGT.
      Nov19  .......... .......... .......... .......... ..........
       jan4  CATTAGTTGC TA.C..... GAAAGGGCAC TCTAATGA.G ACTGCCGGT.
      jan13  CATTAGTTGC WA.C..... GRAAGGGCAC TMTAATGA.G ANTGCCGGT.
      jan10  CATTAGTTGC TA.C..... GAAGGGGCAC TcTAATGA.G ACTGCCGGT.
       Nov4  CATTAGTTGC TA.C..... GAAAGGGCAC TCTAATGA.G ACTGCCGGT.
      jan12  CATTAGTTGC TA.C..... GAAAGGGCAC TMTAATGA.G ACTGCCGGT.
       jan7  CATTAGTTGM TA.N..... GAAAGGGCAC TcTAATGA.G ACTGCCGGT.
      jan21  CATTAGKTGC TA.C..... GAAAGGGCAC TNTAATGA.G ACTGCCGGa.
  Variovorax CATTAGTTGC TA.CA..TTT AGTTGGGCAC TCTAATGA.G ACTGCCGGT.
      april2 CATTAGTTGC TA.C..... GAAAGGGCAC TCTAATGG.G ACTGCCGGT.
   Zoogloea2 CATTAGTTGC TA.C..... GAAAGAGCAC TCTAATGA.G ACTGCCGGT.
     april13 CATTAATtGC CATCA..TTT AGTTGGGCAC TTTAATGG.G ACTGCCGGT.
     april4  CCTTAGTTGC CAGCAT.TTC GGATGGGAAC TMTAAGGA.G ACCGCCGGT.
       jan5  CCTTAGTTGC CAGCAC.TTc GGGTGGGAAC TCTAAGGA.G AcTGCCGGT.
  Legionella CCTTAGTTGC CAGCACGTAA AGGTGGGAAC TCTAAGGA.G ACTGCCGGT.
     april14 CCTTAGTTGC CATCA..TTT AGTTGGGGAC TCTAAGGA.G ACTGCCGGT.
       Nov6  CACTAGTTGC CATCAGGTAA CGCTGGGAAC TCTAGTGA.A ACTGCCGTC.
       Nov9  CACTAGTTGC CATCAGGTAA CGCTGGGAAC TCTAGTGA.A ACTGCCGTC.
       Nov5  CACTAGTTGC CATCANGTAA CGCTGGGAAC TCTAGTGA.A ACTGCCGTC.
     april11 TCTTAGTTGC CATCAGGTTA TGCTGGGGAC TCTAAGAA.G ACTGCCCGC.
     april16 TCTTAGttGC CATCAGGWTA TGcTGGGGAc TcTAaGAA.G ACtGCCCGC.
      jan19  TgTTAGTTGC CATCAGGTTA TGCTGGGGAC TCTAAGAA.G ACTGCCCGC.
      Nov17  TCWTAGTTGC CATCAGGTTA TGCTGGGGAC TCTAAGAA.G ACTGCCCGC.
   Cytophaga GTTTAGTTGC CAGCACGTTA AGGTGGGGAC TCTAAACA.G ACTGCCTGT.
 Flavobacter GTTTAGTTGC CAGCATGTCA TGATGGGGAC TCTAAACA.G ACTGCCTGC.
     april31 GTTTAGTTGC CAGCACGT.A AGGTGGGAAC TCTAAAGA.G ACTGCCGGC.
       jan9  GTTTAGTTGC CAGCACGT.A AGGTGGGAAC TMTAAAGA.G ACTGCCGGC.
     april27 TBTTAGTTGC CAGCATGTCA TGGTGGGGAC TYTAAGAA.G ACTGCCTGC.
      Nov18  MGTCRGKDGC CAGCA..WTC RGKTGGRGAS TSWGACGA.G ACCGCCGGT.
      april5 YTCCAGTTGC CAGCGGTTTG GCCGGG...C ACTCTGGAGA GACTGCCTAC
      Nov14  CAGATGTTAC AATTACTTCT GAT....... .......... ACTGCCCCGC

             1201                                                1250
      Nov12  ATCAAACCGG AGGAAGGTGG GGATGACGTC AAGTCAGCAT GGCCTTTATG
    Nov12re  ATCAAACCGG AGGAAGGTGG GGATGACGTC AAGTCAGCAT GGCCTTTATG
     april23 aTAAGTcG.G AGGAAGGtGT GGAtGACGTC AAGTCcTCAT GGCCCYKACG
      Nov16  ATAAGTCG.G AGGAAGGTGT GGATGACGTC AAGTCCTCAT GGCCCTTACG
     Sphingo ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGACCTTACA
       A8AN3 ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTACG
        jan8 ATAAGCCG.G AGGAAGGTGG GGAWGACGTC AAGTCcTCAT GGCCCTTACA
       jan14 ATAAGCCGAG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTACG
       Brady ATAAGCCGCG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTACG
     april21 aTAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATcAT GGCCCTTACG
        Nov8 ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTACG
     april29 ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTACG
       jan18 ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAW GGCCCTWACG
     april24 ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTACG
     april15 ATAAGCCG.G AGGAAGGTGG GGACGACGTC AAGTCaTCAt GGCCCtTACG
     april26 ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCHTACG
        3M3  ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTACG
```

```
        6M3  ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTACG
    april19  ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTACG
      Nov22  .......... .......... .......... .......... ..........
     april3  ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTACG
    april18  ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTACG
       jan1  ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTATG
    april12  ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTACG
    april10  ATAAGCCG.G AGGAAGGTGG RGATGACGTC AAGTCATCAT GGCCCTtATG
    april20  ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTATG
      Hypho  ATGAGCCGAG AGGAAGGTGG GGATGACGTN AAGTCATCAT GGCCCTTACG
      US353  ATAAGCTGGN AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTACG
       jan6  ATAAGCCG.G AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTATG
       Nov3  ATAAGCCGAG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTACG
  Zoogloea1  ATAAGCCGAG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTACG
    april25  ACAAGCCG.G AGGAAGGCGG GGATGACGTC AAGTCCTCAT GGCCCTTACG
       Nov2  ACAAGCCG.G AGGAAGGTGG GGATAACGTC AAGTCTTCAT GGCCCTTACG
      jan20  TTCAAATCGG AGGAAGGTGG GGATGACGTC AGGTCATCAT GGCCCTTATG
 Polyangium  TTCAAATCGG AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTATG
    april22  GACAAACCGG AGGAAGGCGG GGAKGACGTC AAATCATCAT GCCCCAWaTG
   Bacillus  GACAAACCGG AGGAAGGTGG GGATGACGTC AAATCATCAT GCCCCTTATG
      Lacto  ........GG AGGAAGGTGG GGATGACGTC AAATCATCAT GCCCCTTATG
 Leptothrix  GACAAACCGG AGGAAGGTGG GGATGACGTC AGGTCCTCAT GGCCCTTATG
 Rubrivivax  GACAAACCGG AGGAAGGTGG GGATGACGTC AGGTCATCAT GGCCCTTATG
    Sphaero  GACAAACC.G AGGAAGGTGG GGATGACGTC AGGTCCTCAT GGCCCTTATG
    april17  GACAAACCGG AGGAAGGTGG GGAtGAcGTC AAGTCCTCaT GGCCCTtATA
       jan3  GACAAACCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATA
    april30  GACAAACCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATG
      jan15  GACAAACCGG AGGAAGGWGG GGATGACGTC AAGTCCTCAT GGCCCTTATG
      jan11  GACAAACCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATG
     april6  GACAAACCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATA
     april1  GACAAACCGG AGGAAGGTGG GGATGACGTC AGGTCATCAT GGCCCTTATG
       jan2  GACAAACCGG AGGAAGGTGG GGATGACGTC AAGTCcTCAT GGCCCTTATA
       Nov7  GACAAACCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATA
      jan23  GACAAACCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATA
      Nov19  .......... .......... .......... .......... .........T
       jan4  GACAAGCCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATA
      jan13  GACAAACCGG AGGAAGGTGG GGAWGACGTC AAGTCCTCAT GGCCCTTATA
      jan10  GACAAGCCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATA
       Nov4  GACAAGCCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATA
      jan12  GACAAGCCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATA
       jan7  GACAAGCCGG AGGAAGGTGG GGACGACGTC AAGTCCTCAT GGCCCTKATA
      jan21  GACAAGCCGG AGGAAGGTGG GGACGACGTC AAGTCcTCAT GGCCCTKATA
 Variovorax  GACAAACCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATA
     april2  GACAAACCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATA
  Zoogloea2  GACAAACCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATG
    april13  GACAAACCGG AGGAAGGTGG GGATGACGTC AAGTCCTCAT GGCCCTTATG
    april4   GACAAACCGG AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTATG
       jan5  GACAAACCGG AGGAAGGTGG GGACGAcGTC AAGTCATCAT GGCCCTtACG
 Legionella  GACAAACCGG AGGAAGGCGG GGATGACGTC AAGTCATCAT GGCCCTTACG
    april14  GATGAACCGG AGGAAGGCGG GGACGACGTC AAGTCATCAT GGCCTTTATG
       Nov6  GTAAGACGTG AGGAAGGAGG GGATGATGTC AAGTCATCAT GGCCTTTATG
       Nov9  GTAAGACGTG AGGAAGGAGG GGATGATGTC AAGTCATCAT GGCCTTTATG
       Nov5  GTAAGACGTG AGGAAGGAGG NGATGATGTC CAGTCATCAT GGCCTTAAAG
    april11  GCAAGCGGTG AGGAAGGTGG GGATGACGTC AAGTCATCAC GGCCCTTACG
    april16  GCAAGCGGTG AGGAAGGTGG GGAtGAcGTC AAGTCATCAC GGCCCTtACG
      jan19  GCAAGCGGTG AGGAAGGTGG GGATGACGTC AAGTCATCAC GGCCCTTACG
      Nov17  GCAAGCGGTG AGGAAGGTGG GGATGACGTC AAGTCATCAC GGCCCTTACG
  Cytophaga  GCAAACAGAG AGGAAGGAGG GGACGACGTC AAGTCATCAT GGCCCTTACG
Flavobacter  GNNAGCAGAG AGGAAGGTGG GGACGACGTC AAGTCATCAT GGCCCNTACG
    april31  GACAAGCCGG AGGAAGGCGG GGACGACGTC AAGTCATCAT GGCCCTTATG
```

```
      jan9  GACAAGCCGG AGGAAGGCGG GGACGACGTC AAGTCATCAT GGCCCTTATG
    april27  GCAAGCAGAG AGGAAGGAGG GGATGACGTC AAGTCATCAT GGCCCTTACG
      Nov18  GTTAAACCGG AGGAAGGCRG NGACGASGTM RAGTMATCRK GGCSTKKAWR
     april5  GCAAGTAGAG AGGAAGGTGG GGATGACGTC AAGTCATCAT GGCCCTTACG
      Nov14  CAAAACGGGG AGGAAGGAGG GGATGACGTC CAGTCATCAT GTCCCTTATG

             1251                                                1300
      Nov12  CCTAGGGCGA CACACA.CGC TACAATGGCT GGTACAATGA GCAGCGAACG
    Nov12re  CCTAGGGCGA CACACA.CGC TACAATGGCT GGTACAATGA GCAGCGAACG
    april23  GGTtGGGSTA CACAcG.tGg TACAATGGTG GtGACAATGG G.........
      Nov16  GGTTGGGCTA CACACG.TGC TACAATGGTG GTGACAATGG G.........
    Sphingo  GGCTGGGCTA CACACG.TGC TACAATGGCG GTGACAGTGG GCAGCA.AGC
      A8AN3  CGCTGGGCTA CACACG.TGC TACAATGGCG ACTACAGTGG GCAGCA.ACT
       jan8  CGCTGGGCTA CACACG.tGC TACAATGGCG GTGACAGTGG GCAGCC.ACC
      jan14  GGCCGGGCTA CACACG.TGC TACAATGGTG GTGACAGAGG GAT.......
      Brady  GGCTGGGCTA CACACG.TGC TACAATGGCG GTGACAATGG GATGCGAAGG
    april21  GGcTGGGcTA CAcACG.TGS TACAATGGcG ACCATAAAGG ..........
       Nov8  GGCTGGGCTA CACACG.TGC TACAATGGCG ACCATAAAGG ..........
    april29  GGcTGGGcTA CACACG.TGC TACAATGGCG ACCATAAAGG ..........
      jan18  GGCTGGGCTA CACACG.TGY TACAAWGGCG ACCATAAAGG ..........
    april24  GGSTGGGCTA CACACG.TGC TACAATGGCG ACCATAAAGG ..........
    april15  GGgtGGGVTA CAcACG.tGC TACAATGGCG ACCATaAAGG ..........
    april26  GGSTGGGMTA CACAcG.TGg TACAATGGCG ACCATAAAGG ..........
        3M3  GGCTGGGCTA CACACGTTGC TACAATGGCG GTGACAATGC GCAGCCAACC
        6M3  GGCTGAGCTA CACACGTTGC TACAATGGCG GTGACAATGC GCAGCC.ACC
    april19  GGCTGGGCTA CACACG.TGT TACAATGGCG GTGACAATGc GCAGCC.ACC
      Nov22  .NGTGGGCTA CACACG.TGG TACAATGGCG GTGACAATGC GCAGCC.ACC
     april3  GGCTGGGCTA CACACG.TGC TACAATGGCG GTGACAATGC GCAGCC.ACC
    april18  GGCTGGGCTA CACACG.TGC TACAATGGCG GTGACAATGC GCAGCC.ACC
       jan1  GGCTGGGATA CACACG.TGC TACAATGGCG GTGACAATGC GCAGCC.ACT
    april12  GGCTGGGCTA CACACG.TGC TACAATGGCG GTGACAATGC GCAGCC.ACC
    april10  GGSTGGGATA CACASG.TGG TACAAWGGcG GtGACAATG▌ GCaGCG.aGG
    april20  GGCTGGGVTA CACACG.tGS TACAATGGCG GTGACAATG▌ GCAGCG.AGG
      Hypho  GGCTGGGCTA CACACG.TGC TACAATGGCG GTGACAATG▌ GCAGCA.ACT
      US353  GGCTGGGCTA CACACG.TGC TACAATGGCG GTGACAATG▌ GCAGCA.ACA
       jan6  GGCTGGGCTA CACACG.TGC TACAATGGCG ▌TGACA▌TG▌ GCAGCT.ACA
       Nov3  GGNTGGGCNA CACACG.TGN TACAATGGTG GTGACAGTGG GCAGCG.AGA
  Zoogloea1  GGCTGGGCTA CACACG.TGC TACAATGGTG GTGACAGTGG GCAGCG.AGA
    april25  GGTTGGGcTA CACACG.TGC TACAATGGTG GTGACAATGG GGAGCG.AGG
       Nov2  GGTCGGGCNA CACACG.TAN TACAATGGCA GTGACAATGG GTTGCA.NAG
      jan20  TCCAGGGCTA CACACG.TGC TACAATGGTC GGTACAAAGC GCTGCAAACC
Polyangium  TCCAGGGCTA CACACG.TGC TACAATGGTC GGTACAAACG GTCGCGAACC
    april22  GCCTGGGctA CAcACG.TAC TACAAtGGCT GgtACAACGG GAaGCGAAGc
   Bacillus  ACCTGGGCTA CACACG.TAC TACAATGGCC GGTACAACGG GAAGCGAAGT
      Lacto  ACCTGGGCTA CACACG.TGC TACAATGGAC GGTACAACGA GTTGCGAAAC
Leptothrix  GGTAGGGCTA CACACG.TCA TACAATGGCC GGTACAGAGG GCTGCCAACC
Rubrivivax  GGTAGGGCTA CACACG.TCA TACAATGGCC GGTACAGAGG GCTGCCAACC
    Sphaero  GGTAGGGCTA CACACG.TCA TACAATGGCC GGTACAGAGG GCTGCCAACC
    april17  GGTGGGGCKA CACACG.TCR TACAATGGCC GGTACAAAGG GTAGCCAACC
       jan3  GGTGGGGCTA CACACG.TCA TACAATGGCC GGTACAAAGG GTAGCCAACC
    april30  CGCAGGGCTT CACACG.TCA TACAATGGTC GGTACAGAGG GTCGCCAACC
      jan15  CGCAGGGCTT CACACG.TCA TACAATGGTC GGTACAGAGG GTMGCCAACC
      jan11  SGCAGGGCTT CACACG.TCA TACAATGGTC GGTACAGAGG GTCGCCAACC
     april6  GGTGGGGCTA CACACG.TCA TACAATGGCT GGTACAGAGG GTTGCCAACC
     april1  GGTAGGGCTA CACACG.TCA TACAATGGCC GGTACAGAGG GCTGCCAACC
       jan2  GGTGGGGCTA CACACG.TCA TACAATGGCC GGTACAAAGG GTAGCCAACC
       Nov7  GGTGGGGNTA CACACG.TCA TACAATGGCC GGTACAAAGG GTAGCCAACC
      jan23  GGTGGGGCTA CACACG.TCA TACAATGGCC GGTACAAAGG GTAGCCAACC
      Nov19  GGTGGGGCTA CACACG.TCA TACAATGGCC GGTACAAAGG GTcGCAAACC
       jan4  GGTGGGGCTA CACACG.TCA TACAATGGCT GGTACAGAGG GTTGCCAACC
```

```
      jan13  GGTGGGGCTA CACACG.TCA TACAATGGCT GGTACAGAGG GTTGCCAACC
      jan10  GGTGGGGMTA CACACG.TCA TACAATGGMT GGTACAGAGG GTTGCCAACC
       Nov4  GGTGGGGCTA CACACG.TCA TACAATGGCT GGTACAGAGG GTTGCCAACC
      jan12  GGTGGGGCTA CACACG.TCA TACAATGGCT GGTACAGAGG GTTGCCAACC
       jan7  GGTGGGGMTA CACACG.TCA TACAATGGCT GGTACAGAGG GTTGCCAACC
      jan21  GGTGGGGSTA CACACG.TCA TACAATGGCT GGTACAGAGG GTTGCCAACC
 Variovorax  GGTGGGGCTA CACACG.TCA TACAATGGCT GGTACAAAGG GTTGCCAACC
      april2  GGTGGGGCTA CACACG.TCA TACAATGGCT GGTACAGAGG GTTGCCAACC
  Zoogloea2  GGTAGGGCTT CACACG.TCA TACAATGGTA CATACAGAGG GCCGCCAACC
     april13  GGTAGGGCTT CACACG.TCA TACAATGGTC GGTACAGAGG GTTGCCAAGC
      april4  GCCTGGGSTA CACACG.TGC TACAATGGCT GGTACAGAGG GCTGCAAAAG
       jan5  GCMTGGGCcA CACACG.TGc TACAATGGMT GGTACAGAGG GTCGCGAAGC
 Legionella  GGTAGGGCTA CACACG.TGC TACAATGGGT GATACAGAGG GAAGCGAAGG
     april14  GGTAGGGSTA CACACG.TGC TACAATGGGG cGTACAGAGA GTCGCGAACC
       Nov6  CCCAGGGCTA CACACG.TGC TACAATGGAG TGGACAAAGG GCTGCAACAC
       Nov9  CCCAGGGCTA CACACG.TGC TACAATGGAG TGGACAAAGG GCTGCAACAC
       Nov5  CCCAGGGATA CACACG.TGC NACAATGGAG TGGANAAAGG GNTGCAACAC
     april11  TCCAGGGCTA CACACG.TGC TACAATGGCC GGTACAATGG GCAGCCACAT
     april16  TSCAGGGMTA CACACG.TGG tACAATGGCC GgWACAaTGG GCAGCCACaT
      jan19  TCCAGGGCTA CACACG.TGC TACAATGGCC GGTACAATGG GCAGCCACAT
      Nov17  TCCAGGGCTA CACACG.TGC TACAATGGCC GGTACAATGG GCAGCCACAT
  Cytophaga  TCCGGGGCTA CACACG.TGC TACAATGGAT GGTACAGAGG GCAGCAAGCT
Flavobacter  TCCGGGGCTA CACACG.TGC TACAATGGTC GGTACAGCGG GCAGCTACAC
     april31  CTTTGGGCTA CACACA.TGC TACAATGGCG GATACAATGG GTTGCCAAAC
       jan9  CTTTGGGCTA CACACA.TGC TACAATGGCG GATACAATGG GTTGCCAAAC
     april27  CCCAGGGcTA CACAcG.TGC TACAATGGCG TcTACAAAGT GTTGCCAGTC
      Nov18  TVCRGGGCWG CACACG.TGG TACAATGCGG CGTACAAAGG GTTGCCAACC
      april5  TCCGGGGSTA CACAMG.TGA TACAATGGAT GGCACAGTGG G...CGAGGC
      Nov14  TCCAGGGCTA CACA.GACAC TACAATGGCG ATAACAATGG GTTGCCAAGC

             1301                                                1350
      Nov12  GGTGACCTGG AGCGAATNTC ATCAAAGCCA GTCGTAGTTC AGATTGTAGG
    Nov12re  GGTGACCTGG AGCGAATNTC ATCAAAGCCA GTCGTAGTTC AGATTGTAGG
     april23  .......... ...TTaATCC CCAAAAGCC. ATCTCAGTTc GGATTGGGGT
      Nov16  .......... ...TTAATCC CCAAAAGCC. ATCTCAGTTC GGATTGGGGT
     Sphingo  ACGCGAGTGT GAGCTAATCT CCAAAAGCC. GTCTCAGTTC GGATTGTTCT
      A8AN3  CCGCGAGGGG AAGCTAATCT CCAAAAGTC. GTCTCAGTTC GGATTGTTCT
       jan8  TCGCGAGAGG GAGCTAATCC CAAAAAGCC. GTCTCAGTTC GGATTGTTCT
      jan14  .......... .....AATCC CTAAAAGCC. ATCTCAGTTC AGATCGGGGT
      Brady  .GGCAACCCT TCGCAAATCT CAAAAAGCC. GTCTCAGTTC GGATTGGGCT
     april21  .......... ..TTtAATCC CCAAAAGTc. GTCTCAGTTC AGaTTGTCCT
       Nov8  .......... ..TTTAATCC CCAAAAGTC. GTCTCAGTTC AGATTGTCCT
     april29  .......... ..TTTAATCC CCAAAAGTC. GTCTCAGTTC AGATTGTCCT
      jan18  .......... ..TTTAATCC CCAAAAGTS. GTCTCAGTTC AGATTGTCCT
     april24  .......... ..TTTAATCC CCAAAAGTC. GTCTCAGTTC AGATTGTCCT
     april15  .......... ..ttTAATCC CCAAAAGTg. GTgTCAGTTC AGAWTGTCCT
     april26  .......... ..TTTAATCC CCAAAAGTc. GTCTCAGTTC AGATTGTCCT
         3M3  TAGTAATAGG GAGCTAATCG CAAAAAGCC. GTCTCAGTTC AGATTGAGGT
         6M3  TAGTTAATAG GGGCTAATCG CAAAAAGCC. GTCTCAGTTC AGATTGAGGT
     april19  TCGCGAGAGG GAGcTAATCG CAAAAAGCC. GTCTCAGTTC GGATtGGGCT
      Nov22  TCGCGAGAGG GAGNTAATCG CAAAAAGCC. GTCTCAGTTC GGATTGGGCT
      april3  TAGCAATAGG GAGCTAATCG CAAAAAGCC. GTCTCAGTTC AGATTGGAGT
     april18  TAGCAATAGG GAGcTAaTCG CAAAAAGCC. GTCTCAGTTC AGATTGGAGT
       jan1  CAGCAATGAG GAGCTAATCG CAAAAAGCC. GTCTCAGTTC GGATTGGGGT
     april12  TAGCAATAGG GAGCTAATCG CAAAAAGCC. GTCTCAGTTC AGATTGGAGT
     april10  cgGSAATGCC RAGMTRATST CAAAAaGCC. GTMTCAGTTS GGATKGGGCT
     april20  CAGCAATGCC AAGCTAATCT CAAAAAGCC. GTCTCAGTTC GGATTGGGcT
       Hypho  CAGCAATGGG AAGCTAATCT CAAAAAGCC. GTCTCAGTTC GGATTGGGCT
       US353  CAGCAATGTG AAGCTAATCT CAAAAAGCC. GTCTCAGTTC GGATTGGGCT
       jan6  CAGCAATGTG ATGCTAATCT CTAAAAGCC. GTCTCAGTTC GGATTGGGGT
```

```
      Nov3  CCGNGAGGTC GAGCTAATCT CCAAAAGCC. ATCTCAGTTC GGATTGCACT
 Zoogloea1  CAGCGATGTC GAGCTAATCT CCAAAAGCC. ATCTCAGTTC GGATTGCACT
   april25  CCGCGAGGCC AAGCCAATCT CAAAAAGCC. ATCTCAGTTC GGATTGCACT
      Nov2  TCGCAAGGCN GAGCTAATCC TAAAAAANT. GCCTCAGTTC GGATTGTCCT
     jan20  CGCGAGGGGG AGCCAATCG. CAAAAAACCG ACCTCAGTAC AGATAAGAGT
Polyangium  CGCGAGGGGG TGCTAATCC. GAAAAAACCG GCCTCAGTAC GGATAAGAGT
   april22  TGCGAAGCGG AGCGAATCTT AAG.AATCCA GTCTCAGTTC GGATTGCAGG
  Bacillus  CGCGAGATGG AGCCAATCCT AAGAAAGCCG GTCTCAGTTC GGATTGCAGG
     Lacto  CGCGAGGTCA AGCTAATC.T CTTAAAGCCG TTCTCAGTTC GGATTGCAGG
Leptothrix  CGCGAGGGGG AGCCAATCC. CAGAAAACCG GTCGTAGTCC GGATCGCAGT
Rubrivivax  CGCGAGGGGG AGCCAATCA. CAGAAAACCG GTCGTAGTCC GGATCGCAGT
   Sphaero  CGCGAGGGGG AGCCAATCC. CAGAAAACCG GTCGTAGTCC GGATCGCAGT
   april17  CGCGAGGGGG AGCCAATCC. CACAAAGCCG GTCGTAGTCC GGATSGCAGT
      jan3  CGCGAGGGGG AGCCAATCC. CACAAAGCCG GTCGTAGTCC GGATCGCAGT
   april30  CGCGAGGGGG AGCTAATCC. CATAAAGCCA GTCGTAGTCC GGATCGCAGT
     jan15  CGCGAGGGGG AGCTAATCC. CATAAAGCCA GTCGTAGTCC GGATCGCAGT
     jan11  CGCGAGGGGG AGCTAATCC. CATAAAGCCA GTCGTAGTCC GGATCGCAGT
    april6  CGCGAGGGGG AGCTAATCC. CATAAAGCCA GTCGTAGTCC GGATCGCAGT
    april1  CGCGAGGGGG AGCTAATCT. CAGAAAACCG GTCGTAGTCC GGATCGCAGT
      jan2  CGCGAGGGGG AGCCAATCC. CACAAAGCCG GTCGTAGTCC GGATCGCAGT
      Nov7  CGCGAGGGGG AGCCAATCC. CACAAAGCCG GTCGTAGTCC GGATCGCAGT
     jan23  CGCGAGGGGG AGCCAATCC. CACAAAGCCG GTCGTAGTCC GGATCGCAGT
     Nov19  CGCGAGGGGG AGCCAATCC. ATCAAAGCCG GTcGTAGTCC GGATCGCAGT
      jan4  CGCGAGGGGG AGCCAATCC. CATAAAACCA GTCGTAGTCC GGATCGCAGT
     jan13  CGCGAGGGGG AGCTAATCC. CATAAAACCA GTCGTAGTCC GGATCGCAGT
     jan10  CGCGAGGGGG AGCCAATCC. CATAAAACCA GTBGTAGTCC GGATCGCAGT
      Nov4  CGCGAGGGGG AGCCAATCC. CATAAAACCA GTCGTAGTCC GGATCGCAGT
     jan12  CGCGAGGGGG AGCCAATCC. CATAAAACCA GTCGTAGTCC GGATCGCAGT
      jan7  CGCGAGGGGG AGCCAATCC. CATAAAACCA GTCGTAGTCC GGATCGCAGT
     jan21  CGCGAGGGGG AGCCAATCC. CATAAAACCA GTCGTAGTCC GGATCGCAGT
Variovorax  CGCGAGGGGG AGCTAATCC. CATAAAACCA GTCGTAGTCC GGATCGCAGT
   april2  CGCGAGGGGG AGCTAATCC. CATAAAGCCA GTCGTAGTCC GGATCGCAGT
 Zoogloea2  CGCGAGGGGG AGCTAATCC. CAGAAAGTGT ATCGTAGTCC GGATTGTAGT
   april13  CGCGAGGTGG AGCGAATCT. CACAAAGCCG ATCGTAGTCC GGATTGCAGT
   april4   CGCGAGCTGA AGCTAATCC. TACAAAGCCA GTYGTAGTCC GGATTGGAGT
      jan5  CGCGAGGTGG AGCTAATCC. CAAAAAACCG GTCGTAGTCC GGATCGGAGT
Legionella  GGTGACCTGG AGCTAATCT. TAGAAAGTCG CTCGTAGTCC GGATTGGAGT
   april14  CGCGAGGGGG AGCCAATCT. cGTAAAGCGT CTYGTAGTCC GGATTGGGGT
      Nov6  AGTGATGTGA AGCTAATCCC A.AAAACCAC TTCTCAGTTC AGATTGGAGT
      Nov9  AGTGATGTGA AGCTAATCCC A.AAAACCAC TTCTCAGTTC AGATTGGAGT
      Nov5  AGNGATGTGA AGNTAATCCC A.AAAACCAN TTCTCAGTTC AGATTGGAGT
   april11  GGCAACATGG AGCTAATCTT T.AAAA.CCG GTCTCAGTTC GGATTGAGGT
   april16  GGCAACATGG AGcTAATCTT t.AAAA.CCG GTCTCAGTTC GGATTGAGGT
     jan19  GGCAACATGG AGCTAATCTT T.AAAA.CCG GTCTCAGTTC GGATTGAGGT
     Nov17  GGCAACATGG AGCTAATCTT T.AAAA.CCG GTCTCAGTTC GGATTGAGGT
 Cytophaga  GGCAACAGCA AGCGAATCTC A.AAAAGCCA TTCACAGTTC GGATAGAGGT
Flavobacter AGTAATGTGA TGCCAATCTC T.GAAAGCCG ATCACAGTTC GGATTGAGGT
   april31  TGCGAAGTGG AGCCAATCCC ATCAAAGTCC GTCTCAGTTC GGATTGCAGG
      jan9  TGCGAAGTGG AGCCAATCCC ATCAAAGTCC GTCTCAGTTC GGATTGCAGG
   april27  AGTGATGACG AGCCAATCAC A.AAAAGGAC GTCTCAGTTC GGATTGTAGG
     Nov18  CGTGAGGGGG AACCAATCCC A.AAAAGCGC CGCTCAGTTC GGATTGCAGG
    april5  CGCGAGGCCA AGGTAATCCC C..AAAACCA TTCTCAGTTC GGATTGGAGT
     Nov14  CGCAAGGCGG AGCTAATCCC ATCAAAGATC GTCTCAGTTC AGATTGCAGG

            1351                                                1400
     Nov12  CTGCAACCCG CCTACATGAA GCCGGAGTTG CTAGTAAACG CAGGTCAGCC
   Nov12re  CTGCAACCCG CCTACATGAA GCCGGAGTTG CTAGTAAACG CAGGTCAGCC
   april23  gTGCAACTcG ACCCCaTGAA GTCGGAATCG aTaGTAATCG CGTAACAGCA
     Nov16  CTGCAACTCG ACCCCATGAA GTCGGAATCG CTAGTAATCG CGTAACAGCA
   Sphingo  CTGCAACTCG AGAGCATGAA GGCGGAATCG CTAGTAATCG CGGATCAGCA
```

```
      A8AN3  CTGCAACTCG AGAGCATGAA GGCGGAATCG CTAGTAATCG CGGATCAGCA
       jan8  CTGCAACTCG AGAGCATGAA GGCGGAATCG cTAGTAATCG CGGATCAGCA
      jan14  CTGCAACTCG ACCCCGTGAA GGTGGAATCG CTAGTAATCG TGGATCAGCA
      Brady  CTGCAACTCG AGCCCATGAA GTTGGAATCG CTAGTAATCG TGGATCAGCA
     april21 CtGCAACTCG AGGGCAtGAA GTCGGAATCG NTAGTAATCG CAGATCAGCA
       Nov8  CTGCAACTCG AGGGCATGAA GTCGGAATCG NTAGTAATCG CAGATCAGCA
     april29 CTGCAACTCG AGGGCATGAA GTCGGAATCG CTAGTAATCG CAGATCAGCA
      jan18  CTGCAACTCG AGGGCATGAA GTCGGAATCG YTAGTAATCG CAGATCAGCA
     april24 CTGCAACTMG AGGGCATGAA GTCGGAATCG CTAGTAATCG CAGATCAGCA
     april15 STGCAACTMG AGGGCATGAA GTCGGAATCG TTaGTAATCG CAGATCAGCA
     april26 cTGCAACTcG AGGGCATGAA GTCGGAATCG NTAGTAATCG CAGATCAGCA
        3M3  CTGCAACTCG ACCTCATGAA GTCGGAATCG CTAGTAATCG CGCATCAGCA
        6M3  CTGCAACTCG ACCTCATGAA GTCGGAATCG CTAGTAATCG CGCATCAGCA
     april19 CTGCAACTCG AGCCCATGAA GTTGGAATCG YTAGTAATCG CGCATCAGCA
      Nov22  CTGCAACTCG AGCCCATGAA GTTGGAATCG NTAGTAATCG CGCATCAGCA
      april3 CTGCAACTCG ACTCCATGAA GTCGGAATCG CTAGTAATCG CGCATCAGCA
     april18 CTGCAACTCG ACTCCATGAA GTCGGAATCG CTAGTAATCG CGCATCAGCA
        jan1  CTGCAACTCG ACCCCATGAA GTCGGAATCG CTAGTAATCG CGCATCAGCA
     april12 CTGCAACTCG ACTCCATGAA GTCGGAATCG CTAGTAATCG CGCATCAGCA
     april10 YTGCAAGTCG AGCCCATGAA GTSGGAATCG GTAGTAATMG TGGATCaGCA
     april20 CTGCAACTCG AGCCCATGAA GTCGGAATCG CTAGTAATCG TGGATCAGCA
       Hypho  CTGCAACTCG AGCCCATGAA GTTGGAATCG CTAGTAATCG CGCATCAGCA
       US353  CTGCAACTCG AGCCCATGAA GTTGGAATCG CTAGTAATCG CGCATCAGCA
        jan6  CTGCAACTCG ACCCCATGAA GTCGGAATCG CTAGTAATCG CGCATCAGCA
        Nov3  CTGCAACTCG AGTGCATGAA GTTGGAATCG CTAGTAATCG TGGATCAGCA
  Zoogloea1   CTGCAACTCG AGTGCATGAA GTTGGAATCG CTAGTAATCG CGGATCAGCA
     april25  CTGCAACTcG AGTGCATGAA GTTGGAATCG cTAGTAATCG CGCATCAGCA
        Nov2  CTGCAACTCG AGGGCATGAA GNTGGAATCG CTAGTAATCG CGCAACAGCA
       jan20  STGCAACTCG ACTCCTTGAA GTCGGAATCG CTAGTAATCG CTGATCAGCA
  Polyangium  CTGCAACTCG ACTCTTTGAA GTTGGAATCG CTAGTAATCC CTGATCAGCA
     april22  cTGCAAcTCG CCTGCATGAG GTCGGAaTTG CTAGTAATCG CGGATCAGCA
    Bacillus  CTGCAACTCG CCTGCATGAA GTCGGAATTG CTAGTAATCG CGGATCAGCA
       Lacto  CTGCAACTCG CCTGCATGAA GTTGGAATCG CTAGTAATCG TGGATCAGCA
  Leptothrix  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGCT
  Rubrivivax  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGCT
     Sphaero  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGCT
     april17  STGCAACTgG ACTGCGTGAA GTCGGAATCG CTAGTAATCG TGGATCAGCA
        jan3  CTGCAACTCG AMTGCGTGAA GTCGGAATCG STAGTAATCG TGGATCAGCA
     april30  cTGCAACTcG AcTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGAA
       jan15  CTGCAAcTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGAA
       jan11  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGAA
      april6  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGAA
      april1  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGCT
        jan2  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG TGGATCAGCA
        Nov7  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG TGGATCAGCA
       jan23  CTGCAACTCG ACTGCGTGAA GTCGGAATCG GTAGTAATCG TGGATCAGCA
       Nov19  CTGCAACTNG ACTGCGTGAA GTCGGAATCG NTAGTANTCG TGGATCAGCA
        jan4  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGCA
       jan13  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGCA
       jan10  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGCA
        Nov4  CTGCAANTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGCA
       jan12  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGCA
        jan7  CTGCAACTCG AcTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGCA
       jan21  CTGCAACTCG ACTGCGTGAA GTCGGAATCG cTAGTAATCG CGGATCAGCA
  Variovorax  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG TGGATCAGAA
      april2  CTGCAACTCG ACTGCGTGAA GTCGGAATCG CTAGTAATCG CGGATCAGAA
   Zoogloea2  CTGCAACTCG ACTGCATGAA GTTGGAATCG CTAGTAATCG CGGATCAGCA
     april13  CTGCAACTCG ACTGCATGAA GTCGGAATCG CTAGTAATCG CGGATCAGCA
      april4  CTGCAACTCG ACTCCATGAA GTCGGAATCG CTAGTAATCG CGAATCAGAA
        jan5  cTGCAACTCG ACTCCGTGAA GTCGGAATCG CTAGTAATCG CGAATCAGAA
```

```
Legionella  CTGCAACTCG ACTCCATGAA GTCGGAATCG CTAGTAATCG CGAATCAGCA
   april14  CTGCAACTCG ACTCCATGAA GTTGGAATCG CTAGTAATCG CGAATCAGCA
      Nov6  CTGCAACTCG ACTCCATGAA GCTGGAATCG CTAGTAATCG TATATCAGCA
      Nov9  CTGCAACTCG ACTCCATGAA GCTGGAATCG CTAGTAATCG TATATCAGCA
      Nov5  CTGCAACTCG ACTCCATGAA GCTGGAATCG CTAGTAATCG TATATCAGCA
   april11  CTGCAACTCG ACCTCATGAA GCTGGATTCG CTAGTAATCG CAAATCAGCA
   april16  cTGCAACTCG ACCTCAtGAG GCTgGATTCG CTAGTAATCG CAAATCAGCA
     jan19  STGCAACTCG ACCTCATGAA GCTGGATTCG CTAGTAATCG CAAATCAGCA
     Nov17  STGCAACTCG ACCTCATGAA GCTGGATTCG CTAGTAATCG CAAATCAGCA
  Cytophaga CTGCAACTCG ACCTCTTGAA GTTGGATTCG CTAGTAATCG CGTATCAGCA
Flavobacter CTGCAACTCG ACCTCATGAA GTTGGATTCG CTAGTAATCG CGTATCAGCA
   april31  CTGCAACTCG CCTGCATGAA GTTGGAATCG CTAGTAATcG TGGATCAGCA
      jan9  CTGCAACTcG CCTGCATGAA GTTGGAATCG tTAGTAATCG TGGATCAGCA
   april27  CTGCAACTCG CCTGCATGAA GTTGGAATCG CTAGTAATcG TGGATCAGCA
     Nov18  CTGCAACTCG CCTGCATGAA GCTGGAATSG CTAGTAATCG CGGATCAGGT
     april5 CTGCAACTCG ACTCCATGAA GGTGGAATCG YTAGTAATCG CGGATCAGCA
     Nov14  CTGCAACTCG CCTGTATGAA GTTGGAATTG CTAGTAATCG GAAATCAGC.

            1401                                                1450
     Nov12  ATACTGCGTT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACGTC
   Nov12re  ATACTGCGTT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACGTC
   april23  .tGACGCGGT GAATAcGTTC CgGGGCCtTG TACACACCGC CSGTCACACC
     Nov16  .TGACGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
   Sphingo  .TGCCGCGGT GAATACGTTC CCAGGCCTTG TACACACCGC CCGTCACACC
     A8AN3  .TGCCGCGGT GAATACGTTC CCAGGCCTTG TACACACCGC CCGTCACACC
      jan8  .TGCCGCGGT GAATACGTTC CCAGGCCTTG TACACACCGC CCGTCACACC
     jan14  .TGCCACGGT GAATACGTTC CCGGGCSTTG TACACACCGC CCGTCACACC
     Brady  .CGCCACGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
   april21  .CGCTGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
      Nov8  .CGCTGCGGT GAATACGTTC CCGGGCCTNG TACACACCGC CCGTCACACC
   april29  .CGCTGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
     jan18  .CGcTGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
   april24  .CGCTGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
   april15  .CGCTGCGGT GAATAcGTTC CCGGGCCTtG TACACACCGC CCGTCACACC
   april26  .CGcTgCGGT GAATACGTTC CCGgGCCTTG TACACACCGC CCGTCACACC
       3M3  .TGGCGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
       6M3  .TGGCGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
   april19  .TGGCGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
     Nov22  .TGGCGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
    april3  .TGGCGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
   april18  .TGGCGCGGT GAATACGTTC CCGGGCCTtG TACACACCGC CCGTCACACC
      jan1  .CGGCGCGGT GAATACGTTC CCGGGCMTTG TACACACCGC CCGTCACACC
   april12  .TGGCGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
   april10  .CGCCacGNT GaATAcGTTC cSGGGCCttG tACACACCGC CCGTCACACC
   april20  .CGCCACGGT GaATACGTTC CCGGGCCTtg TACACACCGC CCGTCACACC
     Hypho  .TGGCGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
     US353  .TGGCGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
      jan6  .CGGCGCGGT GAATACGTTC CCGGGCCTTG CACACACCGC CCGTCACACC
      Nov3  .TGCCACGGT GAATACGTTC CCGGGCATNG TACACACCGC CCGTCACACC
 Zoogloea1  .TGCCGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
   april25  .TGGCGCGGT GAATACGTTC CCGGGCCTTG TATACACCGC CCGTCACACC
      Nov2  .TGGCGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACGCC
     jan20  .GGCAGCGGT GAATACGtTC CCGGGCCTtG TACACACCGC CCGTCACACC
Polyangium  .GGCAGGGGT GAATACGTTC CCGGGCNTTG TACACACCGC CCGTCACACC
   april22  .TGCCGCGGT GAATACGTTC CCGGGTcTTG TACACACCGC CCGTCACACC
  Bacillus  .TGCCGCGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
     Lacto  .TGCCACGGT GAATACG... .......... .......... ..........
Leptothrix  .TGCCGCGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
Rubrivivax  .TGCCGCGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
   Sphaero  .TGCCGCGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
```

```
april17  .TGTCACGGT GAATACGTTC CCGGGCCTtG TACACACCGC CCGTCACACC
   jan3  .TGTCACGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
april30  .TGTCGCGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
  jan15  .TGTCGCGGT GAATACGTTC CCGGGTCTTG TACACACCGC CcGTCACACC
  jan11  .TGTCGCGGT GAATACGtTC CCGSGTCTTG TACACACCGC CCGTCACACC
  april6 .TGTCGCGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
  april1 .TGCCGCGGT GAATACGTTC CCGGGTCTTG TACATACCGC CCGTCACACC
   jan2  .TGTCACGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
   Nov7  .TGTCACGGT GAATACGTTC CCGGGTCTCN TACACACCGC CCGTCACACC
  jan23  .TGTCACGGT GAATACGTTC CCGGGTCTTg TACACACCGC CCGTCACACC
  Nov19  .TGTCACGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
   jan4  .TGTCGCGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
  jan13  .TGTcGCGGT GAATACGTTC CCGgGTCTTG TACACACCGC CCGTCACACC
  jan10  .TGTCGCGGT GAATACGTTC CCGGGTCTWG TACACACCGC CCGTCACACC
   Nov4  .TGTCGCGGT GAATACGTTC CCGGGTNTTG TACACACCGC CCGTCACACC
  jan12  .TGTCGCGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
   jan7  .TGTcGCGGC GAATACGTTC CCGGGTcTTG TACACACCGC CCGTCACACC
  jan21  .TGTCGCGGT gaATACGTTC CCGGGTCTtG TACACACCGC CCGTCACACC
Variovorax .TGTCACGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
  april2 .TGTCGCGGC GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
Zoogloea2 .TGTCGCGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
 april13 .TGTCGCGGT GAATACGTTC CCGGGTCTTG TACACACCGC CCGTCACACC
 april4  .TGTCGCGGT GAATACGTTS CCGGSCCTTG TACACACCGC CCGTCACACC
   jan5  .TGTCGCGGW GAGTACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
Legionella .TGTCGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
 april14 TTGTCGCGGT GAATACGTTC CcGGGCCTTG TACACACCGC CCGTCACGTC
   Nov6  ATGATACGGT GAATACGTTC CCGGACCTTG CACACACCGC CCGTCAAGCC
   Nov9  ATGATACGGT GAATACGTTC CCGGACCTTG CACACACCGC CCGTCAAGCC
   Nov5  ATGATACGGT GAATACGTTC CCGGACCTTG CACACACCGC CCGTCAAGCC
 april11 ATGTTGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCAAGCC
 april16 ATGTTGCGGT GAATACGTTC CCGGGCCTtG TACACACCGC CCGTCAAGCC
  jan19  ATgTTGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCAAGCC
  Nov17  ATGTTGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCAAGCC
Cytophaga ATGACGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCAAGCC
Flavobacter ATGACGCGGT GAATACGTTC CCGGGCNTTG TACACACCGC CCGTCAAGCC
 april31 CTGCCACGGT GAATACGTTC CCGGGCCTTG CACACACCGC CCGTCACACC
   jan9  CTGCCACGGT GAATACGTTC CCGGGCCTTG CACACACCGC CCGTCACACC
 april27 CTGCCACGGT GAATACGTTC CCGGGCCTTG CACACACCGC CCGTCACACC
  Nov18  ATGCCGCGGT GAATGTGTTC CTGAGCVTTG TACACACCGC CCGTCAAGCC
  april5 .CGCCGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCAAGCC
  Nov14  GTGTTCCGGT GAATACGTTC TCGGGTCTTG TACACACCGC CCGTCAGGGC

         1451                                                1500
  Nov12  ATGGGAGTTG GTAACACCTG AAGTCGGTGA CTGAACCGCA AGGACAGAGC
Nov12re  ATGGGAGTTG GTAACACCTG AAGTCGGTGA CTGAACCGCA AGGACAGAGC
 april23 ATGGGAGTTG GRWTCACCCG AAGG.SGTGC GCCAACCTCG CAAGAGGGGG
  Nov16  ATGGGAGTTG GGTTCACCCG AAGGCGGTGC GCCAACCTCG CAAGAGGGGG
Sphingo  ATGGGAGTTG GATTCACTCG AAGGCGTTGA GCTAACCCGC AA..GGGAGG
   A8AN3 ATGGGAGTTG GATTCACCCG AAGGCGCTGC GCTAACTCGC AA..GAGAGG
    jan8 ATGGGAGTTG GATTCAcTCG AAGGCGTTGA GCTAACCCGC AA..GGGAGG
   jan14 ATGGGAGTTG GTTTTACCCG AAGGCGMTGC GCTAACCCGC AA..GGGAGG
   Brady ATGGGAGTTG GCTTTACCTG AAGACGGTGC GCTAACCCGC AA..GGGAGG
 april21 ATGGGaGTTG .TTNTACCCG AAGGCAGTGC GCCAACC..G CA..AGGAGG
    Nov8 ATGGGAGTTG .TTATACCCG AAGGCAGTGC GCCAACT..G CA..AGGAGG
 april29 ATGGGAGTTG .TTCTACCCG AAGGCAGTGC GCCAACC..G CA..AGGAGG
   jan18 ATGGGAGTTG .TTATACCCG AAGGCAGTGC GCCAACC..G CA..AGGAGG
 april24 ATGGGAGTTG ATTCTACCCG AAGGCAGTGC GCCAACC..G CA..AGGAGG
 april15 ATGGGAGTTG GtTNTACCCG AAGGCAGTGC GCCAACC..G CA..AGGAGG
 april26 ATGGGAGttG GTTYTACCCG AAGGCAGTGC GCCAACC..G CA..AGGAGG
     3M3 ATGGGAGTTG GTCTTACCCT AAAACGGTGC GCTAACC..G CA..AGGAGG
```

```
          6M3  ATGGGAGTTG GTCTTACCCT AAAACGGTGC GCTAACC..G CA..AGGAGG
      april19  ATGGGAGTTG GCTTTACCCG AAGGCGGTGC GCTAACC..G CA..AGGAGG
        Nov22  ATGGGAGTTG GCTTTACCCG AAGACGGTGC GCTAACC..G CA..AGGAGG
       april3  ATGGGAGTTG GTCATACCCT AAAACGGTGC GCTAACC..G CA..AGGAGG
      april18  ATGGGAGTTG GTCATACCCT AAAACGGTGC GCTAACC..G CA..AGGAGG
         jan1  ATGGGAGTTG GCTTTACCCG AAGGTAGTGC GCTAACC..G CA..AGGAGG
      april12  ATGGGAGTTG GTTTTACCCG AAGACGGTGC GCTAACCCGC AA..GGGAGG
      april10  ATGGGAGTTG GMTTTACCCG AAGACGGTGC GCTAACCAGC AA..TGGAGG
      april20  ATGGGAGTTG GcttTACCCG aAGACGGTGC GCTAACCAGC AA..TGGAGG
        Hypho  ATGGGAGTTG GTTTTACCCG AAGACGGTGC GCTAACC..G CA..AGGAGG
         US353 ATGGGAGTTG GTTTTACCCG AAGACGGTGC GCTAACC..G CA..AGGAGG
         jan6  ATGGGAGTTG GTCATACCCT AAAACGGTGC GCTAACC..G CA..AGGAGG
         Nov3  ATGGGAGTTG GCTTTACCCG AAGGCGCTGT GCTAACC..G CA..AGGAGG
   Zoogloea1   ATGGGAGTTG GTTTTACCCG AAGGCGATGC GCTAACC..G CA..AGGAGG
      april25  ATGGGAGWTG GTTTTACCCG AAGCCGGTGC GCGAACC..G CA..AGGACG
         Nov2  ATGGGAGTTG GTTTTGCCCG AAGCCGGTGC GCTAACC..G CA..AGGAAG
        jan20  ATGGGAGTCA TTWGCTCCAG AAGCGCCTGC GCCAACCGTA ....AGGAGG
   Polyangium  ATGGGAGTCG GTTGCTCCAG AAGTGGCTGA GCCAACCCGC ....AAGGGA
      april22  ACGAGAGTTT ACAACACCCG AAGTCGGTGA GGTAACCGCA ....AGGAGC
     Bacillus  ACGAGAGTTT ACAACACCCG AAGTCGGTGG GGTAACCGCA ....AGGAGC
        Lacto  .......... .......... .......... .......... ..........
   Leptothrix  ATGGGACGGG GTTCCGCCAG AAGTAGTTAG CCTAACCGC. ..........
   Rubrivivax  ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGAGG
      Sphaero  ATGGGAGCGG GTCTCGCCAG AAGTAGTTAG CCTAACCGC. ..........
      april17  ATGGGAGTGG GtTGCTCCAG AAGTAGCTAG TCTAACCGCA ....AGGGGG
         jan3  ATGGGAGTGG GTTGCTCCAG AAGTAGCTAG TCTAACCGCA ....AGGGGG
      april30  ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGAGG
        jan15  ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CcTAACCGCA ....AGGAGG
        jan11  ATGGGAGcGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGAGG
       april6  ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGAGG
       april1  ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGAGG
         jan2  ATGGGAGcGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGGGG
         Nov7  ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGGGG
        jan23  ATGGGAGCGG GWTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGGGG
        Nov19  ATGGGAGCGG GTCTCGCCAG AAGTAGTTAG CCTAACCGC. ..........
         jan4  ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGAGG
        jan13  ATGGGAGcGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGAGG
        jan10  ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGAGG
         Nov4  ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGAGG
        jan12  ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGAGG
         jan7  ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGAGG
        jan21  ATGGGAGC.G GtTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGAGG
   Variovorax  ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CTTAACCGCA ....AGGAGG
       april2  ATGGGAGCGG GTTCTGCCAG AAGTAGTTAG CCTAACCGCA ....AGGAGG
    Zoogloea2  ATGGGAGCGG GTTTTACCAG AAGTAGGTAG CTTCACCGCA ....AGGAGG
      april13  ATGGGAGTGG GTTTCACCAG AAGCAGGTAG CCTAACCGCA ....AGGAGG
      april4   ATGGGAGWRG GTTGTACCAG AAGTGGGTAG CCTAACCGCA ....AGGA.G
         jan5  ATGGGAGTGG GTWGCAAAAG AAGTGGGTAG TCTAACSTTC ....GGGAGG
   Legionella  ATGGGAGTGG GCTGCACCAG AAGTAGATAG TCTAACCTTC ....GGGGGG
      april14  ATGGGAGTGG ATGGTACCAG AAGCGGGTAG CTGAACCGAA ....AGGAAG
         Nov6  ATGGAAGCCG GGTGTACCTA AAGTCGTTAA CCGTAAGGAG ACGCCTAGGT
         Nov9  ATGGAAGCCG GGTGTACCTA AAGTCGTTAA CCGTAAGGAG ACGCCTAGGT
         Nov5  ATGGAAGCCG GGTGTACCTA AAGTCGTTAA CCGTAAGGAG ACGCCTAGGT
      april11  ATGGAAGTCG GGAGTACCTA AAGTCCATAA CCGCAAGGA. TGGCCTAGGT
      april16  ATGGAAGTCG GGAGTACcTA AAGTCCATAA CCGCAAGGA. TGGCCTAGGT
        jan19  ATRGAAGTCG GGAGTACCTA AGGTCCATAA CCGCAAGGAG TGGCCTAGGT
        Nov17  ATGGAAGTCG GGAGTACCTA AAGTCCATAA CCGCAAGGAG TGGCCTAGGT
   Cytophaga   ATGGAAGTTG GGGGTACCTA AAGTACGTAA CCGCAAGGAG CGTCCTAGGG
  Flavobacter  ATGAAAGTTG GGGGTGCCTA AAGCATGTAA CCGCAAGGAG CGTGTTAGGG
      april31  ACGAAAGTC. GTTCTACCCG AAGTGGTTGA GCTAACCCGC AAGGGAGGCA
```

```
      jan9  ACGAAAGTCG GTTCTACCCG AAGTGGTTGA GCTAACCCGC AAGGGAGGCA
   april27  ACGAAAGTC. GTTcTACCCG AAGTGGTTGA GCTAACCCGC AAGGGAGGCA
     Nov18  ACGAAAGGGG GGGGCATCCC AAGTCGCCGA GCCAACCGCA AGGGGGCAGT
    april5  ATGGGAGCCG GGGGCGCCCG AAGCCGGTAG TGATACCGTC GAAGGCGAAA
     Nov14  ATGGGAGTTG CTCATACTCG AAGTCCTACG TTTATCGTGG CCGAAAGTTN

            1501                                                1550
     Nov12  TGCCTCAGGG TGGGGCTGTG ACTGGACGAA ..........  ..........
   Nov12re  TGCCTCAGGG TGGGGCTGTG ACTGGACGAA ..........  ..........
   april23  CAGCCGACCA CGTGGMTC.. ..........  ..........  ..........
     Nov16  CAGCCGACCA CGTGGGCTCA GCGACTGGGK GAA.......  ..........
   Sphingo  CAGGCGACCA CAGTGGGTTT AGCGACTGGG GTGAAGTCGT AACAAGGTAG
     A8AN3  CAGGCGACCA CGGTGGGTTT AGCGACTGGG GTTGAATCGT AACAAGGTAG
      jan8  CAGGCGACCA CAGT.GGTTT AGCGACT...  ..........  ..........
     jan14  CAGGCGACCA CGGTA.GGTC AGCGA.....  ..........  ..........
     Brady  CAGCCGGCCA CGGTAGGGTC AGCGACTGGG GTGAAGTCGT AACAAGGTAG
   april21  CAGCTGACCA CGGTAGGTCA TGAKBGNNGG C.........  ..........
      Nov8  CAGCTGACCA CGGTAGGTCN ATGACTG...  ..........  ..........
   april29  CAGCTGACCA CGGTAGGTCC AATGANT...  ..........  ..........
     jan18  CAGCWGACCA CGGTGAGGT. ..........  ..........  ..........
   april24  CAGCTGACCA CGGTAGGTCC ATGANT....  ..........  ..........
   april15  CAGCTGACCA CgGTAGGTCA WGA.......  ..........  ..........
   april26  CAGCTGACCA CGGTAGGTCC AATGA.....  ..........  ..........
        3M3  CAGCCGGCCA CGGTAAGGTC AGCGACTGGG GTGAAGTCGT AACAAGGTAG
        6M3  CAGCCGGCCA CGGTAAGGTC AGCGACTGGG GTGAAGTCGT AACAAGGTAA
   april19  CAGCCGGCCA CGGTAAGTCA GCGA......  ..........  ..........
     Nov22  CAGCCGGCCA CGGTAAGTCA GCGATT....  ..........  ..........
    april3  CAGCCGGCCA CGGTATGTCN AGCGACTGGG KGMA......  ..........
   april18  CAGCCGGCCA CGGTATGTCC AGCGACTGGG N.........  ..........
      jan1  CAGCTAACCA CGGTAAGTCA GVG.......  ..........  ..........
   april12  CAGCCGGCCA CGGTAAGTCC AGCGACTGGG NGC.......  ..........
   april10  CAGCCGGCCA CGGTAAGTC. ..........  ..........  ..........
   april20  CAGCCGGCCA CGGTAAGTCG AGCGA.....  ..........  ..........
     Hypho  CAGCCGGCCA CGGTNAGGTC AGCGACTGGG G.........  ..........
     US353  CAGCCGGCCA CGGTAAGGTC AGCGACTGGG GTGAAGTCGT AACAAGGTAG
      jan6  CAGCCGGCCA CGGTATGGTC AGCGACTGGG ..........  ..........
      Nov3  CAGGCGACCA CGGTA.GGTC AGCGACTGGG G.........  ..........
 Zoogloea1  CAGTCGACCA CGGTAGGGTC AGCGACTGGG GTGAAGTCGT AACAAGGTAG
   april25  CAGCCGACCA CGGTAAGTCN AGCGACTGGG N.........  ..........
      Nov2  CAGCCGACCA CNGTACG.AT AGCGAGT...  ..........  ..........
     jan20  CAGGTTCCAA GGATGAATGT AAST......  ..........  ..........
Polyangium  GGCAGGCCCC CAAGGAGTGG CTGGTAACTG GGG.......  ..........
   april22  CAGCCGCCGA AGGTGGGGTA GT........  ..........  ..........
  Bacillus  CAGCCGCCGA AGGTGG....  ..........  ..........  ..........
     Lacto  ..........  ..........  ..........  ..........  ..........
Leptothrix  ..........  ..........  ..........  ..........  ..........
Rubrivivax  GCGATTACCA CGGCAGGGTT CGTGACTGGG GTG.......  ..........
   Sphaero  ..........  ..........  ..........  ..........  ..........
   april17  ACG.TTACCA CGGAGTGaTc ATGACN....  ..........  ..........
      jan3  ACG.TTACCA CGGAGTGWTC ATGA......  ..........  ..........
   april30  GCGATTACCA CGGCAGGTTC GTGACT....  ..........  ..........
     jan15  GCGATTACCA CGGCRAGGTT ..........  ..........  ..........
     jan11  GCGATTACCA CGGCAGGT..  ..........  ..........  ..........
    april6  GCGATTACCA CGGCAGGTTC GTGANTGGGK GN........  ..........
    april1  GCGATTACCA CGGCAGGTTC GTGACTGGGK GVA.......  ..........
      jan2  GCGATTACCA CGGCAGGTTC GTGACTGG..  ..........  ..........
      Nov7  GCGATTACCA CGGCAGGTTC GTGACT....  ..........  ..........
     jan23  GCGATTACCA CGGCAGGTTC GWGACT....  ..........  ..........
     Nov19  ..........  ..........  ..........  ..........  ..........
      jan4  GCGATTACCA CGGCA.GGTT CGTGACT...  ..........  ..........
```

```
      jan13  GCGATTACCA CGGCA.GGTT ..........  .......... ..........
      jan10  GCGATTACCA CGGCA.GGTT CGTGA.....  .......... ..........
       Nov4  GCGATTACCA CGGCA.GGTT CGTGANT...  .......... ..........
      jan12  GCGATTACCA CGGCA.GGTT CGWGANT...  .......... ..........
       jan7  GCGATTACCA CGGCA.GGTT ..........  .......... ..........
      jan21  GCGATTACCA CGGCA.GGTT CGTGACT...  .......... ..........
 Variovorax  GCGATTACCA CGGCAGGGTT CGTGACTGGG GTG.......  ..........
      april2 GCG.TTACCA CGGCAGGKTC GTGACTGGGK GMA.......  ..........
  Zoogloea2  GCGCTTACCA CGGTAGGATT CGTGACTGGG GTGAAGTCGT AACAAGGTAG
     april13 GCGCTTACCA CGGTGAGATC ATGA......  .......... ..........
     april4  GCGCTCACCA CGGTATGATT CWTGACTGGG KGVA......  ..........
       jan5  GCGCTCACCA CTTTGTGATC ATG.......  .......... ..........
 Legionella  ACGTTTACCA CGGTGTGGTT CATGACTGGG GTG.......  ..........
     april14 GCGCTTGCCA CGGTATGATT CATGA.....  .......... ..........
       Nov6  AAAACTGTGA CTGGGT....  .......... .......... ..........
       Nov9  AAAACTGTGA TGGGT.....  .......... .......... ..........
       Nov5  AAAACTGGTG AT........  .......... .......... ..........
     april11 AAAACTGTGA TGGGNNMA..  .......... .......... ..........
     april16 AAAAStGKGA SN........  .......... .......... ..........
      jan19  AAAACTGTGA NT........  .......... .......... ..........
      Nov17  AAAACTTGAT GACT......  .......... .......... ..........
  Cytophaga  TAAAACCGAT AACTGGGGCT AAGTCGTAAC AAGGTAGCCG TACCGGAAGG
Flavobacter  CAAAACCGAT AATTGGGGC. ..........  .......... ..........
     april31 GGCT.CCTAA GGTAGGGCTG GTAATT....  .......... ..........
       jan9  GGCTCCCTAA GGTAGGGCTG GTAAWT....  .......... ..........
     april27 GGCT.CCTAA GGTAGGGCTG GTAATT....  .......... ..........
      Nov18  GCCTAAGATG AATCCSTGAK TGGA......  .......... ..........
      april5 CTGTGAT...  .......... ..........  .......... ..........
      Nov14  ATGGTGGCGA CTGGCTTNAA ..........  .......... ..........

             1551                                        1583
       Nov12 .......... .......... ..........  ...
     Nov12re .......... .......... ..........  ...
     april23 .......... .......... ..........  ...
       Nov16 .......... .......... ..........  ...
     Sphingo CCGTAGGGGA ACCTGCGG.. ..........  ...
       A8AN3 CC........ .......... ..........  ...
        jan8 .......... .......... ..........  ...
       jan14 .......... .......... ..........  ...
       Brady CCGTAGGGGA ACCTGCGGCT GGATCACCT.  ...
     april21 .......... .......... ..........  ...
        Nov8 .......... .......... ..........  ...
     april29 .......... .......... ..........  ...
       jan18 .......... .......... ..........  ...
     april24 .......... .......... ..........  ...
     april15 .......... .......... ..........  ...
     april26 .......... .......... ..........  ...
         3M3 CCGTA..... .......... ..........  ...
         6M3 CCGTA..... .......... ..........  ...
     april19 .......... .......... ..........  ...
       Nov22 .......... .......... ..........  ...
      april3 .......... .......... ..........  ...
     april18 .......... .......... ..........  ...
        jan1 .......... .......... ..........  ...
     april12 .......... .......... ..........  ...
     april10 .......... .......... ..........  ...
     april20 .......... .......... ..........  ...
       Hypho .......... .......... ..........  ...
       US353 CCGTAGGGGA AC........ ..........  ...
        jan6 .......... .......... ..........  ...
```

```
      Nov3  .......... .......... .......... ...
 Zoogloea1  CCGTAGGGGA ACCTGCGGCT GGATCACCTC CTT
   april25  .......... .......... .......... ...
      Nov2  .......... .......... .......... ...
     jan20  .......... .......... .......... ...
Polyangium  .......... .......... .......... ...
   april22  .......... .......... .......... ...
  Bacillus  .......... .......... .......... ...
     Lacto  .......... .......... .......... ...
Leptothrix  .......... .......... .......... ...
Rubrivivax  .......... .......... .......... ...
   Sphaero  .......... .......... .......... ...
   april17  .......... .......... .......... ...
      jan3  .......... .......... .......... ...
   april30  .......... .......... .......... ...
     jan15  .......... .......... .......... ...
     jan11  .......... .......... .......... ...
    april6  .......... .......... .......... ...
    april1  .......... .......... .......... ...
     jan2  .......... .......... .......... ...
      Nov7  .......... .......... .......... ...
     jan23  .......... .......... .......... ...
     Nov19  .......... .......... .......... ...
      jan4  .......... .......... .......... ...
     jan13  .......... .......... .......... ...
     jan10  .......... .......... .......... ...
      Nov4  .......... .......... .......... ...
     jan12  .......... .......... .......... ...
      jan7  .......... .......... .......... ...
     jan21  .......... .......... .......... ...
Variovorax  .......... .......... .......... ...
    april2  .......... .......... .......... ...
 Zoogloea2  CCGTATCGGA AGGTGCGGCT GGATCACCTC CTT
   april13  .......... .......... .......... ...
    april4  .......... .......... .......... ...
      jan5  .......... .......... .......... ...
Legionella  .......... .......... .......... ...
   april14  .......... .......... .......... ...
      Nov6  .......... .......... .......... ...
      Nov9  .......... .......... .......... ...
      Nov5  .......... .......... .......... ...
   april11  .......... .......... .......... ...
   april16  .......... .......... .......... ...
     jan19  .......... .......... .......... ...
     Nov17  .......... .......... .......... ...
 Cytophaga  NNNNNNNNGA ATACCTCCTT TCT....... ...
Flavobacter .......... .......... .......... ...
   april31  .......... .......... .......... ...
      jan9  .......... .......... .......... ...
   april27  .......... .......... .......... ...
     Nov18  .......... .......... .......... ...
    april5  .......... .......... .......... ...
     Nov14  .......... .......... .......... ...
```

Appendix G: Pairwise evolutionary distances, obtained from the DISTANCES tool of GCG package, between nucleotide sequences of all the clones analyzed and the selected reference bacteria. Distances were calculated over 1 to 1583 bp, considering all base positions. Jukes-Cantor correction method was used for estimating the number of substitutions per 100 bases

Key for column and row indices:

1 nov12
2 nov12re
3 april23
4 nov16
5 sphingo
6 a8an3
7 jan8
8 jan14
9 brady
10 april21
11 nov8
12 april29
13 jan18
14 april24
15 april15
16 april26
17 3m3
18 6m3
19 april19
20 nov22
21 april3
22 april18
23 jan1
24 april12
25 april10
26 april20
27 hypho
28 us353
29 jan6
30 nov3
31 zoogloea1
32 april25
33 nov2
34 jan20
35 polyangium

36 april22
37 bacillus
38 lacto
39 leptothrix
40 rubrivivax
41 sphaero
42 april17
43 jan3
44 april30
45 jan15
46 jan11
47 april6
48 april1
49 jan2
50 nov7
51 jan23
52 nov19
53 jan4
54 jan13
55 jan10
56 nov4
57 jan12
58 jan7
59 jan21
60 variovorax
61 april2
62 zoogloea2
63 april13
64 april4
65 jan5
66 legionella
67 april14
68 nov6
69 nov9
70 nov5
71 april11
72 april16
73 jan19
74 nov17
75 cytophaga
76 flavobacter
77 april31
78 jan9
79 april27

80 nov18
81 april5
82 nov14

Matrix 1: Part 1

	1	2	3	4	5	6	7	8	9	10	11	12
1	0.00	-0.00	44.73	37.98	41.72	41.09	42.27	38.31	37.99	40.34	39.08	34.55
2		0.00	44.73	37.98	41.72	41.09	42.27	38.31	37.99	40.34	39.08	34.55
3			0.00	4.44	21.55	20.55	22.59	22.75	24.00	24.82	23.20	23.38
4				0.00	32.35	18.85	23.56	27.48	28.53	22.77	20.08	20.26
5					0.00	7.12	9.33	22.01	22.09	23.46	20.03	24.16
6						0.00	6.51	12.57	15.63	15.04	15.22	12.89
7							0.00	23.21	22.83	23.16	19.84	19.32
8								0.00	14.62	23.16	20.46	25.32
9									0.00	19.79	19.24	21.45
10										0.00	5.20	4.11
11											0.00	4.47
12												0.00
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												
26												
27												
28												
29												
30												
31												
32												
33												
34												
35												
36												
37												
38												
39												
40												
41												
42												
43												
44												
45												
46												
47												
48												
49												
50												
51												
52												
53												
54												
55												
56												
57												
58												
59												
60												
61												
62												
63												
64												
65												
66												
67												
68												
69												
70												
71												
72												
73												
74												
75												
76												
77												
78												
79												
80												
81												
82												

Matrix 1: Part 2

	13	14	15	16	17	18	19	20	21	22	23	24
1	38.97	35.42	44.08	36.09	46.42	46.33	42.61	51.15	46.34	41.94	41.53	47.62
2	38.97	35.42	44.08	36.09	46.42	46.33	42.61	51.15	46.34	41.94	41.53	47.62
3	25.99	23.89	26.52	23.60	23.55	24.12	21.02	15.40	23.26	23.83	20.35	24.00
4	22.90	18.56	26.51	20.86	35.40	35.19	17.22	12.34	33.00	21.35	17.06	37.73
5	23.36	23.81	21.77	22.12	37.23	36.85	21.47	19.09	35.12	23.87	20.93	40.18
6	14.57	14.88	17.08	15.60	16.28	17.50	16.31	20.55	17.76	16.94	17.68	14.56
7	22.54	20.76	20.47	21.78	25.31	26.02	19.72	15.85	27.09	23.82	19.04	24.87
8	22.74	24.93	27.78	20.38	23.05	22.80	19.43	19.02	32.62	19.35	17.20	34.63
9	19.79	21.06	23.10	17.73	31.70	30.91	15.33	16.78	29.43	17.56	17.13	32.85
10	6.07	4.50	7.20	8.49	22.33	22.03	13.59	17.89	22.86	20.75	13.74	22.72
11	6.05	3.99	7.00	7.98	17.11	17.40	14.96	17.89	15.13	15.69	14.72	15.13
12	4.25	2.35	6.14	5.78	28.43	28.18	12.03	18.94	27.11	17.01	12.65	27.83
13	0.00	4.45	8.82	9.19	20.69	20.37	14.46	17.95	21.67	20.59	15.56	21.83
14		0.00	6.13	6.72	28.15	27.89	12.45	18.33	26.57	17.99	13.44	27.30
15			0.00	10.73	23.53	24.07	17.07	19.65	21.94	22.73	15.93	21.63
16				0.00	20.24	19.94	13.13	17.89	20.07	14.67	12.35	19.90
17					0.00	2.68	12.15	11.30	18.56	5.71	9.98	24.66
18						0.00	13.23	13.06	19.93	7.10	10.73	26.04
19							0.00	2.30	12.82	12.93	9.24	12.34
20								0.00	10.86	10.86	10.12	10.00
21									0.00	1.29	9.00	9.39

22	0.00	9.49	3.76
23		0.00	8.51
24			0.00

Matrix 1: Part 3

	25	26	27	28	29	30	31	32	33	34	35	36
1	48.43	45.30	46.10	47.08	46.98	45.34	44.61	46.74	47.00	40.44	42.43	31.09
2	48.43	45.30	46.10	47.08	46.98	45.34	44.61	46.74	47.00	40.44	42.43	31.09
3	25.32	23.72	22.08	22.92	22.70	23.03	16.40	18.49	21.19	40.54	41.37	33.15
4	22.55	21.95	32.65	34.21	29.31	21.63	27.85	17.04	20.47	38.12	50.08	29.72
5	21.85	20.92	32.83	34.34	30.83	17.82	21.79	25.25	17.61	43.10	61.03	31.39
6	23.33	14.89	12.87	13.31	15.39	13.52	11.30	16.94	20.88	34.35	36.32	33.61
7	22.99	20.66	22.06	23.26	24.78	18.42	19.50	24.53	17.39	44.97	44.51	32.50
8	18.12	18.76	22.97	22.76	23.03	18.58	22.34	21.09	18.56	36.14	42.98	31.10
9	16.11	12.20	25.38	25.20	20.86	16.74	18.95	17.92	17.02	35.46	60.01	29.98
10	19.94	15.21	18.23	18.00	18.50	20.02	19.04	23.41	18.45	37.49	39.32	30.99
11	22.40	15.48	15.69	15.41	16.25	18.90	16.54	21.23	20.89	35.17	36.45	31.46
12	19.94	13.49	23.84	23.62	24.03	17.77	23.89	20.56	17.43	34.78	43.50	30.53
13	22.04	16.68	16.25	16.25	16.96	20.05	17.31	22.54	18.56	38.38	39.98	29.94
14	20.22	14.93	23.49	23.49	24.77	19.12	23.99	21.78	18.79	35.92	42.59	30.87
15	21.62	17.26	19.61	19.61	20.12	21.77	20.94	25.22	20.37	41.75	43.97	34.21
16	18.17	14.44	17.03	18.14	16.81	20.27	21.46	22.81	18.50	35.62	40.16	32.28
17	17.73	15.20	17.07	19.27	9.23	20.32	27.85	20.36	17.61	35.84	64.30	28.91
18	19.01	16.40	17.48	19.77	9.77	20.85	26.99	20.63	18.58	36.82	63.74	29.87
19	15.85	9.95	10.01	9.60	12.58	18.03	14.37	16.63	17.12	36.78	37.79	29.87
20	17.70	14.01	10.43	10.43	16.71	18.69	17.18	15.32	20.64	36.68	39.46	29.84
21	17.73	14.26	20.68	22.60	18.81	20.58	28.49	20.30	17.61	35.42	51.79	28.58
22	18.37	14.96	8.32	9.77	8.75	20.85	16.79	19.12	17.93	35.80	38.93	28.58
23	16.16	9.98	8.51	9.49	9.74	18.71	16.19	19.50	19.79	33.43	35.26	31.17
24	15.76	12.35	26.78	27.75	19.73	19.28	33.33	19.72	17.29	36.56	57.10	29.23
25	0.00	9.28	14.93	14.62	16.78	19.39	18.37	21.31	25.62	39.83	40.83	36.78
26		0.00	9.93	8.62	11.05	16.71	14.29	17.90	18.04	40.32	40.65	30.09
27			0.00	4.46	6.10	16.66	23.46	17.22	15.71	36.05	59.70	29.33
28				0.00	6.61	16.41	24.51	17.66	15.40	36.05	61.02	29.76
29					0.00	18.55	21.13	20.44	19.23	37.12	42.94	29.44
30						0.00	9.30	17.92	17.34	42.39	42.06	32.95
31							0.00	10.85	16.34	40.42	64.38	29.76
32								0.00	14.17	40.52	41.99	32.06
33									0.00	38.01	38.61	37.25
34										0.00	16.59	32.92
35											0.00	35.31
36												0.00

60	
61	
62	
63	
64	
65	
66	
67	
68	
69	
70	
71	
72	
73	
74	
75	
76	
77	
78	
79	
80	
81	
82	

Matrix 1: Part 4

	37	38	39	40	41	42	43	44	45	46	47	48
1	37.01	28.62	35.92	40.18	37.16	32.93	36.21	37.43	39.00	36.68	38.81	39.10
2	37.01	28.61	35.92	40.18	37.16	32.93	36.21	37.43	39.00	36.68	38.81	39.10
3	38.70	25.24	37.53	40.86	38.16	32.27	35.34	38.35	41.22	37.59	38.00	40.13
4	42.94	20.76	46.64	51.17	48.34	30.59	40.87	33.87	37.97	33.52	44.84	45.81
5	58.89	28.40	59.28	60.28	58.72	30.50	42.23	35.39	37.45	33.64	47.85	48.27
6	26.02	27.69	26.68	29.25	26.17	32.02	29.41	30.71	30.92	31.54	30.51	32.79
7	38.64	29.86	37.62	39.51	38.92	30.67	32.80	36.01	37.45	32.76	37.63	39.12
8	39.05	19.20	39.09	39.52	39.26	28.54	37.40	31.39	33.51	27.93	41.08	40.96
9	58.39	22.73	57.26	58.50	57.58	29.39	34.82	32.30	34.78	31.27	43.26	41.45
10	33.58	24.47	32.69	34.75	35.56	31.59	25.80	33.87	36.26	31.69	32.93	33.51
11	26.32	23.71	31.08	33.63	32.14	32.33	30.06	31.20	34.34	33.55	30.06	31.11
12	38.09	22.96	37.78	38.34	39.95	29.42	31.10	31.17	34.26	30.23	36.81	39.84
13	35.70	27.58	33.93	34.56	36.91	31.90	25.66	35.87	37.04	32.43	32.97	34.17
14	37.32	24.47	37.18	38.71	39.37	28.98	29.27	31.09	35.49	30.86	35.31	37.22
15	40.08	30.00	36.31	36.66	37.61	32.53	31.39	36.95	39.61	34.59	36.01	37.32
16	35.22	26.79	34.66	35.31	36.41	29.42	24.49	33.33	36.84	32.33	34.38	35.23
17	64.48	27.52	60.15	60.48	61.03	28.88	39.38	33.57	35.51	32.76	47.63	47.46
18	63.24	27.69	59.97	60.24	60.51	29.64	39.52	34.56	36.57	33.57	47.56	47.39
19	35.01	29.13	29.37	31.12	30.78	30.15	25.18	31.65	35.19	32.86	29.71	31.40
20	29.39	35.97	31.23	36.32	30.57	35.88	33.19	33.90	34.29	33.40	32.72	33.90
21	45.43	26.28	44.99	45.41	43.88	28.63	41.38	33.05	35.60	31.10	47.92	50.22
22	31.93	26.28	31.94	33.05	32.48	28.97	28.24	33.65	35.92	31.80	34.81	36.01
23	26.71	27.69	28.73	30.24	27.95	29.55	28.35	30.91	33.93	32.50	28.92	30.24
24	50.68	26.28	51.41	51.99	50.92	28.47	43.53	33.11	35.33	31.27	51.27	49.61
25	33.21	33.62	35.40	35.97	34.84	36.09	34.45	37.63	40.64	40.34	35.15	35.97
26	33.08	24.21	32.74	34.35	32.97	30.58	28.80	36.56	38.35	32.67	34.98	36.24
27	57.26	27.52	56.45	56.41	57.36	28.55	35.82	34.09	35.74	32.67	45.29	46.34
28	60.17	26.82	59.77	59.00	60.38	29.56	37.35	34.70	36.06	32.67	45.47	45.58
29	34.73	26.28	35.57	35.74	36.21	29.64	33.34	34.56	36.57	32.86	42.17	42.74
30	31.75	24.75	32.93	33.21	33.27	28.63	31.35	34.61	36.50	32.06	34.16	36.11
31	59.79	24.75	57.81	59.11	57.65	29.22	40.86	32.08	34.24	29.56	40.94	42.35
32	34.55	24.21	33.43	35.39	35.00	31.01	31.99	34.87	36.57	32.41	35.60	37.63
33	31.00	35.18	29.56	31.39	29.04	34.01	32.60	33.09	33.75	33.14	33.79	33.38
34	36.23	20.01	23.69	26.38	25.59	25.46	31.31	27.81	29.53	28.24	29.86	26.38
35	40.98	20.01	32.78	37.77	33.74	25.64	37.11	25.25	28.08	26.61	41.60	38.06
36	7.97	15.68	25.11	27.84	24.66	29.65	28.79	28.26	28.46	28.67	27.42	27.00
37	0.00	9.84	38.50	41.23	38.69	22.18	37.58	30.41	33.24	26.14	38.86	38.73
38		0.00	20.01	19.37	20.12	19.37	18.74	18.74	19.37	18.74	19.37	18.74
39			0.00	3.87	3.89	9.39	23.98	6.58	9.56	9.49	19.40	17.09
40				0.00	5.31	12.83	26.18	8.44	9.90	9.15	19.96	17.17
41					0.00	9.94	25.66	8.61	11.48	10.59	20.11	18.40
42						0.00	3.25	12.26	14.09	15.00	10.68	12.26
43							0.00	16.59	19.49	14.46	26.48	28.47
44								0.00	4.46	3.95	7.58	7.58
45									0.00	5.39	11.01	11.01
46										0.00	7.61	8.37
47											0.00	13.04
48												0.00
49												
50												
51												
52												
53												
54												
55												
56												
57												
58												
59												
60												
61												
62												
63												
64												
65												
66												
67												
68												
69												
70												
71												
72												
73												
74												
75												
76												
77												
78												
79												
80												
81												
82												

Matrix 1: Part 5

	49	50	51	52	53	54	55	56	57	58	59	60
1	35.07	47.21	37.78	34.14	35.88	36.94	41.88	41.07	41.28	44.52	38.66	40.50
2	35.07	47.21	37.78	34.14	35.88	36.94	41.88	41.07	41.28	44.52	38.66	40.50
3	32.68	42.64	38.45	30.23	39.16	38.87	40.97	40.24	39.88	42.82	37.13	38.98
4	28.45	38.82	34.90	27.32	35.54	36.08	50.48	50.42	50.17	43.97	38.20	49.20
5	29.87	38.69	38.60	33.17	33.32	33.23	43.80	42.10	43.80	41.46	33.46	56.91
6	32.52	33.14	31.51	32.51	28.74	31.02	30.51	29.91	29.52	31.43	30.81	29.64
7	29.42	40.09	36.92	33.17	35.19	32.72	39.49	38.70	39.21	41.19	32.27	38.25
8	26.15	38.46	31.83	25.99	29.04	30.32	40.79	39.05	39.86	36.22	31.11	37.64
9	28.03	37.80	32.10	32.51	29.95	32.28	41.25	40.16	40.98	38.25	31.50	57.67
10	28.72	40.03	32.53	31.30	32.82	33.12	36.23	35.46	36.27	38.19	34.47	35.00

11	28.81	33.73	32.01	32.76	33.00	34.45	33.56	33.73	33.73	34.82	36.35	32.17
12	27.33	38.20	33.01	32.03	29.65	31.66	40.28	38.98	40.21	38.93	31.92	38.56
13	30.31	42.00	34.56	33.49	34.04	34.34	36.82	35.57	35.17	39.68	34.17	35.14
14	26.60	37.38	32.93	32.56	31.31	31.93	40.61	39.65	39.85	39.77	33.73	38.93
15	30.99	41.77	37.42	35.53	36.43	35.64	37.42	37.75	37.42	40.64	34.78	36.94
16	28.15	37.84	33.57	31.84	30.55	31.57	37.13	36.26	37.05	39.46	32.01	34.99
17	27.50	37.90	38.23	31.38	33.25	31.68	41.71	39.59	40.91	38.94	31.59	60.24
18	28.28	38.69	38.08	32.86	33.34	32.46	41.87	39.72	41.06	39.21	32.36	60.16
19	27.20	39.25	30.06	32.86	30.90	33.16	32.91	31.47	32.05	36.10	33.67	32.24
20	32.72	34.50	32.72	33.22	35.27	32.63	37.30	36.50	35.27	33.84	35.44	37.56
21	27.58	36.98	41.01	30.89	32.73	31.09	45.72	44.65	44.18	41.65	31.00	45.88
22	27.93	37.32	35.32	30.89	30.55	31.43	33.01	32.56	31.69	36.32	31.33	33.32
23	25.90	31.59	29.66	33.53	29.66	32.81	30.66	29.99	30.66	31.26	32.03	30.24
24	26.73	36.45	41.64	30.57	33.67	31.26	46.54	46.40	45.40	41.67	30.83	50.71
25	35.56	36.38	34.05	41.57	36.09	37.34	36.92	36.50	36.50	38.61	37.89	34.70
26	28.01	39.28	34.44	32.51	34.44	32.98	36.01	35.06	35.38	36.80	33.39	34.32
27	27.50	38.14	35.93	29.61	32.09	31.60	38.60	37.10	37.82	38.43	31.16	56.05
28	27.50	38.83	36.21	30.25	32.64	31.60	39.12	37.60	38.34	38.13	31.16	59.56
29	28.63	37.32	36.65	32.20	33.06	31.77	38.67	36.31	37.29	38.91	31.33	35.97
30	27.58	38.93	34.05	32.86	32.28	32.98	34.05	33.55	34.19	36.50	32.18	33.18
31	27.50	38.59	37.02	32.20	33.14	31.26	45.21	43.45	44.65	39.30	30.83	59.04
32	28.98	41.52	35.51	31.55	32.40	33.42	33.95	33.23	33.72	37.67	33.30	33.95
33	34.20	34.31	34.01	35.57	29.54	31.41	30.90	30.31	29.54	31.81	31.20	29.84
34	27.15	36.59	29.65	27.16	29.11	29.58	31.03	30.20	29.93	34.06	29.83	31.24
35	26.51	35.08	30.03	26.55	28.40	28.92	33.88	33.05	33.88	35.04	30.58	41.57
36	28.26	29.54	29.11	26.69	27.94	27.29	29.65	28.79	27.94	28.56	28.46	27.04
37	21.68	30.89	28.91	23.03	28.87	27.00	35.57	34.32	34.96	36.05	26.50	41.73
38	17.50	18.12	18.12	22.60	20.01	20.01	21.29	20.65	20.01	22.60	21.94	18.12
39	5.96	16.43	11.18	9.43	8.29	8.52	9.09	8.03	8.97	11.42	8.32	15.35
40	8.03	17.46	12.03	9.43	7.94	7.98	8.76	7.91	8.96	11.20	7.91	13.97
41	5.96	16.71	12.45	7.54	9.73	9.06	9.86	8.77	9.37	12.95	8.59	15.51
42	7.02	9.39	8.14	8.48	10.45	12.21	11.52	10.71	10.71	11.40	13.40	11.47
43	5.74	15.49	13.26	7.07	14.74	13.11	26.62	25.53	26.09	21.69	13.34	26.14
44	6.22	16.65	9.08	9.91	9.30	9.21	10.40	10.16	9.95	12.35	10.40	11.02
45	9.54	19.79	12.65	10.39	10.80	10.73	11.72	11.26	11.26	12.18	10.55	12.62
46	9.04	13.34	9.41	10.39	8.64	9.41	9.41	9.15	8.39	9.15	11.52	9.90
47	4.91	14.63	6.58	8.48	8.90	6.50	18.85	18.17	18.07	11.16	8.13	17.41
48	6.44	16.14	9.43	10.39	10.89	8.71	22.43	21.53	22.47	14.71	10.89	20.86
49	0.00	3.17	2.43	4.79	6.48	7.66	7.30	7.00	7.00	6.86	8.61	7.23
50		0.00	12.21	6.15	16.43	12.81	16.72	16.43	16.68	16.92	12.46	15.88
51			0.00	5.24	8.34	8.48	12.86	12.03	12.41	13.67	9.42	10.61
52				0.00	8.01	8.48	9.91	8.95	8.01	8.95	8.52	8.01
53					0.00	4.82	3.59	3.03	3.21	7.30	3.61	6.57
54						0.00	6.25	5.29	5.06	6.50	6.54	6.50
55							0.00	2.96	3.46	6.43	4.56	5.55
56								0.00	2.34	5.86	4.31	4.96
57									0.00	6.23	4.31	5.90
58										0.00	4.85	9.96
59											0.00	6.44
60												0.00
61												
62												
63												
64												
65												
66												
67												
68												
69												
70												
71												
72												
73												
74												
75												
76												
77												
78												
79												
80												
81												
82												

Matrix 1: Part 6

	61	62	63	64	65	66	67	68	69	70	71	72
1	40.18	41.29	33.69	36.53	43.83	35.85	36.83	43.03	43.03	47.36	39.03	41.10
2	40.18	41.29	33.69	36.53	43.83	35.85	36.83	43.03	43.03	47.36	39.03	41.10
3	38.45	38.70	33.78	36.07	39.26	35.05	37.44	40.90	41.37	45.10	40.88	41.32
4	43.61	49.03	29.99	44.75	37.07	41.79	47.26	45.05	45.43	49.44	47.61	38.31
5	47.53	58.53	33.25	42.35	35.68	57.22	50.04	55.35	56.06	61.10	53.40	40.23
6	31.69	27.22	27.70	30.02	30.61	27.75	33.23	38.22	38.36	42.96	35.60	37.23
7	39.30	40.42	35.18	35.36	37.36	34.87	40.64	43.44	44.08	48.32	42.47	40.92
8	40.46	40.32	27.43	40.77	34.21	37.38	47.44	48.19	49.08	51.99	49.58	41.14
9	42.61	55.80	30.07	39.61	35.50	59.98	44.81	48.41	49.08	54.10	44.74	35.60
10	34.53	34.16	29.99	32.42	34.39	32.22	33.24	36.88	37.19	40.32	35.75	38.17
11	30.50	32.18	28.18	29.12	30.67	30.76	32.54	35.10	34.82	37.08	33.59	34.21
12	38.62	38.62	27.56	36.96	32.71	35.13	36.54	42.38	42.88	46.45	40.53	36.11
13	34.34	34.56	29.84	31.27	35.47	31.93	32.81	37.58	38.22	41.13	36.73	40.29
14	36.56	38.71	27.22	36.82	32.95	35.50	36.87	41.85	42.15	46.07	40.66	37.38
15	36.76	35.37	34.30	33.84	37.07	36.15	35.19	41.36	41.47	45.31	41.12	40.54
16	35.39	35.03	28.87	34.00	36.72	32.64	35.07	37.36	37.65	40.37	37.74	36.69
17	42.80	59.09	30.41	41.38	35.92	64.44	43.63	50.74	50.82	56.84	49.86	37.32
18	42.97	59.01	29.93	41.27	36.35	64.21	43.81	50.70	50.78	56.84	49.50	37.75
19	30.34	31.40	28.83	31.46	36.35	32.68	31.25	34.63	34.71	38.60	34.12	36.01
20	33.47	34.50	31.86	34.66	36.86	36.32	35.27	42.46	42.00	45.53	39.10	38.98
21	47.73	46.17	32.22	45.30	34.37	47.61	45.70	49.78	50.29	55.26	49.24	38.08
22	35.49	33.93	31.47	31.11	34.70	34.06	32.20	36.68	37.08	41.15	36.45	38.75
23	29.66	30.58	27.58	26.77	32.50	29.26	31.08	35.71	35.45	38.73	34.64	35.24
24	47.99	52.27	32.20	47.38	33.55	52.99	47.60	52.37	52.44	56.97	50.57	38.44
25	36.09	36.38	32.23	34.41	38.40	35.10	36.32	41.85	41.55	45.16	42.29	42.71
26	35.70	35.29	31.89	34.49	36.49	35.74	35.74	37.68	37.78	41.83	38.44	39.50
27	37.72	55.52	28.72	38.29	34.19	58.55	42.65	49.40	49.48	55.77	47.32	35.92
28	39.65	57.52	28.99	39.34	35.17	61.48	42.91	49.69	49.77	56.52	48.79	36.89
29	37.13	36.79	30.48	35.82	35.35	35.86	36.68	42.13	42.61	47.02	42.91	36.43
30	34.57	33.21	31.80	34.63	37.60	34.86	33.87	42.24	42.24	46.37	39.01	40.02
31	43.80	56.84	28.79	40.47	35.59	60.45	46.58	44.91	45.09	51.87	43.07	38.98
32	36.92	32.58	30.75	32.57	37.70	32.92	32.53	37.53	37.62	41.82	36.01	39.42
33	34.31	30.60	30.02	30.31	34.20	31.77	31.58	36.63	36.76	40.23	34.24	34.39
34	30.48	30.13	27.92	33.35	37.10	33.85	32.86	36.05	36.14	40.46	38.28	40.60
35	34.36	40.42	28.75	40.51	32.66	42.70	39.64	51.50	52.48	58.37	47.38	37.68
36	27.51	29.98	23.73	28.71	30.30	27.36	32.48	42.35	42.01	46.24	38.85	40.27
37	34.13	41.33	27.64	37.14	30.10	39.03	39.59	58.07	58.13	64.93	49.64	41.39
38	20.01	21.94	16.89	18.12	20.65	23.26	21.29	20.01	20.01	25.28	21.41	24.75
39	9.79	17.58	10.11	26.20	24.78	33.33	26.46	49.33	49.92	55.91	44.87	37.14
40	10.70	19.86	11.95	26.35	27.24	34.95	26.93	52.22	52.60	58.91	47.42	39.29
41	10.58	18.12	12.30	27.01	25.70	34.66	29.10	49.86	50.45	56.50	44.48	36.11
42	10.94	15.25	12.59	19.98	20.93	19.63	21.60	34.10	34.57	38.75	33.37	34.34
43	26.28	30.87	17.46	31.83	27.30	32.47	35.58	47.19	48.28	52.54	44.08	38.38
44	8.02	11.24	10.62	22.07	25.48	26.02	19.59	37.47	37.89	42.22	35.65	37.28
45	11.49	13.09	12.85	24.41	28.65	27.49	21.71	41.48	41.60	45.92	40.21	41.32
46	8.39	11.73	12.00	18.75	23.45	23.20	21.48	37.36	37.47	41.35	36.98	37.27
47	11.39	21.31	10.88	32.46	27.83	36.32	34.29	43.71	44.37	48.35	42.60	38.95
48	16.91	23.10	11.09	34.07	28.13	35.13	33.90	42.40	42.86	47.98	43.61	37.61

49	5.43	12.09	11.65	18.29	19.73	20.08	21.52	33.03	32.75	37.00	33.03	32.88
50	15.16	21.66	21.76	28.20	33.49	31.19	31.35	42.21	41.96	46.55	41.59	41.68
51	7.36	14.86	12.17	25.01	30.02	26.03	26.76	39.28	39.88	43.34	38.49	37.71
52	8.95	15.40	11.86	20.77	20.22	21.89	22.46	33.66	33.66	36.36	34.51	36.56
53	8.72	11.30	13.48	21.93	26.71	23.17	21.78	38.64	39.46	43.00	37.23	37.94
54	7.00	12.29	13.87	19.57	24.24	23.08	21.69	37.45	37.82	41.66	38.96	39.93
55	9.77	11.91	14.80	27.02	27.30	28.08	26.92	50.62	51.83	55.62	47.86	39.29
56	8.69	11.13	14.36	26.26	27.60	26.76	27.68	51.21	52.10	55.19	48.12	39.29
57	9.37	11.95	14.33	26.82	27.01	28.98	26.35	49.80	50.50	54.89	46.88	38.61
58	10.39	15.38	16.60	24.97	27.51	30.94	23.75	44.52	45.06	48.20	42.38	42.29
59	8.65	12.17	15.46	19.90	25.73	24.29	20.64	38.05	38.43	41.51	38.43	38.24
60	8.59	14.62	14.43	27.70	28.74	31.98	27.60	50.85	51.23	56.78	47.60	39.22
61	0.00	12.29	11.77	28.81	27.90	29.43	25.55	48.93	49.44	53.81	45.43	39.74
62		0.00	12.38	28.08	29.95	30.02	25.64	50.70	51.38	57.93	46.55	38.61
63			0.00	22.58	24.89	22.09	17.39	38.46	39.17	43.31	36.14	39.45
64				0.00	18.37	21.13	27.73	42.39	43.14	47.89	40.02	36.01
65					0.00	19.11	27.56	44.71	46.37	48.75	40.07	40.86
66						0.00	31.03	46.86	47.80	54.36	44.55	34.33
67							0.00	49.80	50.78	56.96	44.36	36.48
68								0.00	1.96	7.21	24.35	24.55
69									0.00	7.03	24.73	24.83
70										0.00	28.71	28.74
71											0.00	2.79
72												0.00
73												
74												
75												
76												
77												
78												
79												
80												
81												
82												

Matrix 1: Part 7

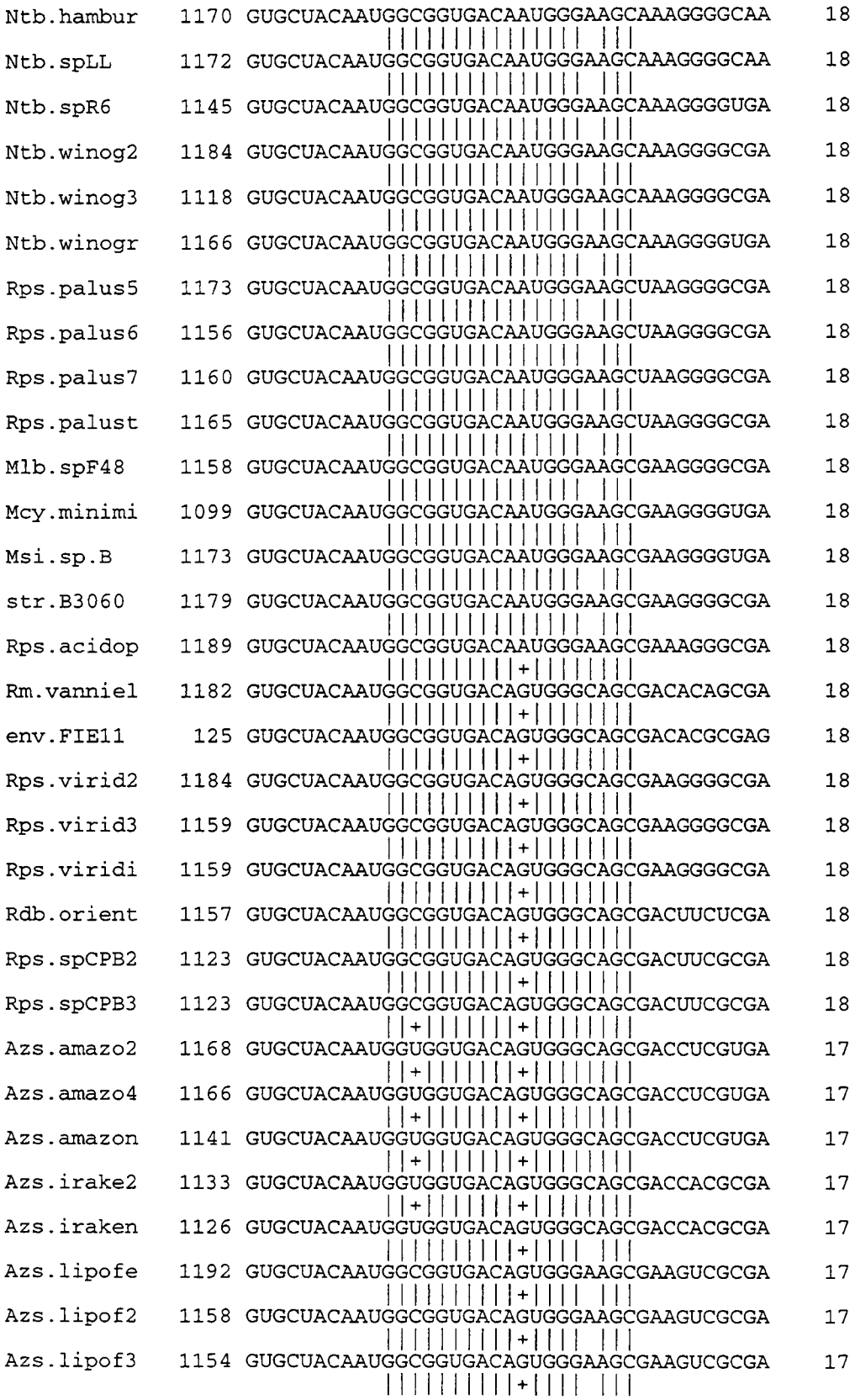
	73	74	75	76	77	78	79	80	81	82
1	39.79	38.66	44.40	42.09	38.95	40.83	42.18	51.71	38.01	37.24
2	39.79	38.66	44.40	42.09	38.95	40.83	42.18	51.71	38.01	37.24
3	41.46	41.59	39.58	38.87	36.30	37.12	39.80	54.02	39.27	47.94
4	47.54	35.74	48.12	45.73	35.85	41.39	35.38	53.05	44.85	43.30
5	57.36	42.18	69.83	69.07	39.28	45.15	39.84	54.57	50.71	52.47
6	36.22	36.84	41.64	38.27	31.00	31.59	32.20	52.93	34.82	44.25
7	43.54	42.74	44.51	41.85	37.70	38.99	40.73	51.18	40.76	48.17
8	52.15	38.64	45.79	46.98	35.85	41.55	34.16	51.77	45.14	49.25
9	48.19	34.55	67.87	65.73	36.51	42.57	34.92	54.91	46.85	46.34
10	36.19	36.88	39.30	37.32	35.84	37.46	35.54	52.99	37.52	41.03
11	33.35	34.65	36.56	36.68	35.33	36.98	32.37	54.75	37.15	39.09
12	41.58	34.12	41.73	41.02	37.39	43.01	33.45	53.57	42.68	39.72
13	37.19	38.94	38.34	38.34	35.88	35.71	34.34	52.35	39.55	43.03
14	40.83	35.29	42.58	41.46	37.59	41.33	33.37	53.93	41.95	38.08
15	40.97	41.10	42.43	41.48	39.71	40.51	39.76	55.35	43.35	45.97
16	38.49	35.92	39.63	38.97	37.66	38.87	36.40	56.92	37.83	38.99
17	52.78	35.53	70.46	71.93	41.42	45.53	39.74	53.68	56.39	54.34
18	52.44	35.92	70.60	72.45	41.30	45.44	39.91	56.13	56.71	54.32
19	34.83	35.57	37.36	35.36	34.40	35.42	36.18	52.80	37.13	42.02
20	39.45	39.71	44.99	41.80	36.14	36.40	39.46	50.49	35.68	44.55
21	49.18	37.87	53.64	50.68	42.47	48.04	40.19	53.34	53.20	53.70
22	36.91	38.31	40.38	38.06	36.53	38.50	37.78	53.34	39.66	42.00
23	34.78	35.66	36.07	36.48	35.87	37.08	35.87	55.22	38.77	41.10
24	52.59	38.75	59.72	57.10	40.44	45.63	39.33	52.93	58.79	57.99
25	42.56	43.17	44.54	46.84	39.97	39.70	40.40	66.68	40.85	49.61
26	39.63	40.45	41.20	40.15	37.28	38.38	39.94	57.39	41.21	46.33
27	50.23	34.25	62.85	64.51	39.67	43.89	38.82	52.59	53.74	55.32
28	51.23	35.15	66.43	66.52	40.53	44.18	39.72	54.23	54.17	55.66
29	42.50	34.71	44.38	43.69	39.28	43.22	39.00	55.56	48.18	52.45
30	39.44	40.65	43.00	38.35	37.81	38.95	40.25	53.89	40.86	47.09
31	51.12	36.76	66.17	64.17	31.55	32.59	36.46	52.05	52.10	55.86
32	36.74	37.47	37.96	36.35	32.84	34.63	37.98	51.72	37.52	43.53
33	34.70	35.30	37.09	36.81	35.38	35.56	34.56	57.51	34.60	41.53
34	38.44	37.63	39.08	38.93	39.72	41.72	36.70	50.27	36.41	46.29
35	51.72	35.28	75.24	74.35	38.31	45.42	33.72	45.38	48.25	51.87
36	39.61	39.80	38.46	39.38	25.09	25.72	32.71	43.91	38.77	36.81
37	55.39	40.24	68.07	73.35	36.96	42.74	35.43	41.51	51.72	47.75
38	22.07	22.07	23.93	21.29	13.24	13.82	16.28	31.70	16.64	20.76
39	49.14	35.24	66.90	66.64	32.38	39.08	31.20	39.26	48.92	46.79
40	51.23	38.28	73.09	70.73	36.59	43.23	35.11	45.56	52.18	50.38
41	49.06	35.24	67.12	66.49	33.77	40.12	32.80	39.26	51.28	46.38
42	33.49	34.78	35.65	36.02	29.06	30.24	31.46	47.76	34.97	35.30
43	46.02	37.43	47.25	46.88	34.04	40.25	35.84	46.23	45.73	50.90
44	37.07	37.53	38.54	39.23	32.07	34.58	34.20	45.07	32.23	36.95
45	41.10	41.23	41.15	43.37	36.43	38.18	38.85	44.74	36.25	41.05
46	37.93	38.85	37.52	39.43	32.48	33.00	33.97	44.58	35.64	38.15
47	46.78	38.60	42.45	42.51	30.73	36.82	34.18	46.56	46.79	49.52
48	46.41	36.41	46.14	44.65	32.26	36.30	34.46	46.06	44.37	49.52
49	33.55	34.89	35.58	34.63	25.79	27.00	30.41	47.58	33.94	34.81
50	42.19	43.45	41.16	41.60	34.28	36.06	36.69	48.61	44.08	45.27
51	39.24	37.43	40.83	40.59	32.10	33.83	34.82	47.07	37.42	39.79
52	36.56	35.19	33.00	35.00	23.73	23.61	28.67	35.00	29.13	33.17
53	38.19	37.34	40.14	40.29	31.88	33.92	37.60	45.73	32.57	38.22
54	39.81	40.74	37.75	40.91	32.10	32.95	34.98	46.43	36.78	39.57
55	47.98	39.56	48.90	49.55	36.48	43.41	37.60	47.25	48.30	51.78
56	48.90	38.28	46.41	46.34	35.58	42.19	36.34	46.23	48.92	50.82
57	46.70	38.28	48.01	48.86	36.05	42.57	36.84	45.73	47.91	51.22
58	43.43	44.43	43.55	45.27	36.50	37.20	40.78	46.43	43.43	47.23
59	39.28	40.60	38.68	39.22	32.09	33.62	36.02	45.40	35.48	38.63
60	51.09	38.53	70.96	71.86	34.48	41.28	34.79	46.37	52.33	50.23
61	45.64	39.33	47.72	47.40	34.77	41.54	34.62	46.74	47.75	50.38
62	50.93	37.65	75.61	73.23	37.42	43.80	36.26	47.58	52.48	49.12
63	37.48	38.12	38.43	37.98	31.57	32.80	35.51	46.70	34.38	38.63
64	41.54	34.93	42.71	41.57	33.95	37.97	34.51	48.39	43.89	51.47
65	40.27	41.12	41.07	41.88	38.31	39.47	40.45	47.37	38.78	45.51
66	48.25	32.49	70.06	71.65	33.95	39.83	33.95	48.72	47.70	48.45
67	46.52	35.36	47.88	49.67	38.09	43.51	34.51	44.08	46.54	49.16
68	30.13	23.21	32.69	34.33	29.47	33.15	26.41	47.55	49.00	48.24
69	30.69	23.72	33.32	34.74	29.53	33.65	26.72	47.22	49.85	49.97
70	35.39	26.41	36.89	38.34	33.85	38.18	30.54	47.25	54.27	53.40
71	7.72	3.02	20.10	20.90	31.17	36.00	27.60	50.65	45.31	47.93
72	3.61	5.06	18.81	20.07	36.24	37.99	32.14	49.17	34.91	40.97
73	0.00	3.61	26.57	27.84	35.77	40.13	28.42	50.04	49.91	47.56
74		0.00	16.70	18.10	34.57	35.88	29.92	51.24	34.06	40.49
75			0.00	12.77	32.56	37.74	29.31	50.17	47.70	54.67
76				0.00	31.75	36.45	29.23	49.56	48.56	56.82
77					0.00	2.30	17.52	39.61	41.95	43.98
78						0.00	18.85	40.23	47.27	50.30
79							0.00	43.14	36.81	39.65
80								0.00	49.70	57.04
81									0.00	51.50
82										0.00

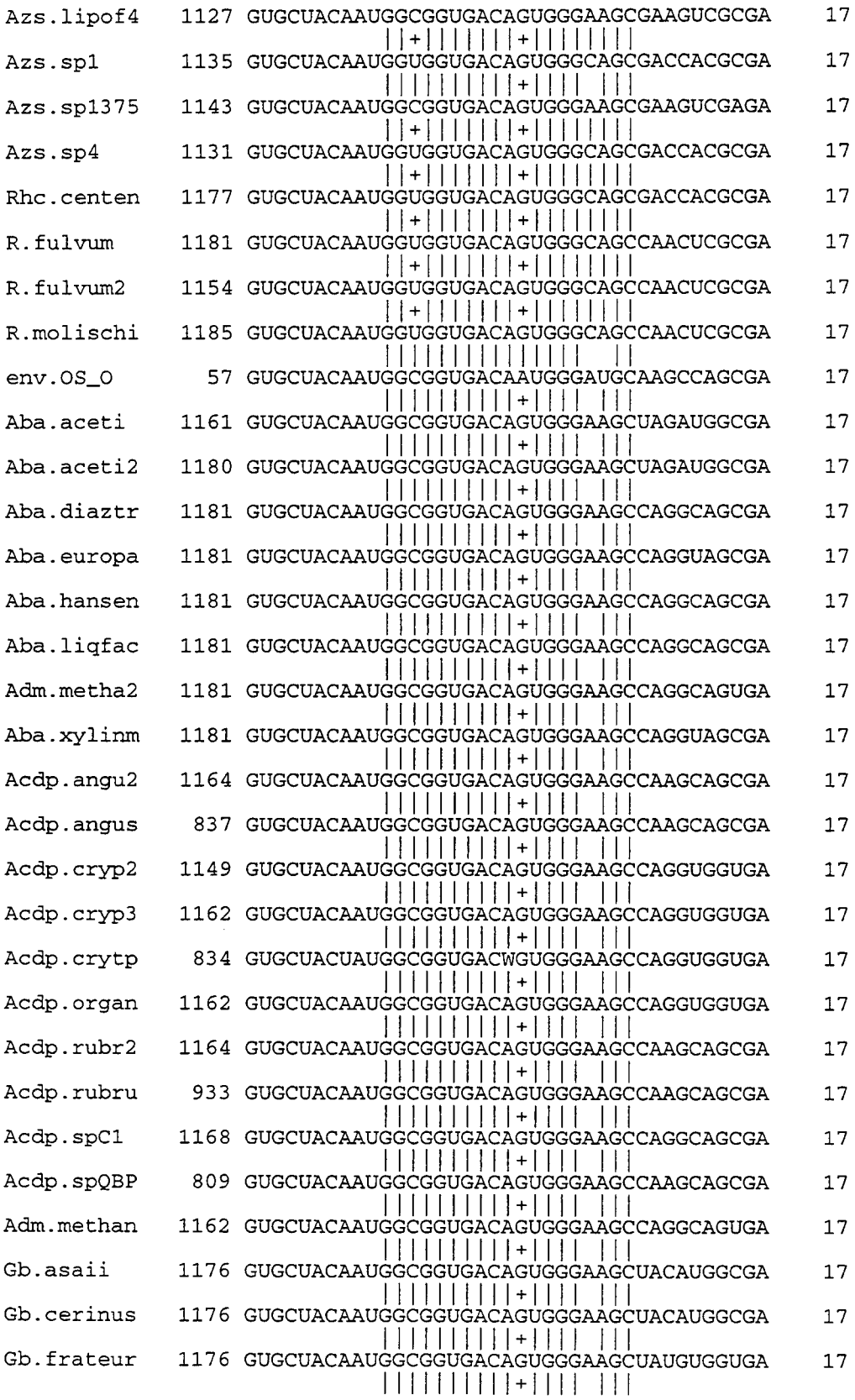
Appendix H: List of ranked best complementarities between the Hyphomicrobium probe sequence and the sequences in the RDP alignment.

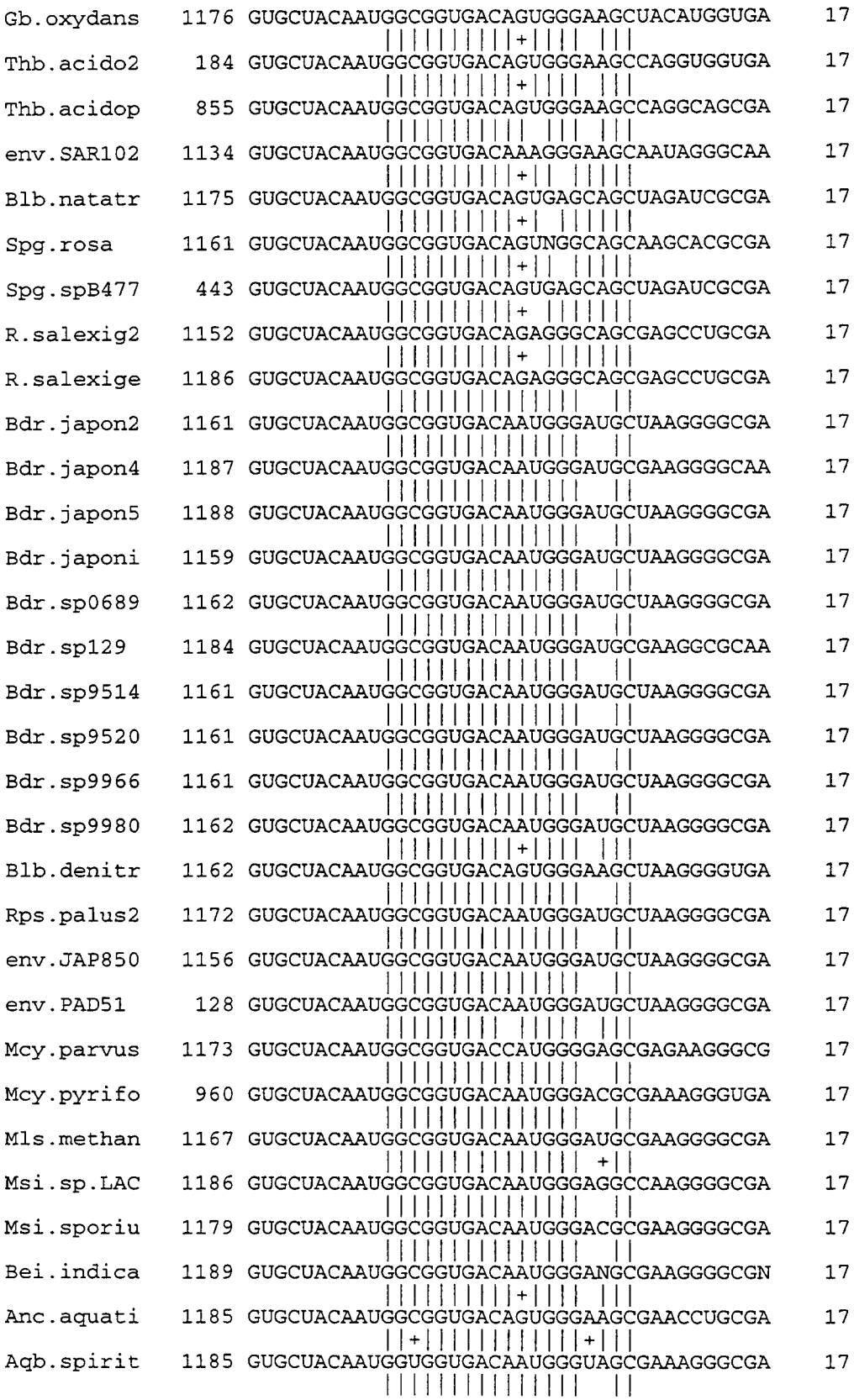
```
5'-3' probe sequence : GCTGCCCATTGTCACCGCC
Td = 81.5 + 16.6 log M + 0.41 [%(G+C)] - 820/n
Max. number of mismatches displayed :     2
[monovalent cations] :   0.300 M

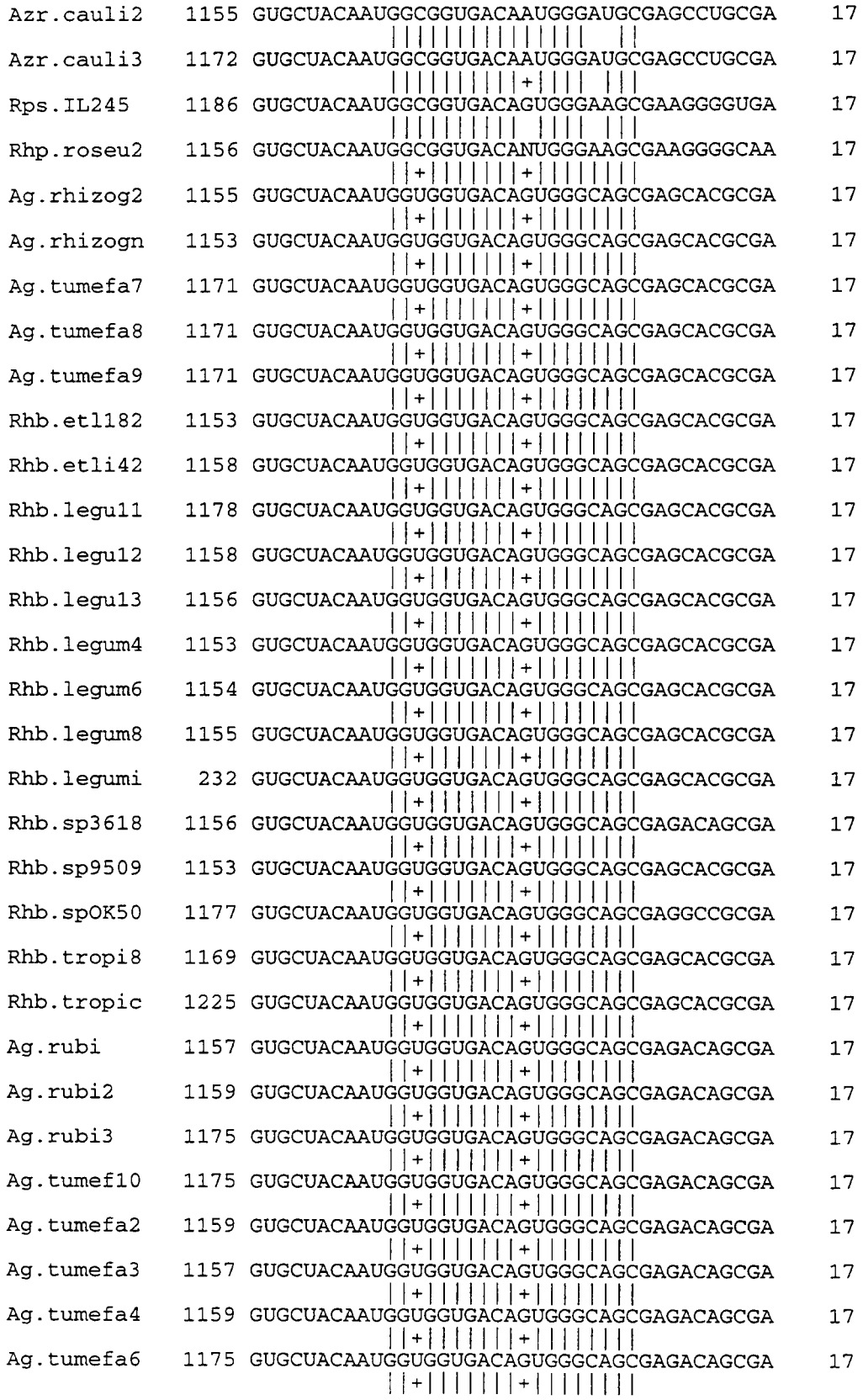

Matches / mismatches (19 is highest score) :
--------------------------------------------
```

	5'end	3' CCGCCACUGUUACCCGUCG 5'	Match	Td
Hyp.vulgar	1139	GUGCUACAAUGGCGGUGACAAUGGGCAGCAACUCAGCAA	19	57.7 C
str.Hyp353	1163	GUGCUACAAUGGCGGUGACAAUGGGCAGCAACACAGCAA	19	57.7 C
Rsc.thsulf	1147	GUGCUACAAUGGCGGUGACAAUGGGAAGCCAGGUCGCGA	18	
Blb.BF14	1173	GUGCUACAAUGGCGGUGACAGUGGGCAGCAAUCCCGCAA	18	
Cau.subvib	1184	GUGCUACAAUGGCGGUGACAGUGGGCAGCAAACUCGCGA	18	
Rhz.subfa2	1178	GUGCUACAAUGGCGGUGACAGUGGGCAGCUAACUCGCGA	18	
Spg.adhae3	1178	GUGCUACAAUGGCGGUGACAGUGGGCAGCAACCUCGCGA	18	
Spg.capsu2	1157	GUGCUACAAUGGCGGUGACAGUGGGCAGCAAGCACGCGA	18	
Spg.capsul	1185	GUGCUACAAUGGCGGUGACAGUGGGCAGCAAGCACGCGA	18	
Spg.macgol	1178	GUGCUACAAUGGCGGUGACAGUGGGCAGCAACCGGGCGA	18	
Spg.mali	488	CGUGCUACAUGGCGGUGACAGUGGGCAGCAAUCCCGCGA	18	
Spg.pruni	488	CGUGCUACAUGGCGGUGACAGUGGGCAGCAAUCCCGCGA	18	
Spg.spB478	440	GUGCUACAAUGGCGGUGACAGUGGGCAGCAAGCACNCGA	18	
Spg.spB522	440	GUGCUACAAUGGCGGUGACAGUGGGCAGCAAGCACGCGA	18	
Spg.spB695	1056	GUGCUACAAUGGCGGUGACAGUGGGCAGCAAGCACGCGA	18	
Spg.spB712	39	GUGCUACAAUGGCGGUGACAGUGGGCAGCAAACUCGCGA	18	
Spg.spF199	1056	GUGCUACAAUGGCGGUGACAGUGGGCAGCAAGCACGCGA	18	
Spg.terrae	1178	GUGCUACAAUGGCGGUGACAGUGGGCAGCAACCUCGCGA	18	
env.PAD23	126	GUGCUACAAUGGCGGUGACAGUGGGCAGCAAACUCGCGA	18	
env.PAD26	126	GUGCUACAAUGGCGGUGACAGUGGGCAGCAAUCUCGCAA	18	
str.B274	2	UGGCGGUGACAGUGGGCAGCCACUACGCGA	18	
Ntb.hambu2	1160	GUGCUACAAUGGCGGUGACAAUGGGAAGCAAAGGGGCAA	18	


Ntb.hambur 1170 GUGCUACAAUGGCGGUGACAAUGGGAAGCAAAGGGGCAA 18
Ntb.spLL 1172 GUGCUACAAUGGCGGUGACAAUGGGAAGCAAAGGGGCAA 18
Ntb.spR6 1145 GUGCUACAAUGGCGGUGACAAUGGGAAGCAAAGGGGUGA 18
Ntb.winog2 1184 GUGCUACAAUGGCGGUGACAAUGGGAAGCAAAGGGGCGA 18
Ntb.winog3 1118 GUGCUACAAUGGCGGUGACAAUGGGAAGCAAAGGGGCGA 18
Ntb.winogr 1166 GUGCUACAAUGGCGGUGACAAUGGGAAGCAAAGGGGUGA 18
Rps.palus5 1173 GUGCUACAAUGGCGGUGACAAUGGGAAGCUAAGGGGCGA 18
Rps.palus6 1156 GUGCUACAAUGGCGGUGACAAUGGGAAGCUAAGGGGCGA 18
Rps.palus7 1160 GUGCUACAAUGGCGGUGACAAUGGGAAGCUAAGGGGCGA 18
Rps.palust 1165 GUGCUACAAUGGCGGUGACAAUGGGAAGCUAAGGGGCGA 18
Mlb.spF48 1158 GUGCUACAAUGGCGGUGACAAUGGGAAGCGAAGGGGCGA 18
Mcy.minimi 1099 GUGCUACAAUGGCGGUGACAAUGGGAAGCGAAGGGGUGA 18
Msi.sp.B 1173 GUGCUACAAUGGCGGUGACAAUGGGAAGCGAAGGGGUGA 18
str.B3060 1179 GUGCUACAAUGGCGGUGACAAUGGGAAGCGAAGGGGCGA 18
Rps.acidop 1189 GUGCUACAAUGGCGGUGACAAUGGGAAGCGAAAGGGCGA 18
Rm.vanniel 1182 GUGCUACAAUGGCGGUGACAGUGGGGCAGCGACACAGCGA 18
env.FIE11 125 GUGCUACAAUGGCGGUGACAGUGGGGCAGCGACACGCGAG 18
Rps.virid2 1184 GUGCUACAAUGGCGGUGACAGUGGGGCAGCGAAGGGGCGA 18
Rps.virid3 1159 GUGCUACAAUGGCGGUGACAGUGGGGCAGCGAAGGGGCGA 18
Rps.viridi 1159 GUGCUACAAUGGCGGUGACAGUGGGGCAGCGAAGGGGCGA 18
Rdb.orient 1157 GUGCUACAAUGGCGGUGACAGUGGGGCAGCGACUUCUCGA 18
Rps.spCPB2 1123 GUGCUACAAUGGCGGUGACAGUGGGGCAGCGACUUCGCGA 18
Rps.spCPB3 1123 GUGCUACAAUGGCGGUGACAGUGGGGCAGCGACUUCGCGA 18
Azs.amazo2 1168 GUGCUACAAUGGUGGUGACAGUGGGGCAGCGACCUCGUGA 17
Azs.amazo4 1166 GUGCUACAAUGGUGGUGACAGUGGGGCAGCGACCUCGUGA 17
Azs.amazon 1141 GUGCUACAAUGGUGGUGACAGUGGGGCAGCGACCUCGUGA 17
Azs.irake2 1133 GUGCUACAAUGGUGGUGACAGUGGGGCAGCGACCACGCGA 17
Azs.iraken 1126 GUGCUACAAUGGUGGUGACAGUGGGGCAGCGACCACGCGA 17
Azs.lipofe 1192 GUGCUACAAUGGCGGUGACAGUGGGAAGCGAAGUCGCGA 17
Azs.lipof2 1158 GUGCUACAAUGGCGGUGACAGUGGGAAGCGAAGUCGCGA 17
Azs.lipof3 1154 GUGCUACAAUGGCGGUGACAGUGGGAAGCGAAGUCGCGA 17


Azs.lipof4 1127 GUGCUACAAUGGCGGUGACAGUGGGAAGCGAAGUCGCGA 17
Azs.sp1 1135 GUGCUACAAUGGUGGUGACAGUGGGCAGCGACCACGCGA 17
Azs.sp1375 1143 GUGCUACAAUGGCGGUGACAGUGGGAAGCGAAGUCGAGA 17
Azs.sp4 1131 GUGCUACAAUGGUGGUGACAGUGGGCAGCGACCACGCGA 17
Rhc.centen 1177 GUGCUACAAUGGUGGUGACAGUGGGCAGCGACCACGCGA 17
R.fulvum 1181 GUGCUACAAUGGUGGUGACAGUGGGCAGCCAACUCGCGA 17
R.fulvum2 1154 GUGCUACAAUGGUGGUGACAGUGGGCAGCCAACUCGCGA 17
R.molischi 1185 GUGCUACAAUGGUGGUGACAGUGGGCAGCCAACUCGCGA 17
env.OS_O 57 GUGCUACAAUGGCGGUGACAAUGGGAUGCAAGCCAGCGA 17
Aba.aceti 1161 GUGCUACAAUGGCGGUGACAGUGGGAAGCUAGAUGGCGA 17
Aba.aceti2 1180 GUGCUACAAUGGCGGUGACAGUGGGAAGCUAGAUGGCGA 17
Aba.diaztr 1181 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAGGCAGCGA 17
Aba.europa 1181 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAGGUAGCGA 17
Aba.hansen 1181 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAGGCAGCGA 17
Aba.liqfac 1181 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAGGCAGCGA 17
Adm.metha2 1181 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAGGCAGUGA 17
Aba.xylinm 1181 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAGGUAGCGA 17
Acdp.angu2 1164 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAAGCAGCGA 17
Acdp.angus 837 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAAGCAGCGA 17
Acdp.cryp2 1149 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAGGUGGUGA 17
Acdp.cryp3 1162 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAGGUGGUGA 17
Acdp.crytp 834 GUGCUACUAUGGCGGUGACWGUGGGAAGCCAGGUGGUGA 17
Acdp.organ 1162 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAGGUGGUGA 17
Acdp.rubr2 1164 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAAGCAGCGA 17
Acdp.rubru 933 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAAGCAGCGA 17
Acdp.spC1 1168 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAGGCAGCGA 17
Acdp.spQBP 809 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAAGCAGCGA 17
Adm.methan 1162 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAGGCAGUGA 17
Gb.asaii 1176 GUGCUACAAUGGCGGUGACAGUGGGAAGCUACAUGGCGA 17
Gb.cerinus 1176 GUGCUACAAUGGCGGUGACAGUGGGAAGCUACAUGGCGA 17
Gb.frateur 1176 GUGCUACAAUGGCGGUGACAGUGGGAAGCUAUGUGGUGA 17


Gb.oxydans 1176 GUGCUACAAUGGCGGUGACAGUGGGAAGCUACAUGGUGA 17
Thb.acido2 184 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAGGUGGUGA 17
Thb.acidop 855 GUGCUACAAUGGCGGUGACAGUGGGAAGCCAGGCAGCGA 17
env.SAR102 1134 GUGCUACAAUGGCGGUGACAAAGGGAAGCAAUAGGGCAA 17
Blb.natatr 1175 GUGCUACAAUGGCGGUGACAGUGAGCAGCUAGAUCGCGA 17
Spg.rosa 1161 GUGCUACAAUGGCGGUGACAGUNGGCAGCAAGCACGCGA 17
Spg.spB477 443 GUGCUACAAUGGCGGUGACAGUGAGCAGCUAGAUCGCGA 17
R.salexig2 1152 GUGCUACAAUGGCGGUGACAGAGGGCAGCGAGCCUGCGA 17
R.salexige 1186 GUGCUACAAUGGCGGUGACAGAGGGCAGCGAGCCUGCGA 17
Bdr.japon2 1161 GUGCUACAAUGGCGGUGACAAUGGGAUGCUAAGGGGCGA 17
Bdr.japon4 1187 GUGCUACAAUGGCGGUGACAAUGGGAUGCGAAGGGGCAA 17
Bdr.japon5 1188 GUGCUACAAUGGCGGUGACAAUGGGAUGCUAAGGGGCGA 17
Bdr.japoni 1159 GUGCUACAAUGGCGGUGACAAUGGGAUGCUAAGGGGCGA 17
Bdr.sp0689 1162 GUGCUACAAUGGCGGUGACAAUGGGAUGCUAAGGGGCGA 17
Bdr.sp129 1184 GUGCUACAAUGGCGGUGACAAUGGGAUGCGAAGGCGCAA 17
Bdr.sp9514 1161 GUGCUACAAUGGCGGUGACAAUGGGAUGCUAAGGGGCGA 17
Bdr.sp9520 1161 GUGCUACAAUGGCGGUGACAAUGGGAUGCUAAGGGGCGA 17
Bdr.sp9966 1161 GUGCUACAAUGGCGGUGACAAUGGGAUGCUAAGGGGCGA 17
Bdr.sp9980 1162 GUGCUACAAUGGCGGUGACAAUGGGAUGCUAAGGGGCGA 17
Blb.denitr 1162 GUGCUACAAUGGCGGUGACAGUGGGAAGCUAAGGGGUGA 17
Rps.palus2 1172 GUGCUACAAUGGCGGUGACAAUGGGAUGCUAAGGGGCGA 17
env.JAP850 1156 GUGCUACAAUGGCGGUGACAAUGGGAUGCUAAGGGGCGA 17
env.PAD51 128 GUGCUACAAUGGCGGUGACAAUGGGAUGCUAAGGGGCGA 17
Mcy.parvus 1173 GUGCUACAAUGGCGGUGACCAUGGGGAGCGAGAAGGGCG 17
Mcy.pyrifo 960 GUGCUACAAUGGCGGUGACAAUGGGACGCGAAAGGGUGA 17
Mls.methan 1167 GUGCUACAAUGGCGGUGACAAUGGGAUGCGAAGGGGCGA 17
Msi.sp.LAC 1186 GUGCUACAAUGGCGGUGACAAUGGGAGGCCAAGGGGCGA 17
Msi.sporiu 1179 GUGCUACAAUGGCGGUGACAAUGGGACGCGAAGGGGCGA 17
Bei.indica 1189 GUGCUACAAUGGCGGUGACAAUGGGANGCGAAGGGGCGN 17
Anc.aquati 1185 GUGCUACAAUGGCGGUGACAGUGGGAAGCGAACCUGCGA 17
Aqb.spirit 1185 GUGCUACAAUGGUGGUGACAAUGGGUAGCGAAAGGGCGA 17


Azr.cauli2 1155 GUGCUACAAUGGCGGUGACAAUGGGAUGCGAGCCUGCGA 17
Azr.cauli3 1172 GUGCUACAAUGGCGGUGACAAUGGGAUGCGAGCCUGCGA 17
Rps.IL245 1186 GUGCUACAAUGGCGGUGACAGUGGGAAGCGAAGGGGUGA 17
Rhp.roseu2 1156 GUGCUACAAUGGCGGUGACANUGGGAAGCGAAGGGGCAA 17
Ag.rhizog2 1155 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Ag.rhizogn 1153 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Ag.tumefa7 1171 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Ag.tumefa8 1171 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Ag.tumefa9 1171 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Rhb.etl182 1153 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Rhb.etli42 1158 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Rhb.legu11 1178 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Rhb.legu12 1158 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Rhb.legu13 1156 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Rhb.legum4 1153 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Rhb.legum6 1154 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Rhb.legum8 1155 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Rhb.legumi 232 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Rhb.sp3618 1156 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACAGCGA 17
Rhb.sp9509 1153 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Rhb.spOK50 1177 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGGCCGCGA 17
Rhb.tropi8 1169 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Rhb.tropic 1225 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA 17
Ag.rubi 1157 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACAGCGA 17
Ag.rubi2 1159 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACAGCGA 17
Ag.rubi3 1175 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACAGCGA 17
Ag.tumef10 1175 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACAGCGA 17
Ag.tumefa2 1159 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACAGCGA 17
Ag.tumefa3 1157 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACAGCGA 17
Ag.tumefa4 1159 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACAGCGA 17
Ag.tumefa6 1175 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACAGCGA 17

```
Ag.tumefac    1187 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACAGCGA    17
Ag.vitis      1154 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Ag.vitis2     1155 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Ag.vitis3     1171 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Azs.sp2       1093 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACAGCGA    17
Blb.aggreg    1143 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Blb.capsul    1150 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Srh.fredi2    1158 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Srh.fredi3    1159 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Srh.fredi5    1184 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Rhb.galeg2    1153 GUGCUACAAUGGUGGUGACAGUGGGCAGCGGAGGAGCGA    17
Rhb.galeg3    1155 GUGCUACAAUGGUGGUGACAGUGGGCAGCGNAGGAGCGA    17
Rhb.galega    1171 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAAGGAGCGA    17
Srh.melil3    1157 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Srh.melil4    1159 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Srh.melil7    1183 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Rhb.sp113     1176 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAAGGAGCGA    17
Rhb.sp7834    1157 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Rhb.spOK55    1177 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAAGGAGCGA    17
Srh.saheli    1158 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Srh.terang    1157 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Srh.xinjia    1159 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
str.B0710      692 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Bar.bacil2    1184 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAAACCGCAA    17
Bar.bacil3    1161 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAAACCGCAA    17
Bar.bacill    1177 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAAACCGCAA    17
Bar.doshia    1176 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGGAG    17
Bar.elizab    1172 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Bar.graham    1191 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Bar.quint2    1166 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
Bar.quinta    1191 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
```

```
Bar.taylor    1188 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Bar.vinso2    1178 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Bar.vinso3    1172 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Bar.vinso4    1184 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Bar.vinson    1165 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Bar.hense2     296 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Bar.hense3    1161 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAAGUCGCGA    17
                   ||+|||||||+||||||||
Bar.hensel    1165 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Bru.aborts    1183 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA    17
                   ||+|||||||+||||||||
Bru.canis     1162 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA    17
                   ||+|||||||+||||||||
Bru.melten    1156 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA    17
                   ||+|||||||+||||||||
Bru.neotom    1163 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA    17
                   ||+|||||||+||||||||
Bru.ovis      1163 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA    17
                   ||+|||||||+||||||||
Bru.suis      1163 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA    17
                   ||+|||||||+||||||||
Myp.dmorph    1159 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Ocb.anthro    1157 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGCACGCGA    17
                   ||+|||||||+||||||||
Zoo.ramig2    1158 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACAGCGA    17
                   ||+|||||||+||||||||
Zoo.ramig5    1178 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACAGCGA    17
                   ||+|||||||+||||||||
Plb.myrsin    1159 GUGCUACAAUGGUGGUGACAGUGGGCAGCGNGACCGCGA    17
                   ||+|||||||+||||||||
Plb.rubiac    1159 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Rhb.ciceri    1158 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Rhb.huaku2    1159 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Rhb.huakui    1159 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Rhb.loti2     1158 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Rhb.loti3     1158 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Rhb.loti4     1159 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Rhb.loti5     1180 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Rhb.sp1148    1184 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Rhb.sp7836    1157 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
Thb.sp051     1157 GUGCUACAUCGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
str.ER2       1183 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17
                   ||+|||||||+||||||||
```

```
Rhb.sp7854    1157 GUGCUACAAUGGUGGUGACAGUGGGCAGCGAGACCGCGA    17


Single insertions / deletions (18 is highest score) :
-----------------------------------------------------

             5'end        3' CCGCCACUGUUACCCGUCG 5'         Match
             -----           |||||| ||||||||||||            -----
Fib.intDR7    1224 UGCUACAAUGGGCGGU-ACAAUGGGCAGCGGGGCCGCGA    18

             5'end        3' CCGCCACUGUUACCCGUCG 5'         Match
             -----           |||||| ||||||||||||            -----
Fib.intNR9    1228 UGCUACAAUGGGCGGU-ACAAUGGGCAGCGGGGCCGCGA    18

             5'end        3' CCGCCACUGUUACCCGUCG 5'         Match
             -----           |||||||||||||||| |||            -----
Afp.cleve     1189 GUGCUACAAUGGCGGUGACAAUGGG-AGCAAAGGGGCGA    18

             5'end        3' CCGCCACUGUUACCCGUCG 5'         Match
             -----           |||||||||||||||| |||            -----
Afp.felis     1189 GUGCUACAAUGGCGGUGACAAUGGG-AGCAAAGGCGCGA    18

             5'end        3' CCGCCACUGUUACCCGUCG 5'         Match
             -----           |||||||||||||||| |||            -----
Mlb.sp.M27    1179 GUGCUACAAUGGCGGUGACARUGGG-AGCGAAGCGCGCG    18

             5'end        3' CCGCCACUGUUACCCGUCG 5'         Match
             -----           |||||| ||||||||||||            -----
env.PAD21      114 CGCUACAAUGGGCGGU-ACAAUGGGCAGCGAAGGGGCGA    18

             5'end        3' CCGCCACUGUUACCCGUCG 5'         Match
             -----           | |||| ||||||||||||            -----
Rht.marin2    1236 UGCUACAAUGGCCGGU-ACAAUGGGCAGCCACCCCGCGA    17

             5'end        3' CCGCCACUGUUACCCGUCG 5'         Match
             -----           | |||| ||||||||||||            -----
Rht.marinu    1236 UGCUACAAUGGCCGGU-ACAAUGGGCAGCCACCCCGCGA    17

             5'end        3' CCGCCACUGUUACCCGUCG 5'         Match
             -----           ||||||| ||+||||||||            -----
str.B712         2             UGGCGGUG-CAGUGGGCAGCAAACUCGCGA    17

             5'end        3' CCGCCACUGUUACCCGUCG 5'         Match
             -----           ||||||||||+|||| |||            -----
Mlb.extor2    1180 GUGCUACRAUGGCGGUGACAGUGGR-AGCGAAGCNGCGA    17

             5'end        3' CCGCCACUGUUACCCGUCG 5'         Match
             -----           ||||||||||+|||| |||            -----
Mlb.zatman    1178 GUGCUACAAUGGCGGUGACAGUGGG-AGCGAAACCGCGA    17
```

```
            5'end        3' CCGCCACUGUUACCCGUCG 5'        Match
            -----           ||||||||||||||| | |           -----
Msi.tricho   1187 GUGCUACAAUGGCGGUGACAAUGGG-AACGACGAMGUGA    17

            5'end        3' CCGCCACUGUUACCCGUCG 5'        Match
            -----           ||||||  ||||  |||||||          -----
env.FL5_2    1117 UGCUACAAUGGGCGGU-ACAAAGGGCAGCGAAGCCGCGA    17

            5'end        3' CCGCCACUGUUACCCGUCG 5'        Match
            -----           ||||||  ||||  |||||||          -----
C.litorale   1225 UGCUACAAUGGGCGGU-ACAACGGGCAGCAAUCUCGCGA    17

            5'end        3' CCGCCACUGUUACCCGUCG 5'        Match
            -----           ||||||  ||||  |||||||          -----
Spl.inuli2   1223 UGCUACAAUGGGCGGU-ACAAAGGGCAGCGAAACCGCAA    17

            5'end        3' CCGCCACUGUUACCCGUCG 5'        Match
            -----           ||||||  ||||  |||||||          -----
Spl.inuli3   1223 UGCUACAAUGGGCGGU-ACAAAGGGCAGCGAAACCGCAA    17

            5'end        3' CCGCCACUGUUACCCGUCG 5'        Match
            -----           ||||||  ||||  |||||||          -----
Spl.inulin   1250 UGCUACAAUGGGCGGU-ACAAAGGGCAGCGAAACCGCAA    17

            5'end        3' CCGCCACUGUUACCCGUCG 5'        Match
            -----           ||||||  ||||  |||||||          -----
Spl.racmi2   1223 UGCUACAAUGGGCGGU-ACAAAGGGCAGCGAAACCGCAA    17
```

Appendix I: Complete sequence of 3M3 16s rRNA.

CAGTGTGATGGATATCTGCAGAATTCGGCTTTTTGATCATGGCTCAGAAC
GAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGCCCCGCAAGGG
GAGTGGCAGACGGGTGAGTAACACGTGGGAACCTTCCCTATAGTACGGA
ATAGCCCAGGGNAAACTTGGAGTAATACCGTATACGCCCGAAAGGGGAA
AGAATTTCGCTATAGGATGGGCCCGCGTAGGATTAGCTAGTTGGTGAGGT
AATGGCTCACCAAGGCGACGATCCTTAGCTGGTTTGAGAGAACGACCAGC
CACACTGGGACTGAGACACGGCCCCAACTCCTACGGGAGGCAGCNAGTGG
GGAATATTGGAACAATGGGGCGCAAAGCCTGAATCCACCATGCGGCGTGA
GTGATGAAGGCTTAGGGGTTGTAAAGNTTTTTGGGCGGGGACGATAATGA
CGGTAACCGCAGAATAAGTCCCGGCTAAATTTCGTGCCAG CAGCCGCGGT
AATACGGAAGGGATAGCGTTGTTCGGAATCACTGGGCGTAAAGCGCACGT
AGGCGGATATGCCAGTCAGGGGTGAAATCCCGGGGCTCAACCTCGGAACT
GCCCTTGATACAGCATGTCTTGAGTCCGATAGAGGTGGGTGGAATTCCTAG
TGTAGAGGTGAAATTCGTAGATATTAGGAAGAACACCGGTGGCGAAGGCG
GCCCACTGGATCGGTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAAC
AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGGATGCTAGCCGTC
GGATAGCTTGCTATTCGGTGGCGCAGCTAACGCATTAAGCATCCCGCCTGG
GGAGTTACGGCCGCAAGGTTAAAACTCAAAGGAATTGACGGGGGCCCGGC
NGCACAAGCGGTGGAGCATGTGGTTTAATTCGACGCAACGCGAAGAACCTT
ACCAGCTCTTGACATTCACTGATTGCCGGTANAGATGCCGGAGTTCCAGCA
ATGGACAGTGGGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGA
TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCATTAGTTGCCATCAT
TCAGTTGGGCACTCTAGTGGGACTGCCGGTGATAAGCCGGAGGAAGGTGGG
GATGACGTCAAGTCATCATGGCCCTTACGGGCTGGGCTACACACGTTGCTAC
AATGGCGGTGACAATGCGCAGCCAACCTAGTAATAGGGAGCTAATCGCAAA
AAGCCGTCTCAGTTCAGATTGAGGTCTGCAACTCGACCTCATGAAGTCGGAA
TCGCTAGTAATCGCGCATCAGCATGGCGCGGTGAATACGTTCCCGGGCCTTG
TACACACCGCCCGTCACACCATGGGAGTTGGTCTTACCCTAAAACGGTGCGC
TAACCGCAAGGAGGCAGCCGGCCACGGTAAGGTCAGCGACTGGGGTGAAGT
CGTAACAAGGTAGCCGTA

Appendix J: Complete sequence of 6M3 16s rRNA.

AGAGTTTGATCATGGCTCAGAACGAACGCTGGCGGCAGGCCTAACACATGCAA
GTCGAACGCCCCGCAAGGGGAGTGGCAGACGGGTGAGTAACACGTGGGAACCT
TCCCTATAGTACGGAATAGCCCAGGGGAAACTTGGAGTAATACCGTATACGCCC
GAAAGGGGAAAGAATTTCGCTATAGGATGGGCCCGCGTAGGATTAGCTAGTTGG
TGAGGTAATGGCTCACCAAGGCGACGATCCTTAGCTGGTTTGAGAGAACGACC
AGCCACACTGGGACTGAGACACGGCCCCAGACTCCTACGGGGAGGCAGCAGTGG
GGAATATTGGACAATGGGCGCAAGCCTGATCAGCCATGCCGCGTGAGTGATGAA
GGCTTAAGGGTTGTAAAGNTTTTTGGCGGGGGACGATAATGACGGTAACCCGCA
GAATAAGTCCCGGCTAAATTCGTGCCAGCAGCCGCGGTAATACGAAGGGGACTA
GCGTTGTTCGGAATCACTGGGCGTAAAGCGCACGTAGGCGGATATGCCAGTCAG
GGGTGAAATCCCGGGGCTCAACCTCGGAACTGCCCTTGATACAGCATGTCTTGAG
TCCGATAGAGGTGGGTGGAATTCCTAGTGTAGAGGTGAAATTCGTAGATATTAGG
AAGAACACCGGTGGCGAAGGCGGCCCACTGGATCGGTACTGACGCTGAGGTGCGA
AAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG
GATGCTAGCCGTCGGATAGCTTGCTATTCGGTGGCGCAGCTAACGCATTAAGCATC
CCGCCTGGGGAGTTACGGCCGCAAGGTTAAAACTCAAAGGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGACGCAACGCGAAGAAACTTACCAG
CTCTTGACATTCACTGATTGCCGGTANAGATGCCGGAGTTCCAGCAATGGACAGTG
GGACAGGTGCTGCATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCC
CGCAACGAGCGCAACCCTCGCCATTAGTTGCCATCATTCAGTTGGGCACTCTAGTGG
GACTGCCGGTGATAAGCCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCT
TACGGGCTGAGCTACACACGTTGCTACAATGGCGGTGACAATGCGCAGCCACCTAG
TTAATAGGGGCTAATCGCAAAAAGCCGTCTCAGTTCAGATTGAGGTCTGCAACTCGA
CCTCATGAAGTCGGAATCGCTAGTAATCGCGCATCAGCATGGCGCGGTGAATACGT
TCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTCTTACCCTAAAAC
GGTGCGCTAACCGCAAGGAGGCAGCCGGCCACGGTAAGGTCAGCGACTGGGGTGAA
GTCGTAACAAGGTAACCGTA

Appendix K: Similarity_Rank results for 3M3 and 6M3 clones of *Hyphomicrobium* on submission to RDP database.

1395 **3M3** Unknown

S_ab	Olis	Short-ID	Full name
0.640	1374	str.Hyp353	str. US-353.
0.638	1315	Hyp.vulgar	Hyphomicrobium vulgare str. MC-750.
0.583	216	Thb.acido2	Thiobacillus acidophilus.
0.583	218	Cau.cresc5	Caulobacter crescentus str. CB13.
0.583	218	Cau.cresc6	Caulobacter crescentus str. CB15.
0.577	267	env.PAD26	str. PAD26.
0.577	562	Spg.spB522	Sphingomonas sp. str. B522.
0.568	1222	Rhp.roseu2	Rhodoplanes roseus str. 941.
0.566	267	env.PAD53	str. PAD53.
0.564	1193	Rps.spCPB2	Rhodopseudomonas sp. str. CP-B-2.
0.564	1193	Rps.spCPB3	Rhodopseudomonas sp. str. CP-B-3.
0.562	1017	Srh.fredi4	Sinorhizobium fredii.
0.561	1336	Rhp.roseus	Rhodoplanes roseus str. 941.
0.561	1329	Rdb.orient	Rhodobium orientis str. MB312.
0.558	1325	Rhp.elegan	Rhodoplanes elegans str. AS130.
0.557	1365	Rhb.sp7854	Rhizobium sp.
0.557	1365	Rhb.sp7836	Rhizobium sp.
0.557	1211	Rps.ANTP1	Rhodopseudomonas sp. str. ANT-P-1.
0.556	1366	Azr.cauli2	Azorhizobium caulinodans.
0.556	599	Spg.mali	Sphingomonas mali str. Y-347.

1368 **6M3** Unknown

S_ab	Olis	Short-ID	Full name
0.658	1374	str.Hyp353	str. US-353.
0.652	1315	Hyp.vulgar	Hyphomicrobium vulgare str. MC-750.
0.593	1193	Rps.spCPB2	Rhodopseudomonas sp. str. CP-B-2.
0.593	1193	Rps.spCPB3	Rhodopseudomonas sp. str. CP-B-3.
0.590	1329	Rdb.orient	Rhodobium orientis str. MB312.
0.579	1222	Rhp.roseu2	Rhodoplanes roseus str. 941.
0.578	218	Cau.cresc5	Caulobacter crescentus str. CB13.
0.578	218	Cau.cresc6	Caulobacter crescentus str. CB15.

0.576 599 Spg.mali Sphingomonas mali str. Y-347.
0.573 1336 Rhp.roseus Rhodoplanes roseus str. 941.
0.573 1017 Srh.fredi4 Sinorhizobium fredii.
0.573 1256 str.B3060 str. B-3060.
0.573 1396 Azr.cauli3 Azorhizobium caulinodans.
0.573 1344 Bru.canis Brucella canis.
0.573 1325 Rhp.elegan Rhodoplanes elegans str. AS130.
0.571 1211 Rps.ANTP1 Rhodopseudomonas sp. str. ANT-P-1.
0.571 1404 Zoo.ramig5 Zoogloea ramigera.
0.569 267 env.PAD26 str. PAD26.
0.568 1365 Rhb.sp7854 Rhizobium sp.
0.568 1365 Rhb.sp7836 Rhizobium sp.

Appendix L: Align_Sequence results for 3M3 and 6M3 clones of *Hyphomicrobium* on submission to RDP database.

3M3

```
str.Hyp353    0 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CUCAGAACGAACGCUGGCGG
3M3           1 CAGUGUGAUGGAUAUCUGCAGAAUUCGGCUUUUUGAUCAUGGCUCAGAACGAACGCUGGCGG
+++++++++1    1 >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
str.Hyp353   21 CAGGCCUAACACAUGCAAGUCGAACGCUGUAGCAAUACAGAGUGGCAGACGGGUGAGUAACA
3M3           63 CAGGCCUAACACAUGCAAGUCGAACGCCCCGCAAGGG--GAGUGGCAGACGGGUGAGUAACA
+++++++++1   42 ------------------------------++++++++++-----------------------
str.Hyp353   83 CGUGGGAAUCUUCCUAUCGGUACGGAAUAGCUCAGGG-AAACUUGGGGUAAUACCGCAUACG
3M3          123 CGUGGGAACCUUCCCUAUAGUACGGAAUAGCCCAGGGNAAACUUGGAGUAAUACCGUAUACG
+++++++++1   54 ------------------------------------@------------------------
str.Hyp353  144 CCCUUC-GGGGNAAAGAUUUAUCGCCGAUAGAUGAGCCCGCGUCUGAUUAGCUAGUUGGUGA
3M3          185 CCCGAAAGGGGAAAGAAUUUCGCUAUAG--GAUGGGCCCGCGUAGGAUUAGCUAGUUGGUGA
+++++++++1   55 ---+++@-------------++++++++++-----------------------------------
str.Hyp353  205 GGUAAUGGCUCACCAAGGCGACGAUCAGUAGCUGGUCUGAGAGGAUGACCAGCCACACUGGG
3M3          245 GGUAAUGGCUCACCAAGGCGACGAUCCUUAGCUGGUUUGAGAGAACGACCAGCCACACUGGG
+++++++++1   69 ------------------------------------------------------------------
str.Hyp353  267 ACUGAGACACGGCCCAGACUCCUACGGGAGGCAGCA-GUGGGGAAUAUUGGA-CAAUGGGCG
3M3          307 ACUGAGACACGGCCCCAACUCCUACGGGAGGCAGCNAGUGGGGAAUAUUGGAACAAUGGGGC
+++++++++1   69 ------------------------------------@---------------@-------++
str.Hyp353  327 C--AAGCCUGAUCCAGCCAUGCCGCGUGAGUGACGAAGGUCUUCGGAUUGUAAAGCUCUUUU
3M3          369 GCAAAGCCUGAAUCCACCAUGCGGCGUGAGUGAUGAAGGCUUAGGGGUUGUAAAGNUUUUUG
+++++++++1   74 +@@-------------------------------------------------------------
str.Hyp353  387 GGCGGGGACGAUAAUGACGGUACCCGCAGAAUAAGCCCCGGCUAAC-UUCGUGCCAGCAGCC
3M3          431 GGCGGGGACGAUAAUGACGGUAACCGCAGAAUAAGUCCCGGCUAAAAUUUCGUGCCAGCAGCC
+++++++++1   76 ------------------------------------------+@---------------
str.Hyp353  448 GCGGUAAUACGAAGGGGGCUAGCGUUGUUCGGAAUCACUGGGCGUAAAGCGCACGUAGGCGG
3M3          493 GCGGUAAUACGGAAGGGA-UAGCGUUGUUCGGAAUCACUGGGCGUAAAGCGCACGUAGGCGG
+++++++++1   78 ----------+++++++-----------------------------------------------
str.Hyp353  510 AUUCGUAAGUCAGGGGUGAAAUCCCGGGGCUCAACCUCGGAACUGCCUUUGAUACUGACAGU
3M3          554 AUAUGCCAGUCAGGGGUGAAAUCCCGGGGCUCAACCUCGGAACUGCCCUUGAUACAGCAUGU
+++++++++1   86 ------------------------------------------------------------------
str.Hyp353  572 CUUGAGUCCGGAAGAGGUGAGUGGAAUUCCUUGUGUAUAGGUGAAAUUCGUAGAUAUUAGGA
3M3          616 CUUGAGUCCGAUAGAGGUGGGUGGAAUUCCUAGUGUAGAGGUGAAAUUCGUAGAUAUUAGGA
+++++++++1   86 ------------------------------------------------------------------
str.Hyp353  634 AGAACACCAGUGGCGAAGGCGGCUCACUGGUCCGGUACUGACUCUGAGGUGCGAAAGCGUGG
3M3          678 AGAACACCGGUGGCGAAGGCGGCCCACUGGAUCGGUACUGACGCUGAGGUGCGAAAGCGUGG
+++++++++1   86 ------------------------------------------------------------------
str.Hyp353  696 GGAUUAAACAGGAUUAGAUACCCUG-UAGUCCACGCCGUAAACUAUGGAUGCUAGCCGUCGG
3M3          740 GGAGCAAACAGGAUUAGAUACCCUGGUAGUCCACGCCGUAAACGAUGGAUGCUAGCCGUCGG
+++++++++1   86 ------------------------@-----------------------------------------
str.Hyp353  757 NAAGCUUGCUUGUCGGUGGCGCAGCUAACGCUUUAAGCAUCCCGCCUGGGGAGU-ACGGCCG
3M3          802 AUAGCUUGCUAUUCGGUGGCGCAGCUAACGCAUUAAGCAUCCCGCCUGGGGAGUUACGGCCG
+++++++++1   87 ------------------------------------------------------@-------
str.Hyp353  818 CAAGNUUAAAACUCAAAGGAAUUGACGGGGGCCCG----CACAAGCGGUGGAGCAUGUGGUU
3M3          864 CAAGGUUAAAACUCAAAGGAAUUGACGGGGGCCCGGCNGCACAAGCGGUGGAGCAUGUGGUU
+++++++++1   88 ------------------------------------@@@@----------------------
str.Hyp353  876 UAAUUCGACGCAACGCNAAGAACCUUACCAGCUCUUGACAUUUACGGACCGUUUCCAGAGAU
3M3          926 UAAUUCGACGCAACGCGAAGAACCUUACCAGCUCUUGACAUUCACUGAUUGCCGGUANAGAU
+++++++++1   92 ------------------------------------------+++++++++++++++++++
str.Hyp353  938 GGAUUCAUCCUAGCANAUAGGCCGUAGGACAGGUGCUGCGUGGCUGUCGUCAGCUCGUGUCG
3M3          988 GCCGGAGUUCCAGCAAUGGACAGUG-GGACAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCG
+++++++++1  113 ++++++++++++++++++++++++++-----------------------------------------
str.Hyp353 1000 UGAGAUGUUGGGUUAAGUCCCGCAACGAGCGCAACCCUCGCCAUUAGUUGCCAUCAUUUAGU
3M3         1049 UGAGAUGUUGGGUUAAGUCCCGCAACGAGCGCAACCCUCGCCAUUAGUUGCCAUCAUUCAGU
+++++++++1  138 ------------------------------------------------------------------
```

```
str.Hyp353 1062 UGGGCACUCUAGUGGGACUGCCGGUGAUAAGCUGGNAGGAAGGUGGGGAUGACGUCAAGUCA
3M3         1111 UGGGCACUCUAGUGGGACUGCCGGUGAUAAGCCGG-AGGAAGGUGGGGAUGACGUCAAGUCA
+++++++++1  138 ------------------------------++++----------------------------
str.Hyp353 1124 UCAUGGCCCUUACGGGCUGGGCUACACACGU-GCUACAAUGGCGGUGACAAUGGGCAG-CAA
3M3         1172 UCAUGGCCCUUACGGGCUGGGCUACACACGUUGCUACAAUGGCGGUGACAAUGCGCAGCCAA
+++++++++1  142 ------------------------------@----------------------+++++@---
str.Hyp353 1184 CACAGCAAUGUGAAGCUAAUCUCAAAAAGCCGUCUCAGUUCGGAUUGGGCUCUGCAACUCGA
3M3         1234 CCUAGUAAUAGGGAGCUAAUCGCAAAAAGCCGUCUCAGUUCAGAUUGAGGUCUGCAACUCGA
+++++++++1  149 ------------------------------------------------------------
str.Hyp353 1246 GCCCAUGAAGUUGGAAUCGCUAGUAAUCGCGCAUCAGCAUGGCGCGGUGAAUACGUUCCCGG
3M3         1296 CCUCAUGAAGUCGGAAUCGCUAGUAAUCGCGCAUCAGCAUGGCGCGGUGAAUACGUUCCCGG
+++++++++1  149 ------------------------------------------------------------
str.Hyp353 1308 GCCUUGUACACACCGCCCGUCACACCAUGGGAGUUGGUUUUACCCGAAGACGGUGCGCUAAC
3M3         1358 GCCUUGUACACACCGCCCGUCACACCAUGGGAGUUGGUCUUACCCUAAAACGGUGCGCUAAC
+++++++++1  149 ------------------------------------------------------------
str.Hyp353 1370 CGCAAGGAGGCAGCCGGCCACGGUAAGGUCAGCGACUGGGGUGAAGUCGUAACAAGGUAGCC
3M3         1420 CGCAAGGAGGCAGCCGGCCACGGUAAGGUCAGCGACUGGGGUGAAGUCGUAACAAGGUAGCC
+++++++++1  149 ------------------------------------------------------------
str.Hyp353 1432 GUAGGGGAAC
3M3         1482 GUA~~~~~~~
+++++++++1  149 ---<<<<<<<
```

6M3

```
str.Hyp353    0 ~~~~~~~~~~~~~~~CUCAGAACGAACGCUGGCGGCAGGCCUAACACAUGCAAGUCGAACGC
6M3           1 AGAGUUUGAUCAUGGCUCAGAACGAACGCUGGCGGCAGGCCUAACACAUGCAAGUCGAACGC
+++++++++1    1 >>>>>>>>>>>>>>>----------------------------------------------------
str.Hyp353   48 UGUAGCAAUACAGAGUGGCAGACGGGUGAGUAACACGUGGGAAUCUUCCUAUCGGUACGGAA
6M3           63 CCCGCAAGGG--GAGUGGCAGACGGGUGAGUAACACGUGGGAACCUUCCCUAUAGUACGGAA
+++++++++1    16 ++++++++++++----------------------------------------------------
str.Hyp353  110 UAGCUCA-GGGAAACUUGGGGUAAUACCGCAUACGCCCUUC-GGGGNAAAGAUUUAUCGCCG
6M3           123 UAGCCCAGGGGAAACUUGGAGUAAUACCGUAUACGCCCGAAAGGGGAAAGAAUUUCGCUAUA
+++++++++1    27 ----+++@----------------------------+++@-------------+++++++
str.Hyp353  170 AUAGAUGAGCCCGCGUCUGAUUAGCUAGUUGGUGAGGUAAUGGCUCACCAAGGCGACGAUCA
6M3           185 G--GAUGGGCCCGCGUAGGAUUAGCUAGUUGGUGAGGUAAUGGCUCACCAAGGCGACGAUCC
+++++++++1    43 +++----------------------------------------------------------
str.Hyp353  232 GUAGCUGGUCUGAGAGGAUGACCAGCCACACUGGGACUGAGACACGGCCC-AGACUCCUAC-
6M3           245 UUAGCUGGUUUGAGAGAACGACCAGCCACACUGGGACUGAGACACGGCCCCAGACUCCUACG
+++++++++1    45 ----------------------------------------------@----------@
str.Hyp353  292 GGGAGGCAGCAGUGGGGAAUAUUGGACAAUGGGCGCAAGCCUGAUCCAGCCAUGCCGCGUGA
6M3           307 GGGAGGCAGCAGUGGGGAAUAUUGGACAAUGGGCGCAAGCCUGAUC-AGCCAUGCCGCGUGA
+++++++++1    47 ----------------------------------------------+-----------
str.Hyp353  354 GUGACGAAGGUCUUCGGAUUGUAAAGCUCUUUUGGCGGGGACGAUAAUGACGGUA-CCCGCA
6M3           368 GUGAUGAAGGCUUAAGGGUUGUAAAGNUUUUUGGCGGGGGACGAUAAUGACGGUAACCCGCA
+++++++++1    48 ----------------------------------------------------@------
str.Hyp353  415 GAAUAAGCCCCGGCUAACUUCGUGCCAGCAGCCGCGGUAAUACGAAGGGGGCUAGCGUUGUU
6M3           430 GAAUAAGUCCCGGCUAAAUUCGUGCCAGCAGCCGCGGUAAUACGAAGGGGACUAGCGUUGUU
+++++++++1    49 ----------------------------------------------------------
str.Hyp353  477 CGGAAUCACUGGGCGUAAAGCGCACGUAGGCGGAUUCGUAAGUCAGGGGUGAAAUCCCGGGG
6M3           492 CGGAAUCACUGGGCGUAAAGCGCACGUAGGCGGAUAUGCCAGUCAGGGGUGAAAUCCCGGGG
+++++++++1    49 ----------------------------------------------------------
str.Hyp353  539 CUCAACCUCGGAACUGCCUUUGAUACUGACAGUCUUGAGUCCGGAAGAGGUGAGUGGAAUUC
6M3           554 CUCAACCUCGGAACUGCCCUUGAUACAGCAUGUCUUGAGUCCGAUAGAGGUGGGUGGAAUUC
+++++++++1    49 ----------------------------------------------------------
str.Hyp353  601 CUUGUGUAUAGGUGAAAUUCGUAGAUAUUAGGAAGAACACCAGUGGCGAAGGCGGCUCACUG
6M3           616 CUAGUGUAGAGGUGAAAUUCGUAGAUAUUAGGAAGAACACCGGUGGCGAAGGCGGCCCACUG
+++++++++1    49 ----------------------------------------------------------
str.Hyp353  663 GUCCGGUACUGACUCUGAGGUGCGAAAGCGUGGGGAUUAAACAGGAUUAGAUACCCUG-UAG
6M3           678 GAUCGGUACUGACGCUGAGGUGCGAAAGCGUGGGGAGCAAACAGGAUUAGAUACCCUGGUAG
+++++++++1    49 ----------------------------------------------------------@---
```

```
str.Hyp353  724 UCCACGCCGUAAACUAUGGAUGCUAGCCGUCGGNAAGCUUGCUUGUCGGUGGCGCAGCUAAC
6M3         740 UCCACGCCGUAAACGAUGGAUGCUAGCCGUCGGAUAGCUUGCUAUUCGGUGGCGCAGCUAAC
+++++++++1   50 ----------------------------------------------------------
str.Hyp353  786 GCUUUAAGCAUCCCGCCUGGGGAGU-ACGGCCGCAAGNUUAAAACUCAAAGGAAUUGACGGG
6M3         802 GCAUUAAGCAUCCCGCCUGGGGAGUUACGGCCGCAAGGUUAAAACUCAAAGGAAUUGACGGG
+++++++++1   50 ------------------------@---------------------------------
str.Hyp353  847 GGCCCGCACAAGCGGUGGAGCAUGUGGUUUAAUUCGACGCAACGCNAAGAACCUUACCAGCU
6M3         864 GGCCCGCACAAGCGGUGGAGCAUGUGGUUUAAUUCGACGCAACGCGAAGAAACUUACCAGCU
+++++++++1   51 ----------------------------------------------------------
str.Hyp353  909 CUUGACAUUUACGGACCGUUUCCAGAGAUGGAUUCAUCCUAGCANAUAGGCCGUAGGACAGG
6M3         926 CUUGACAUUCACUGAUUGCCGGUANAGAUGCCGGAGUUCCAGCAAUGGACAGUG-GGACAGG
+++++++++1   51 ---------++++++++++++++++++++++++++++++++++++++++++++++-------
str.Hyp353  971 UGCUGCGUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGCGCA
6M3         987 UGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAUGUUGGGUUAAGUCCCGCAACGAGCGCA
+++++++++1   97 ----------------------------------------------------------
str.Hyp353 1033 ACCCUCGCCAUUAGUUGCCAUCAUUUAGUUGGGCACUCUAGUGGGACUGCCGGUGAUAAGCU
6M3         1049 ACCCUCGCCAUUAGUUGCCAUCAUUCAGUUGGGCACUCUAGUGGGACUGCCGGUGAUAAGCC
+++++++++1   97 ----------------------------------------------------------+
str.Hyp353 1095 GGNAGGAAGGUGGGGAUGACGUCAAGUCAUCAUGGCCCUUACGGGCUGGGCUACACACG-UG
6M3         1111 GG-AGGAAGGUGGGGAUGACGUCAAGUCAUCAUGGCCCUUACGGGCUGAGCUACACACGUUG
+++++++++1   99 +++----------------------------------------------------@--
str.Hyp353 1156 CUACAAUGGCGGUGACAAUGGGCAGCAACACAGCAAUGUGAAGCUAAUCUCAAAAAGCCGUC
6M3         1172 CUACAAUGGCGGUGACAAUGCGCAGCCACCUAGUUAAUAGGGGCUAAUCGCAAAAAGCCGUC
+++++++++1  102 ----------------------------------------------------------
str.Hyp353 1218 UCAGUUCGGAUUGGGCUCUGCAACUCGAGCCCAUGAAGUUGGAAUCGCUAGUAAUCGCGCAU
6M3         1234 UCAGUUCAGAUUGAGGUCUGCAACUCGACCUCAUGAAGUCGGAAUCGCUAGUAAUCGCGCAU
+++++++++1  102 ----------------------------------------------------------
str.Hyp353 1280 CAGCAUGGCGCGGUGAAUACGUUCCCGGGCCUUGUACACACCGCCCGUCACACCAUGGGAGU
6M3         1296 CAGCAUGGCGCGGUGAAUACGUUCCCGGGCCUUGUACACACCGCCCGUCACACCAUGGGAGU
+++++++++1  102 ----------------------------------------------------------
str.Hyp353 1342 UGGUUUUACCCGAAGACGGUGCGCUAACCGCAAGGAGGCAGCCGGCCACGGUAAGGUCAGCG
6M3         1358 UGGUCUUACCCUAAAACGGUGCGCUAACCGCAAGGAGGCAGCCGGCCACGGUAAGGUCAGCG
+++++++++1  102 ----------------------------------------------------------
str.Hyp353 1404 ACUGGGGUGAAGUCGUAACAAGGUAGCCGUAGGGGAAC
6M3         1420 ACUGGGGUGAAGUCGUAACAAGGUAACCGUA~~~~~~~
+++++++++1  102 ---------------------------------<<<<<<<
```

VITA

Prakash Koderi Narayana Karanth was born in Byatarayanapura, Bangalore City, Karnataka State, INDIA on December 13, 1968. After getting his primary school education in Sri Vinayaka Vidya Kendra, he graduated from Gandhinagar Higher Secondary School in 1984. He got his Pre-University degree from Hyderabad Karnataka Education Society, Bangalore in 1986. He received the Bachelor of Science degree in Dairy Technology from the Dairy Science College, University of Agricultural Sciences, Bangalore in 1992. In August 1994, he was enrolled in the University of Tennessee, Knoxville for his graduate studies in the Biotechnology Program. He worked under the guidance of Dr. Gary Sayler (Director, Center for Environmental Biotechnology), for his Master of Science degree in Life Sciences.