The Full Genome Sequence of an Antarctic Microbe Constructed Using a Rapid, Portable Sequencer and a Hybrid Assembly

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Introduction

Blood Falls in the McMurdo Dry Valleys, Antarctica is home to a diverse makeup of microbial life. These microbes and their activities hold clues to ecosystem function and adaptation to extreme conditions. The genomes of these microbes can be sequenced to unlock these clues. A *Shewanella* sp. was isolated from Blood Falls and, because of its cultivability, serves as a model organism to study. In this investigation, the genome of the *Shewanella* strain BF02_SheW was sequenced and analyzed to explore the lifestyle of Antarctic extremophiles. Here a genome sequencing pipeline for field use was developed. Ultimately, we aim to modify our pipeline for remote genome sequencing to extract information from microbial field samples in remote locations allowing us to understand microbial life in environmental conditions.

**Figure 1** (right): Blood Falls, Taylor Glacier, Antarctica. Location from which *Shewanella* strain BF02_SheW was cultivated.1

Oxford Nanopore Sequencing

Genomic DNA was extracted from a pure culture of *Shewanella* strain BF02_SheW using the MoBio PowerSoil DNA isolation kit. An average DNA concentration was found to be 28.9 ng/µl with an optical density at 260/280 of 2.15 and an OD 260/230 of 2.01. Gel electrophoresis showed the average base length of the genomic DNA to be approximately 9-10 kb. The recommended DNA quality for the Oxford Nanopore Technologies (ONT) pipeline is OD 260/280 of 1.8 and OD 260/230 of 2.0-2.2, an average fragment size to be >50kb, and 400 ng of genomic material to be loaded into the flowcell.

Despite the genomic material failing to meet the ONT guidelines for sequencing, the material was prepared according to the ONT SKQ-RAD02 Rapid Sequencing protocol and loaded into the FLOWMIN106 (R9.4 SpotON) flowcell. Using the ONT MinKNOW software and the Metrichor live basecalling function, the sample was sequenced for 26 hours.

**Figure 2** (above): a) The Oxford Nanopore open to show the flowcell; b) the Oxford Nanopore closed next to a penny to show size.

Hybrid Genome Assembly

**Figure 3**: MinKNOW interface showing individual pores while sequencing the genomic data.

**Figure 4**: Nanopore sequencing graphic.

**Figure 5**: Illumina HiSeq next to person for size comparison.

**Figure 6**: A graphic depicting Illumina sequencing.5

**Figure 7**: STEM image of the *Shewanella* strain BF02_Shew isolate showing a flagella, extracellular vesicles, and potentially nanowires.

**Figure 8**: Quast visualization of the *Shewanella* strain BF02_SheW genome.

**Figure 9**: Annotation data generated by GhostKOALA software classifying gene function.

**Figure 10**: Genes associated with polar flagella assembly and function. Green boxes indicate genes identified by GhostKOALA.7

**Figure 11**: Two component regulatory system leading to flagellar assembly. Red boxes indicate identified genes.5

Links Between Phenotype and Genotype

As seen in Figure 12, *Shewanella* strain BF02_SheW is a cold tolerant organism, and grows more quickly at 10 °C and 15 °C. The *flagL* gene, found in this genome, is related to cold and pressure tolerance in microorganisms. The gene has the potential to be horizontally transferred from *Martinsonella* to *Shewanella* strain BF02_SheW.

**Figure 12**: Growth curve of *Shewanella* strain BF02_SheW in marine broth.5

**Figure 13** (left): Test tube showing the crystal violet stained biofilm of *Shewanella* strain BF02_SheW.5

**Figure 14**: The two component regulatory system coding for quorum sensing.7

**Figure 15**: Biofilm Formation workflow. Dysregulation of the response regulator can have implications for the growth of *Shewanella* strain BF02_SheW.

**Figure 16**: A graphic depicting quorum sensing and the *Shewanella* strain BF02_SheW biofilm formation.

**Figure 17**: Diagram illustrating the quorum sensing system and its association with *Shewanella* strain BF02_SheW biofilm formation.

**Figure 18**: The *Shewanella* strain BF02_SheW biofilm is influenced by the quorum sensing system.

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References:

2. Oxford Nanopore Technologies (nanoporetech.com)
3. Illumina (youtube.com/user/IlluminaInc)