May 18, 1966

To the Graduate Council:

I am submitting herewith a thesis written by Garland S. Teague, Jr., entitled "Submerged Culture Production of 5'-Phosphodiesterase by Streptomyces albus." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology.

Major Professor

We have read this thesis and
recommend its acceptance:

John T. Smith

Jeanine L. Collins

Ivan E. McCarty

Accepted for the Council:

Dean of the Graduate School

SUBMERGED CULTURE PRODUCTION OF 5'-PHOSPHODIESTERASE BY *STREPTOMYCES ALBUS*

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Garland S. Teague, Jr.

June 1966

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CHAPTER I

INTRODUCTION

The flavor enhancing properties of certain 5'-ribonucleotides have been known for some time. In 1913, Kodama (20)* reported on the seasoning effect of inosine-5-phosphate (5'-IMP). This compound was also identified as one of the important beef flavor precursors by Batzer and LandMann (3). Kuninaka (21) found that guanosine-5-phosphate (5'-GMP) and xanthine-5-phosphate (5'-XMP) also had a flavoring effect similar to 5'-IMP.

Snake venom (16) and intestional mucosa (6) were known as sources of 5'-phosphodiesterase capable of hydrolyzing the phosphate ester linkages of ribonucleic acid (RNA) to produce 5'-ribonucleotides. Recently, it has been discovered that many microorganisms (22,23,25,26,27) produce 5'-phosphodiesterase. Therefore, it has become possible to use certain microorganisms as sources of 5'-phosphodiesterase for use in production of 5'-ribonucleotides from RNA. With this in mind, a strain of Streptomyces albus, known to produce 5'-phosphodiesterase in submerged culture (23), was studied to determine the effects of various factors (pH, temperature, and nutrients) on growth and enzyme production and to scale up production from laboratory to pilot-plant quantity.

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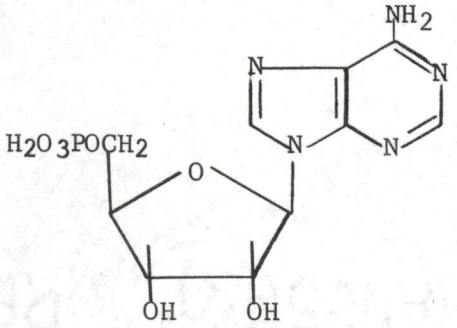
*Numbers in parentheses refer to similarly numbered items in the Bibliography.

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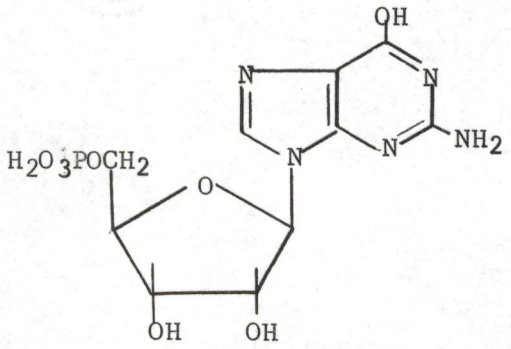
REVIEW OF THE LITERATURE

I. CHEMISTRY OF 5'-RIBONUCLEOTIDES

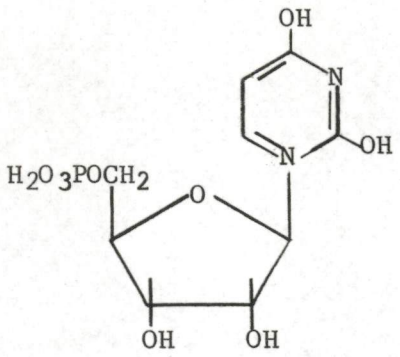
The 5'-ribonucleotides are compounds which consists of ribose, purine, and pyrimidine bases, and phosphoric acid. The structures, characterized by the periodate oxidation method (18) and confirmed by chemical synthesis (2) are shown as follows:



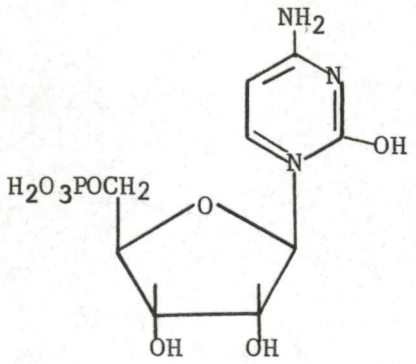
Adenosine-5-phosphate (5'-AMP)



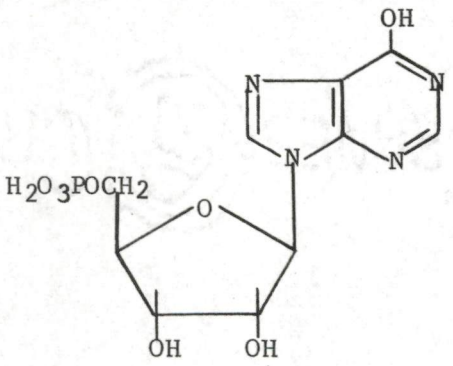
Guanosine-5-phosphate (5'-GMP)



Uridine-5-phosphate (5'-UMP)



Cytidine-5-phosphate (5'-CMP)



Inosine-5-phosphate (5'-IMP)

Inosine-5-phosphate is formed by the deamination of adenosine-5-phosphate (32). The strain of Streptomyces albus used in this study produces an adenylic acid deaminase capable of catalyzing such a reaction (23).

II. CHEMISTRY OF RIBONUCLEIC ACID

The qualitative composition of RNA may be summarized as follows (33):

Adenine (A)

Guanine (G)

Cytosine (C)

Uracil (U)

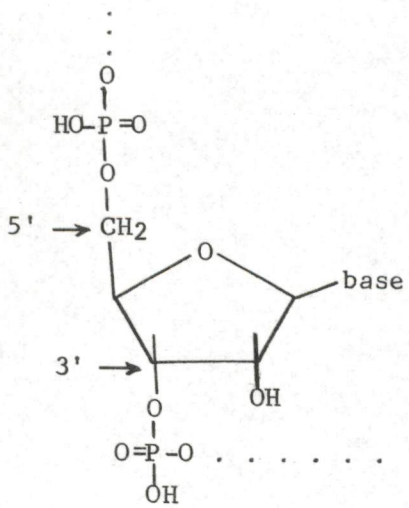
D-Ribose

Phosphate.

The sequence is presented below:

ApUpGpApCpUpAp . . . . .

The phosphate groups are bound to the C5' position of one ribose and the C3' position of the adjacent ribose forming diester linkages in the following manner:



III. HYDROLYSES OF RIBONUCLEIC ACID

Acid Hydrolysis

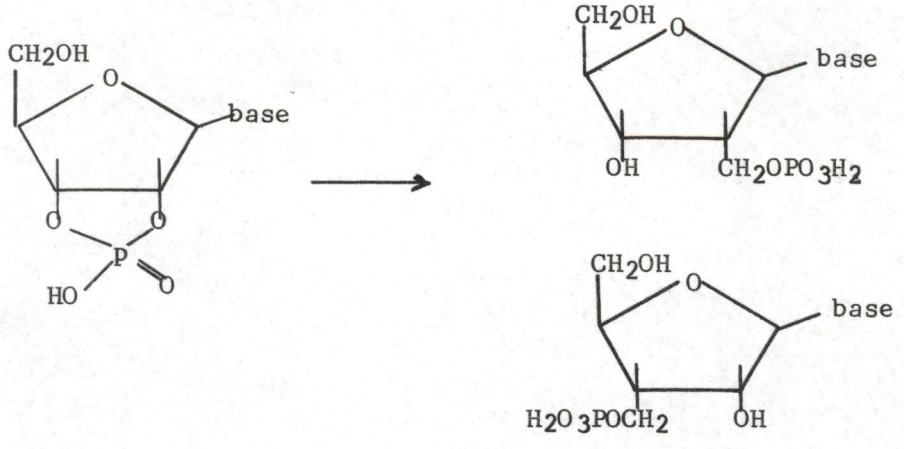
Complete acid hydrolysis of nucleic acids yields a mixture of purines and pyrimidines, ribose, and phosphoric acid. Nucleosides and nucleotides can be isolated after partial hydrolysis. The nucleosides contain a base and ribose, whereas the nucleotides contain phosphoric acid in addition to a base and ribose (33). This hydrolytic scheme is illustrated as follows:

base-sugar-phosphate $\xrightarrow[\text{hydrolysis}]{\text{complete acid}}$ bases, sugar, phosphate

base-sugar-phosphate $\xrightarrow[\text{hydrolysis}]{\text{partial acid}}$ nucleosides, nucleotides

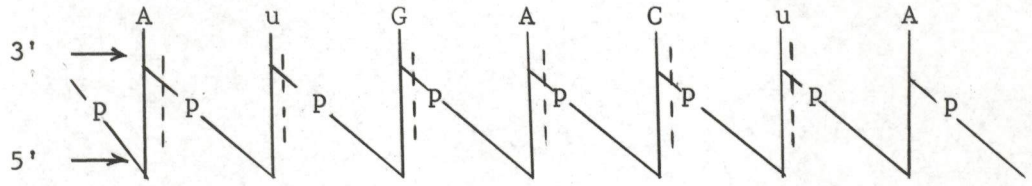
Alkaline Hydrolysis

Alkaline hydrolysis degrades RNA to a mixture of 2' and 3' nucleotides. This is a two-step reaction involving the intermediate formation of a phosphate triester followed by a hydrolytic splitting of one bond to leave a cyclic diester (33):



Hydrolysis by 5'-Phosphodiesterase

This enzyme splits the C3'-phosphate linkage to yield 5'-ribonucleotides. This is shown schematically as follows (5):



IV. 5'-PHOSPHODIESTERASE

Occurrence

A group of phosphodiesterases, which hydrolyze only one ester linkage in a diesterified phosphoric acid, are known to be quite widespread in plant and animal tissues (32). 5'-Phosphodiesterases have been found in rattlesnake and viper venon (16), intestional mucosa (6), rice bran (31), and various microorganisms (22,23,25,26,27). Many tissues contain both phosphodiesterase and phosphomonoesterase. In a study of the venoms of twelve different snakes, Gulland and Jackson (12) found that seven of the venoms contained both phosphomonoesterase and phosphodiesterase, whereas five contained phosphodiesterase only. It has not been possible to remove the phosphomonoesterase, but its activity can be inhibited by use of sodium arsenate (17).

Microbial 5'-Phosphodiesterase

A study of the distribution of 5'-phosphodiesterases in microorganisms made by Ogata et al. (26) found that the enzymes were produced by various microorganisms belonging to Streptomyces, Bacillus,

Fungi imperfecti such as Fusarium and Helminthosporium, and Ascomycetes such as Neurospora, Glomerella, and Aspergillus. Nakao and Ogata (25) found that Aspergillas quernicus possessed a phosphomonoesterase and an adenylic acid deaminase in addition to a phosphodiesterase. The formation of 5'-nucleotides or 3'-nucleotides depends on the range of pH used for hydrolysis. At a pH lower than 6, 3'-nucleotides were formed; while at a pH higher than 8.5, 5'-nucleotides were produced. Plackett (27) reported that Astercoccus mycodes produced a 5'-phosphodiesterase in culture broth when incubated at pH 8 and 37°C. in the presence of 0.3 N. NaCl. Kuninaka et al. (22) found that Penicillium citrinum was capable of hydrolyzing RNA into 5'-ribonucleotides. The highest activity was found at pH 5 and 60°C.

Lu (23) found that Streptomyces albus produced both a 5'-phosphodiesterase and an adenylic acid deaminase when grown in submerged culture.

V. SCALE-UP TECHNOLOGY

Preservation of Microorganisms

The aim of all methods of maintaining cultures is to store them under conditions which will keep biological activity at a minimum, while ensuring that the organisms remain alive and retain the desired properties. For routine use, organisms are maintained in open stock cultures on agar media. They are stored at low temperatures and are transferred at least once every six months (28).

Media

The medium should provide adequate amounts of all essential nutrients for growth and synthesis of the desired product. Utilizable and inexpensive sources of carbon are of major importance. Sugars are most commonly used in laboratory-scale work, but economic considerations largely preclude the use of pure sugars as a carbon source on an industrial scale. The nitrogen source in most industrial media consists of inexpensive proteinaceous material such as soybean, yeast extract, malt extract, urea, casein, fish meal, or meat scraps. The mineral salt composition can be very critical. Salts such as potassium or sodium phosphates and magnesium sulfates are commonly added to supply the macronutrient elements. Trace elements have also been found to be essential for maximum production of certain microbial products. Certain organic compounds, or growth factors, are required for growth of some microorganisms. This is usually not a problem as ample quantities of these nutrients are usually present in carbohydrate or nitrogenous materials used in the growth media (28).

Sterilization

One of the basic requirements for the propagation of pure cultures is the need to use sterile apparatus and media. The items for which special provision must be made are the culture vessels, media, and air supply. Contamination may arise from insufficient sterilization of either of these items or from outside as a result of faulty technique. Culture vessels and media are usually heat sterilized.

They may be treated as a unit or sterilized separately (14). The most practical method of air sterilization involves filtration with glass wool, slag wool, or cotton wool (15).

pH

With nearly all microorganisms, there is a narrow pH range which is most favorable for growth. Outside this range, growth is restricted and at extremes of acidity or alkalinity, there are deleterious effects on the organism. The optimum pH for growth varies with each organism and depends upon the growth conditions. Bacteria, in general, grow best in media of pH near 7.0, with limits between 5.0 and 8.0 (28). Most actinomycetes prefer an alkaline medium. For most soil species the limiting pH is 5.0. With most strains on most media, there is a tendency for the medium to become more alkaline during growth (29).

Temperature

The majority of microorganisms grow best in the range from 25° to 40°C. But it is often found that the optimum temperature for product formation is not the same as that at which the organism grows most rapidly (28). Actinomycetes usually grow best at 30° to 37°C., with an upper limit of 42°C. for most forms (29).

Aeration

Submerged growth of microorganisms is obtained by agitating or shaking the culture or by bubbling in air. The most convenient method is to shake the culture using a reciprocating or a rotary shaker. For

larger batches, submerged propagators are used.

Oxygen is essential for organisms growing aerobically, and it must be supplied continuously even in batch cultures. The oxygen requirement of a culture will vary during growth. If a sufficient supply of oxygen is not maintained during each stage, growth and product formation may be inhibited. The amount of oxygen required will depend on both the organism and the desired product. The oxygen requirement for maximum product formation may differ from the maximum demand of the organism (4).

The degree of aeration obtained depends upon several factors. The physical and chemical properties of culture fluids have an influence. Particular attention has been given to the effect of microbial cell matter in the culture (4,10). The shape and design of the culture vessels also affect aeration efficiency. Corman (8) and Shu (30) showed that vertical identations around the base of shake flasks increased oxygen absorption rates. In submerged propagators, aeration efficiency is influenced by the type of sparger used to disperse the air bubbles and by the position of the baffles. Generally, aeration rates of one volume per volume per minute are sufficient (10). However, air flow in volumes per volume of medium per minute is often unrelated to actual aeration efficiency, which is the amount of oxygen dissolved in the medium (34).

Agitation is considered essential for efficient aeration. Agitation improves aeration efficiency by increasing the interfacial area, by dispersing the air stream into currents which delay the escape of

air bubbles from the medium, and by breaking up cell clumps (10).

One of the greatest problems in aerating and shaking cultures is the tendency of the culture to foam. Foaming can be a serious problem, as it can lead to contamination and loss of material. Media containing yeast extract, molasses, peptone, or corn steep liquor foam readily because of the foam stabilizing properties of the polypeptides in these materials. Foaming is controlled by adding some form of nontoxic antifoam to the culture. Oils, alcohols derived from fatty acids, polyglycol and silicone oils are commonly used. Animal and vegetable oils often act as nutrients, while others, such as silicone oils, are not metabolized (10).

It is desirable to use a minimum of antifoam agent due to its undesirable effect on the oxygen absorption rate (8). Deindoerfer and Gaden (9) found that a concentration of 0.25 per cent antifoam agent (3 per cent Alkaterge C in lard oil) reduced the oxygen absorption coefficient almost 50 per cent.

Recovery of Products

The first step in recovering a product from a microbial culture is to remove the microbial cell material. The techniques used depend upon the type of microorganism. Bacteria and yeast are best removed by centrifugation, while filamentous organisms are usually separated using rotary filters (28).

CHAPTER III

MATERIALS AND METHODS

I. 5'-PHOSPHODIESTERASE ACTIVITY

Enzyme Preparation

The medium for 5'-phosphodiesterase production by Streptomyces albus was prepared as follows (23):

glucose	5.0%	peptone	0.50%
yeast extract	0.5%	$MgSO_4 \cdot 7H_2O$	0.05%
beef extract	0.5%	$CaCl_2 \cdot 2H_2O$	0.01%
soytone	0.5%		

The pH of the medium was adjusted to 7.0 with 1 N. NaOH. Thirty (30) ml. portions were poured into 250 ml. Hinton shake-flasks and sterilized at 250°F. for 15 minutes.

Inoculation was carried out by transferring one loopful of sporulating culture, grown on Emerson's medium, to each flask. The inoculated flasks were placed on a rotary shaker (150 r.p.m.) for 72 hours at 30°C. The culture broth was centrifuged, and the supernatant was used as the enzyme preparation.

Measurement of Enzyme Activity

McDonald's method (24), as modified by Fiske-Subbarow (11), Allen (1), and Lu (23), was used to determine 5'-phosphodiesterase

activity. To obtain 5'-ribonucleotides, a reaction mixture was prepared as follows:

2 per cent RNA (tris buffer, pH 9.0) . . . . . 0.9 ml.

0.1 M. Na_2HAsO_4. . . . . 0.4 ml.

Enzyme preparation . . . . . 0.2 ml.

The mixture was incubated at 37°C. for 16 hours. The reaction was terminated by heating for 10 minutes in a water bath at 100°C. One ml. of 1 N. NaOH was added to 1 ml. of the RNA digest and heated at 100°C. for 5 minutes in order to degrade the remaining RNA into 2' and 3'-nucleotides. The pH of the RNA digest was adjusted to 7.0 with 0.5 N. HCl.

The 5'-nucleotides, produced from the hydrolysis of RNA, were subjected to a highly specific preparation of bull semen 5'-nucleotidase (7), prepared by the simplified method of Heppel and Hilmore (13), by preparing the following reaction mixture:

RNA digest . . . . . 0.2 ml.

0.4 M. tris buffer containing 0.02 M.

$MgCl_2$ . . . pH 8.5 . . . . . 0.2 ml.

5'-nucleotidase. . . . . 0.1 ml.

To release the inorganic phosphate, the reaction-mixture was incubated at 37°C. for 3 hours. The reaction was terminated by adding 1 ml. 6 N. H_2SO_4 to the mixture. A blank was prepared by adding 6 N. H_2SO_4 before addition of the substrate.

The amount of inorganic phosphate released was determined by the following procedure: one ml. of 18 per cent sodium metabisulfite

was added, diluted to 5 ml. with distilled water, and held for 5 minutes to reduce arsenate to arsenite; one ml. of amidol reagent (0.4 g. amidol and 8 g. $NaHSO_3$ in 100 ml. of distilled water), followed by 3.3 per cent ammonium molybdate were added, and the total volume made to 10 ml. The color reaction was allowed to develop for five minutes (19), and the optical density was measured at 660 mμ. with a Spectronic-20. The optical density was related to amount of inorganic phosphate released by means of a standard phosphate curve.

II. EFFECT OF pH AND TEMPERATURE ON 5'-PHOSPHODIESTERASE ACTIVITY

Effect of pH

The effect of pH on 5'-phosphodiesterase activity was determined by adjusting the pH of the initial reaction mixture to different pH values and measuring enzymatic activity at each pH. For this study, pH levels of 5.0, 6.0, 7.0, 8.0, 8.5, 9.0, 9.5, and 10.0 were used.

Effect of Temperature

The effect of temperature on 5'-phosphodiesterase activity was studied by incubating the reaction mixture at temperatues of 30°C., 37°C., 45°C., and 60°C. and measuring the enzymatic activity at each of these temperatures.

III. EFFECT OF DIFFERENT CONDITIONS ON GROWTH AND 5'-PHOSPHODIESTERASE PRODUCTION BY STREPTOMYCES ALBUS IN HINTON FLASKS

Sample Preparation

A medium for growing the organism and producing 5'-phosphodiesterase was prepared with the following composition (23):

carbohydate	5.0%	peptone	0.5%
yeast extract	0.5%	$MgSO_4 \cdot 7H_2O$	0.05%
meat extract	0.5%	$CaCl_2 \cdot 2H_2O$	0.01%
soytone	0.5%		

The pH of the medium was adjusted to the desired value, and a 30 ml. portion of the medium was poured into 250 ml. Hinton flasks and sterilized at 250°F. for 15 minutes. The organisms were grown on a rotary shaker for 72 hours at the desired temperature. A 10 ml. aliquot of culture broth was withdrawn and centrifuged. The supernatant was used as the enzyme preparation, and the cellular matter was used to prepare a cell slurry. The cell slurry was dried in an oven at 80°C. for 24 hours to obtain the dry weight of the cellular material which was used as a measure of growth.

Effect of pH

The effect of pH on growth and 5'-phosphodiesterase production was studied by adjusting the pH of the growth medium to different values and determining how growth and enzyme production were affected at each of these pH levels. For this work, pH values of 5.0, 6.0,

7.0, 8.0, and 9.0 were used.

Effect of Temperature

The effect of temperature on growth and enzyme production was studied by growing the organisms under different temperature conditions and establishing how the temperature variations affected growth and 5'-phosphodiesterase production. The temperatures used for this study were 12°C., 22°C., and 35°C. A refrigerated room was used to obtain a temperature of 12°C., while an insulated box, equipped with a thermostatically controlled heater, was used for a temperature of 35°C. Room temperature was fairly constant around 22°C.

Effect of Carbon Source

Several aspects of carbon source were studied. Glucose and starch in concentrations of 5 per cent in the growth medium were compared to determine which was the best source of carbon for Streptomyces albus. The effect of different levels of glucose on growth and 5'-phosphodiesterase production also was studied. For this study, glucose concentrations of 2.5 per cent, 5.0 per cent, 7.5 per cent, and 10.0 per cent, were used in the growth medium.

Effect of Ribonucleic Acid

Since the production of many enzymes can be increased by the presence of their specific substrates, RNA was added to the growth medium of Streptomyces albus in an attempt to increase 5'-phosphodiesterase production in Hinton flasks.

IV. EFFECT OF DIFFERENT CONDITIONS ON GROWTH AND 5'-PHOSPHODIESTERASE PRODUCTION BY STREPTOMYCES ALBUS IN SUBMERGED CULTURE

Sample Preparation

A medium of the following composition was prepared in 1,000 ml. amounts:

glucose	5.0%	peptone	0.5%
yeast extract	0.5%	$MgSO_4 \cdot 7H_2O$	0.05%
meat extract	0.5%	$CaCl_2 \cdot 2H_2O$	0.01%
soytone	0.5%		

The pH was adjusted to the desired value, and the medium was autoclaved at 250°F. for 20 minutes.

The propagator was sterilized at 250°F. for 1 hour, and 5,000 ml. of medium was added aseptically. Inoculation was carried out with approximately 100 ml. of broth culture which had been grown on a rotary shaker for 72 hours. The propagator was connected to air source, and the fermentation was continued for 48 hours. (See Plate I.)

After 48 hours of growth, a 10 ml. aliquot was withdrawn and centrifuged. The supernatant was used as the enzyme preparation, and the cellular material was used to prepare a cell slurry, which was dried to determine the dry matter weight of the sample.

Effect of pH

The effect of pH on growth and 5'-phosphodiesterase production in submerged culture was studied by adjusting the pH of the growth

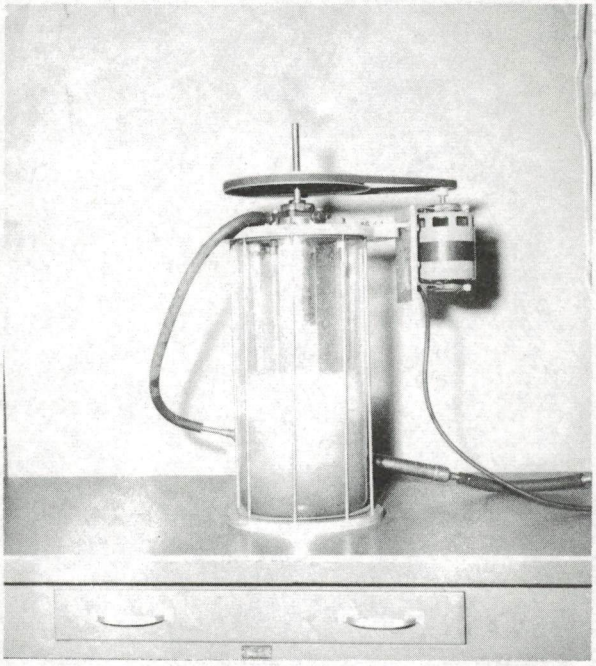


PLATE I

SUBMERGED CULTURE PROPAGATOR

medium to values of 5.0, 6.0, 7.0, 8.0, and 9.0 and measuring how growth and enzyme production varied at each pH level.

Effect of Carbon Source

The availability of carbon source as a possible limiting factor was studied by using growth medium containing glucose at levels of 2.5 per cent, 5.0 per cent, 7.5 per cent, and 10.0 per cent. The effect of glucose level on both growth of the organisms and production of 5'-phosphodiesterase was determined.

Effect of Aeration

The effect of the aeration rate on growth and enzyme production in submerged culture was studied by using three different aeration rates (.03 cu. ft./min., 2.24 cu. ft./min., and 4.80 cu. ft./min.) and by measuring how growth and 5'-phosphodiesterase yield varied with aeration rate. A rate of .03 cu. ft./min. was obtained with an aspirator, while rates of 2.24 cu. ft./min. and 4.80 cu. ft./min. were obtained by using a portable air compressor. The flow rates were determined by using a water displacement method.

Effect of Ribonucleic Acid

RNA was added to the growth medium in an effort to increase production of 5'-phosphodiesterase under conditions of submerged culture growth.

CHAPTER IV

RESULTS AND DISCUSSION

I. EFFECT OF pH AND TEMPERATURE ON 5'-PHOSPHODIESTERASE ACTIVITY

Effect of pH

The effect of pH on 5'-phosphodiesterase activity is shown in Table I. The analysis of variance shows a highly significant difference in enzymatic activity due to pH. Duncan's Multiple Range Test indicates that the optimum pH for enzymatic activity was 9.0. Although activity decreased at higher and lower pH levels, there was considerable activity throughout the range of pH from 5.0 to 10.0. These data indicate that the reaction rate of the enzyme is affected by pH but the enzyme is not inactivated within this range. Lu (23) reported that the optimum pH for 5'-phosphodiesterase activity was 9.1, with sharp decreases in reaction rate at higher and lower pH levels.

Effect of Temperature

Table II presents the effect of temperature on 5'-phosphodiesterase activity. The analysis of variance shows a highly significant difference in enzymatic activity due to the effect of temperature. As shown by Duncan's Multiple Range Test, there was a significant difference in activity among each of the four temperatures (30°C., 37°C., 45°C., and 60°C.). Up to 45°C. there was an increase in reaction rate as expected,

TABLE I

EFFECT OF pH ON 5'-PHOSPHODIESTERASE ACTIVITY
(μM. Pi RELEASED)

	pH							
Replicates	5.0	6.0	7.0	8.0	10.0	8.5	9.5	9.0
1	19.0	22.0	23.0	25.0	26.5	22.0	30.0	35.0
2	20.0	18.0	21.0	23.0	21.5	26.5	30.5	28.5
3	18.0	22.0	19.0	24.0	24.0	25.0	29.0	32.0
Total	57.0	62.0	63.0	72.0	72.0	73.5	89.5	95.5
Mean	19.0	20.7	21.0	24.0	24.0	24.5	29.8	31.8
Statistical Significance 0.05 Level	———	———	———	———	———	———		

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	7	59.89	14.97**
Replicate	2	6.01	1.50 N. S.
Error	14	4.00	

TABLE II

EFFECT OF TEMPERATURE ON 5'-PHOSPHODIESTERASE ACTIVITY (μM. Pi RELEASED)

Replicates	Temperature 60°C.	30°C.	37°C.	45°C.
1	16.0	30.0	31.0	34.5
2	17.0	25.0	31.0	34.5
3	15.0	28.0	31.0	33.0
Total	48.0	83.0	93.0	102.0
Mean	16.0	27.7	31.0	34.0
Statistical Significance 0.05 Level	___	___	___	___

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	3	186.33	85.08**
Replicate	2	1.53	0.70 N. S.
Error	6	2.19	

but at 60°C. there was a decrease in enzymatic activity, probably due to thermal denaturation of the enzyme protein.

II. EFFECT OF DIFFERENT CONDITIONS ON GROWTH AND 5'-PHOSPHODIESTERASE PRODUCTION BY *STREPTOMYCES ALBUS* IN HINTON FLASKS

Effect of pH

The effect of pH of the growth medium on 5'-phosphodiesterase production by *Streptomyces albus* is presented in Table III. The analysis of variance shows a highly significant difference in enzyme yield due to the effect of the pH of the medium. Duncan's Multiple Range Test indicates a significantly greater enzyme yield in growth medium of pH 7.0 to 8.0, with no significant difference in yield between these two pH levels. The yield of 5'-phosphodiesterase decreased at a more significant rate in growth medium of acid pH than in medium of alkaline pH. This decrease in available enzyme may have been caused by an inhibition of enzyme production, by a partial denaturation of the enzyme, or by a combination of both.

Table IV presents the effect of the pH of the medium on growth of *Streptomyces albus*. The analysis of variance shows that the pH of the medium had a highly significant effect on growth in Hinton flasks. No growth occurred at pH 5.0. Duncan's Multiple Range Test indicates that there was no significant difference in growth from pH 6.0 to 9.0. Apparently, under growth conditions in Hinton flasks, the organisms can adjust to pH differences within the range of pH 6.0 to 9.0 with no

TABLE III

EFFECT OF pH ON 5'-PHOSPHODIESTERASE PRODUCTION (μM. Pi RELEASED) BY *STREPTOMYCES ALBUS* IN HINTON FLASKS

	pH				
Replicates	5.0	6.0	9.0	8.0	7.0
1	00.0	19.5	25.0	26.0	28.5
2	00.0	18.0	26.0	27.5	32.0
Total	00.0	37.5	51.0	53.5	60.5
Mean	00.0	18.8	25.5	26.8	30.3
Statistical Significance 0.05 Level					

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	4	291.06	170.21**
Replicate	1	2.00	1.18 N. S.
Error	4	1.72	

TABLE IV

EFFECT OF pH ON GROWTH (MG./ML. DRY WEIGHT) OF *STREPTOMYCES ALBUS* IN HINTON FLASKS

	pH				
Replicates	5.0	9.0	6.0	8.0	7.0
1	00.00	16.83	20.09	20.15	24.92
2	00.00	17.45	19.24	19.68	18.44
Total	00.00	34.24	39.33	39.83	43.36
Mean	00.00	17.14	19.67	19.92	21.68
Statistical Significance 0.05 Level					

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	4	158.91	48.01**
Replicate	1	5.16	1.56 N. S.
Error	4	3.31	

significant effect on growth but are unable to survive in a medium of pH 5.0.

There was a tendency for the pH of the medium to increase slightly during growth. This is a characteristic of most strains of actinomycetes (29) and is probably due to the excretion of metabolic by-products during growth.

Effect of Temperature

Table V presents the effect of temperature on 5'-phosphodiesterase production by Streptomyces albus in Hinton flasks, and Table VI shows the effect of temperature on growth of the organisms. In each case, the analysis of variance shows a highly significant difference due to the effect of the temperature conditions under which the cultures were grown. The Duncan's Multiple Range Tests indicate that there was significantly less growth and 5'-phosphodiesterase production at 12°C. than at 22°C. or 35°C. but no significant difference existed between 22°C. and 35°C. This type of growth response to temperature was expected due to the growth characteristics of actinomycetes (29). The results also tend to indicate that 5'-phosphodiesterase production by Streptomyces albus is dependent on the extent of growth.

Effect of Carbon Source

The influence of the carbon source on 5'-phosphodiesterase production in Hinton flasks is presented in Table VII, while Table VIII presents the effect of the source of carbon on growth of Streptomyces albus. In each table, the analysis of variance shows no significant

TABLE V

EFFECT OF TEMPERATURE ON 5'-PHOSPHODIESTERASE PRODUCTION (μM. Pi RELEASED) BY *STREPTOMYCES ALBUS* IN HINTON FLASKS

Replicates	Temperature 12°C.	22°C.	35°C.
1	11.0	33.5	33.5
2	13.0	33.0	34.0
Total	24.0	66.5	67.5
Mean	12.0	33.3	33.8
Statistical Significance 0.05 Level			

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	2	308.30	390.25**
Replicate	1	0.67	0.85 N. S.
Error	2	0.79	

TABLE VI

EFFECT OF TEMPERATURE ON GROWTH (MG./ML. DRY WEIGHT) OF STREPTOMYCES ALBUS IN HINTON FLASKS

	Temperature		
Replicates	12°C.	22°C.	35°C.
1	7.10	20.15	20.94
2	7.56	20.40	20.50
Total	14.66	40.55	41.44
Mean	7.33	20.28	20.72
Statistical Significance 0.05 Level			

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	2	151.69	1051.73**
Replicate	1	0.00	0.00 N. S.
Error	2	0.11	

TABLE VII

EFFECT OF CARBON SOURCE ON 5'-PHOSPHODIESTERASE PRODUCTION (μM. Pi RELEASED) BY *STREPTOMYCES ALBUS* IN HINTON FLASKS

Replicates	Carbon Source (5%) Potato Starch	Glucose
1	34.5	34.0
2	34.5	37.0
Total	69.0	71.0
Mean	34.5	35.5
Statistical Significance 0.05 Level		

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	1	1.00	0.57 N. S.
Replicate	1	2.25	1.28 N. S.
Error	1	1.75	

TABLE VIII

EFFECT OF CARBON SOURCE ON GROWTH (MG./ML. DRY WEIGHT) OF STREPTOMYCES ALBUS IN HINTON FLASKS

Replicates	Carbon Source (5%) Glucose	Potato Starch
1	17.20	18.04
2	22.30	24.98
Total	39.50	43.02
Mean	19.75	21.51
Statistical Significance 0.05 Level		

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	1	3.10	3.69 N. S.
Replicate	1	36.24	43.14 N. S.
Error	1	0.84	

difference due to the type of carbohydrate used as a source of carbon. There is no difference in growth or enzyme production between the medium containing 5 per cent potato starch and medium with 5 per cent glucose. Apparently Streptomyces albus can utilize either starch or glucose as a source of carbon for the synthesis of cellular components. The ability to use starch as a carbon source is true of most soil Streptomyces (29) and is of economic significance, since sugar is too expensive to use for work beyond laboratory scale.

Effect of Glucose Level

The effect of the glucose level of the growth medium on 5'-phosphodiesterase production by Streptomyces albus is shown in Table IX, while Table X presents the effect on growth. In each case, the analysis of variance shows no significant difference in either growth or enzyme production among the four different levels of glucose (2.5 per cent, 5.0 per cent, 7.5 per cent, and 10 per cent) used in the growth medium. These data indicate that during the 72-hour growth period in Hinton flasks, the amount of glucose available as a source of carbon is not a limiting factor for either growth or enzyme production. The accumulation of metabolic by-products or a need for increased aeration probably become limiting before a decrease in amount of utilizable carbon becomes critical. These results strengthen evidence which indicates that 5'-phosphodiesterase production by Streptomyces albus is closely related to the amount of growth.

TABLE IX

EFFECT OF GLUCOSE LEVEL ON 5'-PHOSPHODIESTERASE PRODUCTION (μM. Pi RELEASED) BY *STREPTOMYCES ALBUS* IN HINTON FLASKS

	Per Cent Glucose			
Replicates	10.00	7.5	2.5	5.0
1	19.0	32.0	35.0	32.0
2	20.5	32.0	30.5	39.0
Total	39.5	64.0	65.5	71.0
Mean	19.8	32.0	32.8	35.5
Statistical Significance 0.05 Level				

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	3	97.92	8.64 N. S.
Replicate	1	2.00	0.35 N. S.
Error	3	11.33	

TABLE X

EFFECT OF GLUCOSE LEVEL ON GROWTH (MG./ML. DRY WEIGHT) OF STREPTOMYCES ALBUS IN HINTON FLASKS

	Per Cent Glucose			
Replicates	10.0	7.5	2.5	5.0
1	8.79	14.95	14.30	17.16
2	14.23	11.14	14.54	17.81
Total	23.02	26.09	28.84	34.97
Mean	11.51	13.05	14.42	17.49
Statistical Significance 0.05 Level				

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	3	12.92	1.80 N. S.
Replicate	1	0.79	0.11 N. S.
Error	3	7.17	

III. EFFECT OF DIFFERENT CONDITIONS ON GROWTH AND PRODUCTION OF 5'-PHOSPHODIESTERASE BY STREPTOMYCES ALBUS IN SUBMERGED CULTURE

Effect of pH

Table XI presents the effect of pH of the growth medium on production of 5'-phosphodiesterase by Streptomyces albus in submerged culture. The analysis of variance shows a highly significant difference in enzyme production in medium of different pH values. Duncan's Multiple Range Test indicates that maximum 5'-phosphodiesterase production was obtained in medium of pH 7.0 to 8.0, with no difference between these two pH levels. Enzyme yield decreased at both higher and lower pH levels, but the decrease was more marked in acid medium than in alkaline medium. This decrease in enzyme yield at pH levels above and below pH 7.0 to 8.0 also occurred when Streptomyces albus was grown in Hinton flasks and, as previously mentioned, may have been caused by inhibition of enzyme production, by a partial denaturation of the enzyme, or by both.

The effect of the pH of the medium on growth in submerged culture is presented in Table XII. A highly significant difference in growth due to the pH of the medium is shown by the analysis of variance. Duncan's Multiple Range Test indicates a significant difference in growth at each pH level, with maximum growth in medium of pH 7.0. Growth was less in medium of acid pH than in medium of alkaline pH; no growth was obtained at pH 5.0. Since there was no significant

TABLE XI

EFFECT OF pH ON 5'-PHOSPHODIESTERASE PRODUCTION (μM. Pi RELEASED) BY STREPTOMYCES ALBUS IN SUBMERGED CULTURE

	pH				
Replicates	5.0	6.0	9.0	8.0	7.0
1	00.0	19.5	30.0	35.5	37.0
2	00.0	21.0	32.0	35.0	36.5
Total	00.0	40.5	62.0	70.5	73.5
Mean	00.0	20.3	31.0	35.3	36.8
Statistical Significance 0.05 Level					

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	4	463.04	671.07**
Replicate	1	0.62	0.90 N. S
Error	4	0.69	

TABLE XII

EFFECT OF pH ON GROWTH (MG./ML. DRY WEIGHT) OF STREPTOMYCES ALBUS IN SUBMERGED CULTURE

	pH				
Replicates	5.0	6.0	9.0	8.0	7.0
1	00.00	16.10	20.05	22.95	24.22
2	00.00	17.54	21.35	23.25	24.70
Total	00.00	33.64	41.40	46.20	48.92
Mean	00.00	16.82	20.70	23.10	24.46
Statistical Significance 0.05 Level	———	———	———	———	———

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	4	197.79	998.83**
Replicate	1	1.24	6.89 N. S
Error	4	0.18	

difference in growth in the range from pH 6.0 to 9.0 in Hinton flasks, it may be concluded that the pH of the growth medium was of more significance for growth by submerged culture than for growth in Hinton flasks. This difference in the effect of the pH of the growth medium on Streptomyces albus in Hinton flasks and in submerged propagators is probably due to the difference in aeration rate between these two methods. Oxygen is probably a very critical limiting factor for organisms growing in Hinton flasks, whereas a sufficient supply of oxygen is undoubtedly available during submerged culture growth. Therefore, pH may exert a greater influence on the activity of Streptomyces albus growing by submerged propagation than on cultures growing in Hinton flasks.

Effect of Glucose Level

Table XIII presents the effect of the glucose level of the growth medium on production of 5'-phosphodiesterase by Streptomyces albus in submerged propagators. The analysis of variance shows a highly significant difference in enzyme production among the four levels of glucose (2.5 per cent, 5.0 per cent, 7.5 per cent, and 10.0 per cent) used in the medium. Duncan's Multiple Range Test indicates that, among these four glucose levels, maximum 5'-phosphodiesterase production was obtained at 5.0 per cent glucose. No significant difference in yield was found between 2.5 per cent and 10.0 per cent glucose.

TABLE XIII

EFFECT OF GLUCOSE LEVEL ON 5'-PHOSPHODIESTERASE PRODUCTION (μM. Pi RELEASED) BY *STREPTOMYCES ALBUS* IN SUBMERGED CULTURE

	Per Cent Glucose			
Replicates	2.5	10.0	7.5	5.0
1	25.0	22.0	27.0	35.5
2	26.0	23.0	28.0	37.5
Total	51.0	45.0	55.0	73.0
Mean	25.5	25.5	27.5	36.5
Statistical Significance 0.05 Level				

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	3	218.00	558**
Replicate	1	3.12	24*
Error	3	0.38	

The effect of the glucose level of the growth medium on proliferation of Streptomyces albus is shown in Table XIV. A highly significant difference in growth among the four levels of glucose is indicated by the analysis of variance. Maximum growth was obtained in the range of 2.5 per cent to 5.0 per cent glucose, with Duncan's Multiple Range Test indicating no significant difference in growth between these two levels of glucose. There was less growth in medium containing 7.5 per cent and 10.0 per cent glucose. This decrease in growth in medium containing higher concentrations of glucose may have been due to an osmotic effect or to a breaking down of glucose molecules during the increased period (20 minutes) of heat sterilization used for the larger batches of medium involved in growth by submerged culture propagation.

The effect of the glucose level of the growth medium on proliferation and 5'-phosphodiesterase production by Streptomyces albus in submerged propagation is in contrast to the effect on cultures in Hinton flasks. In Hinton flasks there was no significant difference in either growth or enzyme production among the four levels of glucose while in submerged culture, the glucose level of the medium had a significant effect on both growth and 5'-phosphodiesterase production. Apparently under conditions of growth in submerged culture, the amount of available carbon source is more critical than under growth conditions in Hinton flasks. This difference in the effect of glucose level is probably due to an increase in growth at the higher levels of aeration used in submerged propagation. With increased growth, the amount

TABLE XIV

EFFECT OF GLUCOSE LEVEL ON GROWTH (MG./ML. DRY WEIGHT) OF STREPTOMYCES ALBUS IN SUBMERGED CULTURE

	Per Cent Glucose			
Replicates	10.0	7.5	2.5	5.0
1	17.20	20.40	22.20	23.95
2	17.64	20.50	23.40	24.25
Total	34.84	40.90	45.60	48.20
Mean	17.42	20.45	22.80	24.10
Statistical Significance 0.05 Level				

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	3	17.17	107.31**
Replicate	1	0.51	3.19 N. S.
Error	3	0.16	

of utilizable carbon source available could possibly be a limiting factor for both growth and 5'-phosphodiesterase production.

Effect of Aeration Rate

Table XV shows the effect of the aeration rate on the production of 5'-phosphodiesterase in submerged culture. A highly significant difference in enzyme yield due to variations in air supply is shown by the analysis of variance. Duncan's Multiple Range Test indicates that there was significantly greater 5'-phosphodiesterase production at aeration rates of 2.24 cu. ft./min. and 4.80 cu. ft./min. than at a rate of 0.03 cu. ft./min. but there was no difference in enzyme yield between these two higher rates. The increase in aeration rate from 2.24 cu. ft./min. to 4.80 cu. ft./min. probably failed to increase 5'-phosphodiesterase production by Streptomyces albus because the supply of oxygen may have already been above the critical level for the culture. When the oxygen supply is above this critical level, additional oxygen will not result in an increase in product formation (10).

The effect of the aeration rate on growth of Streptomyces albus is presented in Table XVI. The analysis of variance shows a highly significant difference in growth among the three aeration rates. Duncan's Multiple Range Test indicates a significant difference in growth at each level of aeration, with maximum growth at the highest rate (4.80 cu. ft./min.). As the aeration of the cultures was increased, the amount of medium lost by evaporation also increased. After 48 hours of growth, there was no evaporation at an aeration rate

TABLE XV

EFFECT OF AERATION RATE (CU. FT./MIN.) ON 5'-PHOSPHODIESTERASE PRODUCTION (uM. Pi RELEASED) BY STREPTOMYCES ALBUS IN SUBMERGED CULTURE

	Aeration Rate		
Replicates	0.03	2.24	4.80
1	18.0	36.5	38.5
2	17.0	35.5	36.7
Total	35.0	72.0	75.5
Mean	17.5	36.0	37.5
Statistical Significance 0.05 Level			

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	2	247.17	457.72**
Replicate	1	2.67	4.94 N. S.
Error	2	0.54	

TABLE XVI

EFFECT OF AERATION RATE (CU. FT./MIN.) ON GROWTH (MG./ML. DRY WEIGHT) OF STREPTOMYCES ALBUS IN SUBMERGED CULTURE

	Aeration Rate		
Replicates	0.03	2.24	4.80
1	11.76	24.22	34.68
2	11.54	24.70	34.67
Total	23.30	48.92	69.35
Mean	11.65	24.46	34.68
Statistical Significance 0.05 Level	———	———	———

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	2	266.20	3802.86**
Replicate	1	0.01	0.14 N. S.
Error	2	0.07	

of 0.03 cu. ft./min., but at the higher rates there was considerable evaporation. This evaporation was equivalent to 30 per cent of volume at an aeration rate of 2.24 cu. ft./min. and to approximately 50 per cent of volume at 4.80 cu. ft./min. Therefore, loss of liquid may account for part of the apparent increase in cellular matter and enzyme production at higher aeration rates. By taking into consideration the concentration effect due to evaporation and by assuming a direct relationship, the adjusted mean values in Table XV would be 25.0 μM. Pi release with an aeration rate of 2.24 cu. ft./min. and 19.5 μM. Pi released with a rate of 4.80 cu. ft./min. In Table XVI, the adjusted dry weight mean values would be 17.19 mg./ml. with a rate of 2.24 cu. ft./min. and 17.34 mg./ml. with a rate of 4.80 cu. ft./min.

IV. EFFECT OF RIBONUCLEIC ACID ON PRODUCTION OF 5'-PHOSPHODIESTERASE BY STREPTOMYCES ALBUS

Table XVII shows the effect on production of 5'-phosphodiesterase by Streptomyces albus in Hinton flasks when ribonucleic acid (0.10 per cent) was added to the growth medium. The effect on enzyme production in submerged culture is presented in Table XVIII. In both cases, there was no significant difference in 5'-phosphodiesterase production due to the presence of ribonucleic acid in the growth medium. These results indicate that 5'-phosphodiesterase production was not increased by the addition of its specific substrate (RNA) to the medium. Therefore, it may be concluded that RNA did not induce an increase in enzyme synthesis.

TABLE XVII

EFFECT OF RIBONUCLEIC ACID ON 5'-PHOSPHODIESTERASE PRODUCTION (μM. Pi RELEASED) BY STREPTOMYCES ALBUS IN HINTON FLASKS

	Growth Medium (% RNA)	
Replicates	0.10	0.00
1	30.5	32.0
2	32.5	31.0
Total	63.0	63.0
Mean	31.0	31.0
Statistical Significance 0.05 Level		

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	1	0.00	0.00 N. S.
Replicate	1	0.00	0.00 N. S.
Error	1	2.50	

TABLE XVIII

EFFECT OF RIBONUCLEIC ACID ON 5'-PHOSPHODIESTERASE PRODUCTION (μM. Pi RELEASED) BY *STREPTOMYCES ALBUS* IN SUBMERGED CULTURE

	Growth Medium (% RNA)	
Replicates	0.10	0.00
1	35.0	36.0
2	35.5	35.0
Total	70.5	71.0
Mean	35.3	35.5
Statistical Significance 0.05 Level		

Summary of Analysis of Variance

Source	D. F.	M. S.	F Ratio
Treatment	1	0.04	0.06 N. S.
Replicate	1	0.04	0.06 N. S.
Error	1	0.61	

CHAPTER V

SUMMARY

This study was made to determine how different conditions affected growth and 5'-phosphodiesterase production by Streptomyces albus in 30 ml. Hinton flasks and in 5 liter submerged culture propagators. The effect of pH and temperature on 5'-phosphodiesterase activity were also studied. Enzymatic activity was measured by using a colorimetric procedure. Growth was measured by determining cell matter dry weight.

From the data, the following conclusions may be made:

1. The optimum conditions of pH and temperature for 5'-phosphodiesterase activity were pH 9.0 and 45°C., respectively.

2. The optimum pH for 5'-phosphodiesterase yield in Hinton flasks was pH 7.0 to 8.0, while there was no significant difference in growth in medium ranging from pH 6.0 to 9.0. No growth was obtained at pH 5.0.

3. Growth and production of 5'-phosphodiesterase were reduced at 12°C., and there were no significant differences between 22°C. and 35°C.

4. Streptomyces albus utilized starch or glucose as a carbon source with no significant effect on either growth or production of 5'-phosphodiesterase.

5. In the range of 2.5 per cent to 10.0 per cent, the level of glucose in the growth medium had no significant effect on either growth or production of 5'-phosphodiesterase in Hinton flasks.

6. The optimum pH for 5'-phosphodiesterase yield in submerged culture was pH 7.0 to 8.0, while growth was maximum in medium of pH 7.0.

7. Maximum 5'-phosphodiesterase production was obtained in submerged culture with a medium containing 5 per cent glucose, while no significant difference in growth between 2.5 per cent and 5.0 per cent glucose was observed.

8. Growth and production of 5'-phosphodiesterase were less at an aeration rate of 0.03 cu. ft./min. than at 2.24 cu. ft./min. or 4.80 cu. ft./min. There was significantly greater growth at 4.80 cu. ft./min. than at 2.24 cu. ft./min., but there was no significant difference in enzyme production.

9. Production of 5'-phosphodiesterase was not increased by the addition of ribonucleic acid to the growth medium.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Allen, R. J. L. 1940. The estimation of phosphorous. Biochem. J. 34, 858.

2. Baddiley, J. A., A. M. Michaelson, and A. R. Todd. 1947. Synthesis of adenosine triphosphate. Nature 161, 761.

3. Batzer, O. F., and W. A. LandMann. 1962. Identification of some beef flavor precursors. J. Agr. Food Chem. 10, 94.

4. Brierley, M. R., and R. Steel. 1958. Agitation-aeration in submerged fermentation. II. Effect of solid dispersed phase on oxygen absorption in a fermentor. Appl. Microbiol. 7, 57.

5. Cohn, E. E., and P. K. Stumpf. 1964. "Outlines of Biochemistry." 1st ed. John Wiley and Sons, Inc., New York, p. 89.

6. Cohn, W. E. 1957. Nucleotides by ion exchange chromatography. "Methods in Enzymology." Vol. III. (S. P. Colowick and N. O. Kaplan, eds.) Academic Press, Inc., New York, p. 738.

7. Cohn, W. E., and E. Volkin. 1955. Nucleoside-5'-phosphates from ribonucleic acid. Nature 167, 483.

8. Corman, J., et al. 1957. Oxygen absorption rates in laboratory and pilot plant equipment. Appl. Microbiol. 5, 313.

9. Deindoerfer, G. 1955. Effects of physical properties on oxygen transfer in penicillin fermentation. Appl. Microbiol. 3, 253.

10. Finn, R. K. 1954. Agitation-aeration in the laboratory. Bact. Rev. 18, 254-270.

11. Fiske, C. H., and Y. Subbarow. 1925. The colorimetric determination of phosphorous. J. Biol. Chem. 66, 375.

12. Gulland, J. M., and E. M. Jackson. 1938. Phosphoesterases of bone and snake venoms. Biochem. J. 32, 590.

13. Heppel, L. A., and R. J. Hilmore. 1951. Purification and properties of 5'-nucleotidase. J. Biol. Chem. 188, 655.

14. Herold, M., and J. Necosek. 1957. Protected fermentation. Advanc. Appl. Microbiol. I, 3.

15. Humphrey, A. E. 1958. Air sterilization. Advanc. Appl. Microbiol. II, 301.

16. Hurst, R. O., and G. C. Butler. 1951. The chromatographic separation of phosphatase in snake venom. J. Biol. Chem. 193, 91.

17. Hurst, R. O., J. A. Little, and G. C. Butler. 1951. The enzymatic degradation of thymonucleic acid. J. Biol. Chem. 188, 705.

18. Khym, J., and W. E. Cohn. 1960. Characterization and some chemical reactions of periodate oxidized nucleosides. J. Am. Chem. Soc. 82, 6380.

19. Kitson, M. 1944. Further studies of the molybdenum blue reaction. Ind. Eng. Chem. Anal. Ed. 16, 466.

20. Kodama. 1913. On procedure of separating inosinic acid. J. Tokyo Chem. Soc. 34, 751.

21. Kuninaka, A. 1960. Studies on the taste of ribonucleic acid derivatives. J. Agr. Chem. Soc. Japan 34, 489.

22. Kuninaka, A., et al. 1959. Studies on 5'-phosphodiesterase in microorganisms. Part I. Formation of nucleotide-5'-monophosphates from yeast RNA by penicillium citrinum. Bull. Agr. Chem. Soc. Japan 23, 239.

23. Lu, J. Y. 1964. A 5'-phosphodiesterase of the streptomyces species. M. S. Thesis, The University of Tennessee, Knoxville, Tennessee.

24. McDonald, M. R. 1955. Ribonucleases. "Methods in Enzymology." Vol. II. (S. P. Colowick and N. O. Kaplan, eds.) Academic Press, Inc., New York, p. 427.

25. Nakoa, Y., and K. Ogata. 1963. Degradation of nucleic acids and their related compounds by microbial enzymes. Part V. Degradation of RNA by Aspergillus quercinas. Agr. Biol. Chem. Japan 27, 199.

26. Ogata, K., et al. 1963. Degradation of nucleic acids and their related compounds by microbial enzymes. Part I. On the distribution of extracellular enzymes capable of degrading RNA into 5'-mononucleotides in microorganisms. Agr. Biol. Chem. Japan 27, 110.

27. Plakett, P. 1957. Depolymerization of RNA by extracts of Astercoccus mycodes. Biochem. Biophys. Acta 26, 664.

28. Rose, A. H. 1961. "Industrial Microbiology." 1st ed. Butterworths, Washington, pp. 34-48, 74-79, 85-89.

29. Skinner, C. E., C. W. Emmons, and H. M. Tsuchiya. 1961. "Henrici's Molds, Yeasts, and Actinomycetes." 2nd ed. John Wiley and Sons, Inc., New York and London, p. 362.

30. Shu, P. 1953. Oxygen uptake in shake flash fermentations. J. Agr. Food Chem. 1, 1119.

31. Tauber, H. 1949. "Chemistry and Technology of Enzymes." 1st ed. John Wiley and Sons, Inc., New York and London, p. 32.

32. West, E. S., and W. R. Todd. 1955. "Text of Biochemistry." 2nd ed. The Macmillan Company, New York, pp. 389, 447.

33. White, A. P., P. Handler, E. L. Smith, and D. Stetten. 1959. "Principals of Biochemistry." 2nd Ed. McGraw-Hill Book Co., New York, pp. 189-191.

34. Wise, W. S. 1951. The measurement of the aeration of culture media. J. Gen. Microbiol. 5, 167.

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