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Dorothy Marie Nickless for the Master of Science
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Title: Growth Conditions Affecting the Expression of Amylase in *Bacillus* species

Abstract approved: Scott S. Cupper
(Thesis Advisor Signature)

Microorgansims must be able to respond effectively to nutrient availability in their environment in order to replicate and survive. Enzymes allow microorganisms to catalyze metabolic reactions after nutrients have been taken in. Enzyme synthesis depends on the presence or absence of nutrients and allows microorganisms to utilize energy and replicate effectively through enzyme repression or induction. One example of such an enzyme is amylase, an extracellular enzyme capable of hydrolyzing the alpha-1,4 glycosidic linkages of starch, produced by the genus *Bacillus*. Among the different *Bacillus* species, amylase gene and protein sequence exhibits variability. Hence, this study investigated and proved that different growing environments affect amylase expression from *B. coagulans, B. megaterium, B. sphaericus, B. subtilis,* and *B. thuringiensis*. These findings may allow for a better understanding of amylase expression in the food and biotechnological industries. In these industries, microbial sources receive most of the attention due to their inexpensive cost production. However, commercial production levels are variable which further illustrates the need to examine growth conditions. The present studies further allowed for the development of a

laboratory experiment addressing genetic regulation of the amylase gene in *Bacillus* species that can be performed in a high school or college laboratory setting.

GROWTH CONDITIONS AFFECTING THE EXPRESSION OF AMYLASE IN *BACILLUS* SPECIES

A Thesis

Presented to

The Department of Biological Sciences

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Dorothy Marie Nickless

May 2001

Thesis
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Approved by Major Advisor

Approved by Committee Member

Approved by Committee Member

Approved for Department of Biological Sciences

Approved for the Graduate Council

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PREFACE

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INTRODUCTION

Living organisms have evolved the ability to organize their various macromolecules and chemical processes into defined structures which ultimately results in their replication. These processes are collectively referred to as metabolism and cells are constantly undergoing change as a result of these metabolic processes (22, 32).

Microbial cells, which are composed of a wide array of chemical structures, must be able to respond effectively to environmental changes. Once nutrients from the environment are taken in, catabolic and anabolic pathways are activated to convert them into the basic constituents that make up the cell (22). Since the basic building blocks of a cell are not always present in its surroundings, microorganisms must control their vast number of catabolic and anabolic pathways in response to nutrient availability (22). Regulation of these pathways is essential for conservation of microbial energy (32). For example, although organisms possess the genetic information needed to utilize numerous energy sources, enzymes for a particular nutrient are only expressed when the energy source is present in its surroundings. Similarly, it would be wasteful for a microorganism to synthesize the enzymes required to manufacture a certain end product if that end product were already present in adequate amounts. Thus, regulation of both catabolic and anabolic pathways maximize the cell's efficiency of operation and is essential to the existence of life (32).

I. Metabolic regulation. The common feature of all metabolic reactions is that they are catalyzed by enzymes (28). However, the numerous enzymes in a cell are not present in constant quantities at all times throughout the life cycle and their synthesis in many cases is the result of an environmental stimulus (28).

A. Enzyme repression. Enzyme repression is very common in prokaryotes. Generally, this system regulates the biosynthetic processes of a cell through inhibition of gene expression, resulting in decreased enzyme synthesis (22, 28). This is a

specific effect, with enzyme repression occurring only on the enzymes of the product in question.

B. Enzyme induction. Enzyme induction is a process that regulates catabolic pathways. Induction acts by turning on the transcription of a gene or genes in response to a molecule in the environment (28). This process, like enzyme repression, is also specific and has no effect on enzymes of unrelated catabolic pathways.

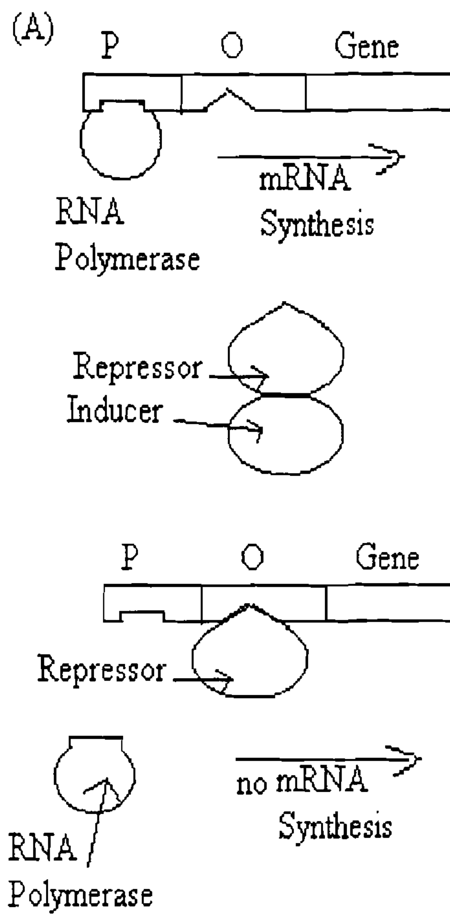
Benefits of enzyme repression and enzyme induction are to allow bacteria the ability to reproduce at their maximum rate in any environment and effectively use available energy (22). As illustrated in Figure 1, enzyme induction and repression involve similar genetic elements: a regulatory gene that synthesizes a repressor protein, an operator region which binds the repressor protein, and a promoter that binds RNA polymerase (22).

II. Enzymes. Enzymes are defined as protein catalysts that have specificity for the reaction catalyzed and the molecules acted upon (32). Enzyme specificity is based to a great extent on the three-dimensional structure of the active site present on the enzyme molecule (28). The active site is the area on the enzyme surface into which the substrate molecule fits and is acted on to yield a product (28). Some enzymes are pure proteins, but many consist of a protein combined with a much smaller nonprotein molecule referred to as a coenzyme or cofactor (28). The coenzyme or cofactor assists the protein portion, called the apoenzyme, in accepting or donating atoms when needed (32). When united, the two portions form a complete enzyme, the holoenzyme (32). Coenzymes can be in various forms of vitamins or ions, but cofactors in forms of metals (28).

III. Extracellular enzymes. Extracellular enzymes are secreted by a microorganism into its extracellular medium (9). In many cases, these enzymes allow microorganisms the ability to utilize high molecular weight compounds that otherwise may have been inaccessible by digesting them into smaller, usable forms (26). The exact mechanism for the synthesis of many extracellular enzymes in bacteria is poorly understood (18).

FIG. 1. Regulation of mRNA synthesis. O, operator; P, promoter.

A. Enzyme Induction. When an inducer is present, it binds to a repressor and the repressor-inducer complex cannot bind to the operator. This allows RNA polymerase to bind to the promoter and start transcription. When an inducer is absent, the repressor binds to the operator preventing RNA polymerase binding to the promoter and hence, no mRNA synthesis occurs. B. Enzyme Repression. When a corepressor is present, it interacts with a repressor and the resulting complex binds to the operator, preventing RNA polymerase from binding to the promoter. Transcription is turned off. When a corepressor is absent, the repressor cannot bind to the operator, allowing RNA polymerase to bind to the promoter and transcription may ensue. Some genes are not inducible or repressible and are synthesized continuously. Genes that are always turned on are referred to as constitutive.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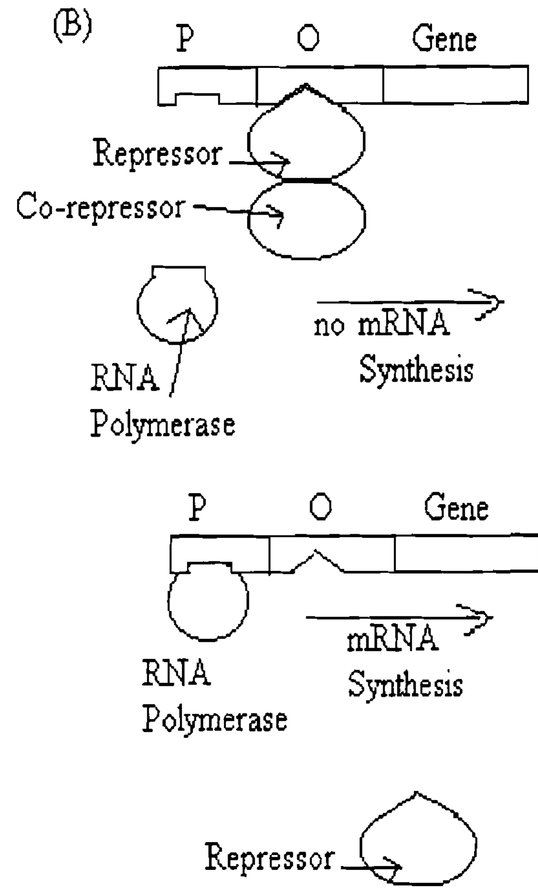
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IV. Amylase. Amylase is an extracellular enzyme which is capable of hydrolyzing the alpha-1,4 glycosidic linkages of starch (9). In plants, starch consists of a mixture of amylose (15-30%) and amylopectin (70-85%) (1, 33). Amylose is predominantly a linear polysaccharide composed of alpha-1,4 and some alpha-1,6 linked glucose residues whereas amylopectin has a greater tree-like structure due to the presence of more alpha-1,6 versus alpha-1,4 linked glucose residues (33). One form of amylase, alpha-amylase, is useful in the initial step of starch hydrolysis (1). Alpha-amylase is capable of hydrolyzing starch interiorly yielding glucose molecules which are transported across the cell membrane for use in various metabolic reactions (7). In addition to alpha-amylase (1, 6), amylase exists in a variety of forms including beta-amylases (2, 6), isoamylases and pullulanases (2), glucoamylases (2), and cyclodextrin glucotransferases (2). Commercially, amylases have many uses, including the desizing of fabrics (1), production of syrups (2), adhesives (19), detergents (1), pharmaceuticals (19), and in sewage treatment (1).

Amylases of different specificities are synthesized and secreted extracellularly by a wide variety of microorganisms including gram negative and gram positive bacteria, yeast, and fungi (2, 4). Amylase production is minimal among gram negative bacteria, but is quite abundant in gram positive bacteria, particularly in the genus *Bacillus* (2). The genus *Bacillus* belongs to the family *Bacillaceae* and is ubiquitous in nature, inhabiting soil, water, and airborne dust (14). The amylase gene and its corresponding protein sequence exhibits little sequence homology among the various *Bacillus* species (5, 13, 21, 23, 25, 35).

V. Objectives. Based on the variability of the amylase gene and its protein product, it was theorized that expression of the gene may be variable among different *Bacillus* species and growth conditions would likely affect its production. Hence, the first objective was to determine what effect growth conditions had on amylase expression in different *Bacillus* species. In the course of these experiments, it was concluded that these

principles could be explored at the high school level. Consequently, the second objective was to design a laboratory experiment that would the address genetic regulation of amylase expression in *Bacillus* that could be performed on a high school level.

MATERIALS AND METHODS

I. Bacterial strains and growth conditions. *Bacillus* species used in this study were obtained from Presque Isle Cultures (Presque Isle, PA). Organisms were routinely propagated on tryptic soy agar (TSA) plates at 37° C and stored at 4° C. Media and solutions used in this investigation are listed in Table 1.

II. Amylase assay. Bacterial supernatant was examined by the amylase assay. The specific conditions for the assay were determined by trail and error and the procedure listed proved most reproducible. Twenty microliters of supernatant was placed into a 4 mm well created in 20 ml of solidified starch agar contained in a Petri dish. The starch agar plate containing the supernatant was subsequently incubated at room temperature for approximately 16 h. Amylase activity was determined by flooding the agar plate with Gram's iodine. A clear halo forming almost immediately around the well indicated starch digestion by amylase. A summary of amylase activity for all *Bacillus* species tested is illustrated in Table 2. Since assays were performed in triplicate, amylase activity was present in three forms maximal, variable, or an absence. Maximal activity is defined as 3 out of 3 (100%) positive assays. While variable activity is 1 out of 3 or 2 out of 3 (33% - 66%) positive assays. Finally, an absence of amylase activity describes 0 out of 3 positive assays.

III. Effect of growth conditions on amylase activity. Bacterial cultures demonstrating amylase activity were aseptically inoculated into 3 ml of each brain heart infusion broth (BHI), Luria-Bertani broth (LB), minimal media broth (MM), tryptic soy broth (TSB), and 2XYT broth contained in a 16 X 150 mm test tube. The inoculated media was incubated at either 30° C, 37° C, or 42° C for 16 h with 250 rpm continuous shaking in a New Brunswick Series 25 Incubator Shaker (Edison, New Jersey). An additional culture was incubated at 37° C for 16 h without shaking. An aliquot of each culture was transferred to a sterile 1.5 ml microcentrifuge tube and centrifuged at 13,000 rpm for 5 min in a Beckman Microfuge Lite Centrifuge (Palo Alto, CA) to

precipitate bacterial cells. Serial two-fold dilutions were prepared from each supernatant using sterile TSB. Amylase activity in each diluted sample was determined using the amylase assay described above.

TABLE 1. Growth media and solutions used in this study [a]

Name	Ingredients
Brain Heart Infusion Broth (1 L)	37 g Brain Heart Infusion Media
Luria-Bertani Broth (1 L)	10 g Tryptone 5 g Yeast Extract 10 g Sodium Chloride
Minimal Media Broth (1 L)	1 g Dextrose 7 g Dipotassium Phosphate 2 g Monopotassium Phosphate 0.5 g Sodium Chloride 0.1 g Magnesium Sulfate 1 g Ammonium Sulfate
Starch Agar (1 L)	25 g Starch Media
Tryptic Soy Agar (1 L)	30 g Tryptic Soy Media 20 g Agar
Tryptic Soy Broth (1 L)	30 g Tryptic Soy Media
2XYT Broth (1 L)	16 g Tryptone 10 g Yeast Extract 5 g Sodium Chloride
Gram's Iodine (300 ml)	10 ml Ethanol (95%) 1 g Iodine 2 g Potassium Iodide

[a] Media and media components were purchased from Fisher Scientific Co.(St. Louis, MO).

TABLE 2. Summary of amylase activity from *Bacillus* species

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus circulans	-	-	-	-	-	-	-	-
Bacillus coagulans	+	+	+	+	+	+	+	+
Bacillus kaustophilus	-	-	-	-	-	-	-	-
Bacillus lichenformis	-	-	-	-	-	-	-	-
Bacillus macerans	v	v	v	v	v	v	v	v
Bacillus megaterium	+	+	+	+	+	+	+	+
Bacillus polymyxa	+	+	+	+	+	+	+	v
Bacillus pomolis	-	-	-	-	-	-	-	-
Baciilus sphaericus	+	+	+	+	+	+	+	v
Bacillus sterothermophile	-	-	-	-	-	-	-	-
Bacillus subtilis	+	+	+	+	+	+	+	v
Bacillus thuringiensis	+	+	+	+	+	+	+	+

[a] Organisms were propagated in tryptic soy broth (TSB) at 37° C with shaking at 250 rpm. A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile TSB was used as a negative control which tested (-) for amylase activity. Data are the result of three experiments.

IV. Effect of various sugars on amylase expression. Pure cultures of each organism demonstrating amylase activity were aseptically inoculated into 3 ml TSB contained in a 16 x 150 mm test tube with and without various sugars at a final concentration of 0.2%, 1.0%, and 2.0%. Inoculated cultures were incubated at 37° C for 16 h with 250 rpm continuous shaking. An aliquot of each culture was transferred to a sterile 1.5 ml microcentrifuge tube and centrifuged at 13,000 rpm for 5 min to precipitate bacterial cells. The supernatant was examined for amylase activity as described above.

V. Growth curve. Pure cultures of *Bacillus* species demonstrating amylase activity were aseptically inoculated into 25 ml of TSB contained in a 125 ml Erlynmeyer flask and propagated at 37° C for approximately 16 h with shaking at 250 rpm. An appropriate amount of this culture based on the ABS_{600} reading (determined using a Beckman DU 530 Life Science UV/Vis Spectrophotometer (Fullerton, CA)) was used to inoculate 100 ml of TSB contained in a 500 ml Erlynmeyer flask to an initial ABS_{600} of 0.05. This culture was incubated at 37° C with shaking. At 1h, 2h, 3h, 4h, 5h, 6h, 8h, 10h, 14h, and 18h post inoculation, the ABS_{600} was determined. Amylase activity was determined from serial two-fold dilutions of culture supernatant as indicated above.

VI. Enzyme stability. Pure cultures of each bacteria showing amylase activity were aseptically inoculated into 3 ml TSB contained in a 16 x 150 mm test tube and propagated at 37° C for 16 h with 250 rpm continuous shaking. The effect of various environmental conditions on the stability of amylase was investigated as described below:

A. Temperature. Aliquots of supernatant from each organism were incubated at 4° C, 30° C, 37° C, 60° C, and 100° C for 10 min. After incubation, amylase activity was determined as described above.

B. Salt concentration. Using a 5 M stock solution of sodium chloride, an appropriate amount was added to bacterial supernatants to achieve final salt concentrations of 1 M, 0.75 M, 0.50 M, and 0.25 M. After incubation at room temperature for 10 min, amylase activity was determined as described above.

VII. Ammonium sulfate fractionation. Pure cultures of each *Bacillus* species demonstrating amylase activity were aseptically inoculated into 25 ml of TSB contained in a 125 ml Erlynmeyer flask and propagated overnight at 37° C with shaking at 250 rpm. An appropriate amount of this culture based on the ABS_{600} reading was used to inoculate 100 ml TSB contained in a 500 ml Erlynmeyer flask to an initial ABS_{600} of 0.05. This culture was incubated at 37° C with shaking at 250 rpm for an appropriate period of time to achieve maximal amylase production (determined from growth curve data). Cells were precipitated by centrifugation for 5 min at 4,000 x g and the supernatant divided up into 10 ml aliquots. Solid ammonium sulfate was added to reach a final concentration of 20% - 80% with constant stirring at 4° C. An aliquot was removed and transferred to an 1.5 ml microcentrifuge tube. After centrifugation for 5 min, the resulting precipitate was resuspended in 200 ml of TSB. Serial two-fold dilutions were performed on the resuspended precipitate using TSB. Amylase activity was determined using the amylase assay described above.

VIII. Calculation of activity units (AU). Activity units (AU) are an arbitrary term used to quantify the amylase activity and are defined as the reciprocal of the highest dilution giving a zone of clearing. For example, if 20 µl of supernatant gave a zone of clearing at a 1:4 dilution, then 4 AU/20 µl = 20 AU/100 µl = 200 AU/ml.

RESULTS

I. Screening of *Bacillus* species for amylase activity. Twelve different *Bacillus* species were propagated in tryptic soy broth (TSB) and screened for amylase activity. Table 2 indicates that six species were positive for amylase activity and one species demonstrated variable activity. The six positive species were used in subsequent experiments. The five negative species could be due to the gene for amylase not being present or expressed.

II. Effect of various sugars on amylase expression. Various *Bacillus* species were grown in tryptic soy broth (TSB) with and without different concentrations of sugars. Sugars used were fructose, galactose, glucose, maltose, lactose, and sucrose at final concentrations of 0.2%, 1.0%, and 2.0%. Amylase activity was not affected by a 0.2% sugar concentration among the *Bacillus* species tested (Table 3). However, at 1.0% and 2.0% sugar concentrations amylase activity was affected to varying degrees (Tables 4, 5). For example, amylase activity in *B. coagulans* was repressed at both 1.0% and 2.0% fructose, glucose, and maltose whereas in *B. megaterium*, 1.0% glucose did not cause repression, but a 2.0% concentration did. The remaining *Bacillus* species tested exhibited similar levels of activity regardless of the sugar concentration used.

III. Enzyme stability. The effect of various environmental conditions on the stability of amylase from crude supernatants was investigated.

A. Temperature. Aliquots of supernatant from each *Bacillus* species were incubated at 4° C, 30° C, 37° C, 50° C, and 100° C to evaluate the effect of temperature on amylase activity (Table 6). Amylase activity was completely inhibited at 100° C in all *Bacillus* species while 50° C inhibited amylase activity in *B. polymyxa, B. subtilis,* and *B. thuringiensis*. Variable to optimal levels of amylase activity was observed in

*B. coagulans,B. sphaericus,*and *B. megaterium* at 50° C. Amylase activity was not inhibited at 4° C, 30° C, and 37° C in all the *Bacillus* species tested.

B. Salt concentration. Aliquots of crude supernatant from each *Bacillus* species were subjected to varying concentrations of sodium chloride (1.0M, 0.75M, 0.50M, and 0.25M) to evaluate the effect on amylase activity (Table 7). Amylase from *B. coagulans* was not affected by the varying concentrations of sodium chloride. *Bacillus sphaericus* and *B. megaterium* showed variable amylase activity (33% - 66%) at 1.0M and 0.75M sodium chloride, but no inhibition at sodium concentrations less than 0.75M. *Bacillus polymyxa, B. subtilis,* and *B. thuringiensis* exhibited variable amylase activity (33% - 66%) at all concentrations of sodium chloride examined.

IV. Effect of growth conditions on amylase activity. To determine if different growth conditions affected amylase activity, the *Bacillus* species demonstrating amylase activity were propagated at different temperatures (30° C, 37° C, or 42° C) and in different growth media (BHI, LB, MM, TSB, and 2XYT). Activity units (AU) were calculated allowing amylase activity to be quantified.

A. Growth at 37° C with 250 rpm shaking. Brain heart infusion media and TSB allowed maximal amylase activity (data not shown) for all *Bacillus* species tested. However, propagation in LB, MM, and 2XYT resulted in varying degrees of activity (Tables 8, 9, 10). Specifically, slightly lower amounts of amylase activity were observed in LB and MM (Table 8, 9). Amylase production was significantly inhibited in *B. subtilis* upon propagation in 2XYT media whereas other species were unaffected (Table 10). All data are summarized in Table 11.

B. Growth at 37° C without 250 rpm shaking. Amylase activity from all *Bacillus* species tested was variable in BHI, LB, MM, TSB, and 2XYT, suggesting

shaking is critical to achieve maximal amylase activity (Tables 12 - 16). Propagation in BHI resulted in minimal activity in *B. subtilis* and *B. thuringiensis,* whereas the other species exhibited significant variability (Table 12). Propagation in LB media provided varying levels of amylase activity in all species examined, except *B. thuringiensis,* which exhibited near maximal activity (Table 13). Table 14 illustrates that overall MM provided little to no amylase activity in all species examined. Growth in TSB and 2XYT also provided similar results to that of BHI with minimal activity occuring in *B. subtilis* (Tables 15, 16). All data are summarized in Table 17.

C. Growth at 30° C with 250 rpm shaking. Brain heart infusion broth allowed maximal production of amylase in all *Bacillus* species tested (data not shown). Similar results were obtained in all *Bacillus* species propagated in TSB with the exception of *B. megaterium* which exhibited variable activity (Table 18). Amylase activity was significantly decreased upon growth in LB and MM, with the exception of *B. sphaericus* which still retained near maximal activity (Tables 19, 20). Table 21 demonstrates that 2XYT resulted in predominently maximal activity with some variable activity in all species examined. All data are summarized in Table 22.

D. Growth at 42° C with 250 rpm shaking. Amylase from all *Bacillus* species tested was at optimal to variable levels in all media tested except MM (Tables 23 - 27). Amylase activity in supernatant from cells grown in BHI, LB, TSB, and 2XYT was maximal in all species, except for a few species which showed some variability (Tables 23, 24, 26, 27). In contrast, propagation in MM signficantly lowered amylase activity in all species examined (Table 25). All data are summarized in Table 28.

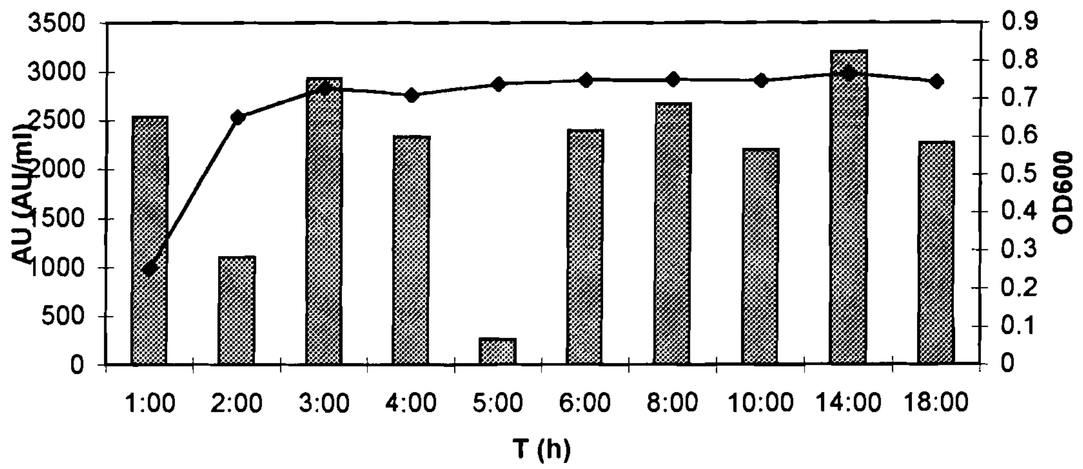
V. Growth curves. To investigate amylase production in conjunction with cell growth, ABS_{600} readings were determined at different time intervals on all *Bacillus* species exhibiting amylase activity. All *Bacillus* species examined showed similar growth curve patterns, but different patterns of amylase activity. *Bacillus megaterium*, *B. polymyxa,*

B. subtilis, and *B. thuringiensis* had an initial increase in amylase activity, while *B. coagulans* and *B. sphaericus* demonstrate an initial decrease in activity (Figures 2A – 2F). In all *Bacillus* species examined, amylase activity could be detected at all time points although at varying levels.

VI. Ammonium sulfate fractionation. *Bacillus* species exhibiting amylase activity were subjected to ammonium sulfate fractionation to determine the quantity of solid ammonium sulfate necessary to precipitate amylase from the supernatant. Amylase from *B. megaterium*, *B. subtilis*, and *B. thuringiensis* was precipitated during the entire fractionation process in maximal amounts (Tables 29 – 31). Amylase produced by *B. coagulans* was precipitated using 20% - 60% ammonium sulfate with a maximal amount of activity (Table 32). Amylase from *B. polymyxa* and *B. sphaericus* could not be precipitated using ammonium sulfate fractionation (data not shown). All data are summarized in Table 33.

FIG. 2. Growth and amylase production. A. *B. coagulans*; B. *B. megaterium*; C. *B. polymyxa*; D. *B. sphaericus*; E. *B. subtilis*; and F. *B. thuringiensis*. Each organism was grown in tryptic soy broth (TSB) at 37° C with continuous shaking at 250 rpm. At timed intervals, aliquots were removed and amylase activity determined as described in the Materials and Methods. Data are the mean of three experiments. (Dark bars represent activity units while triangles connected by a line indicate growth curve data.)

A.



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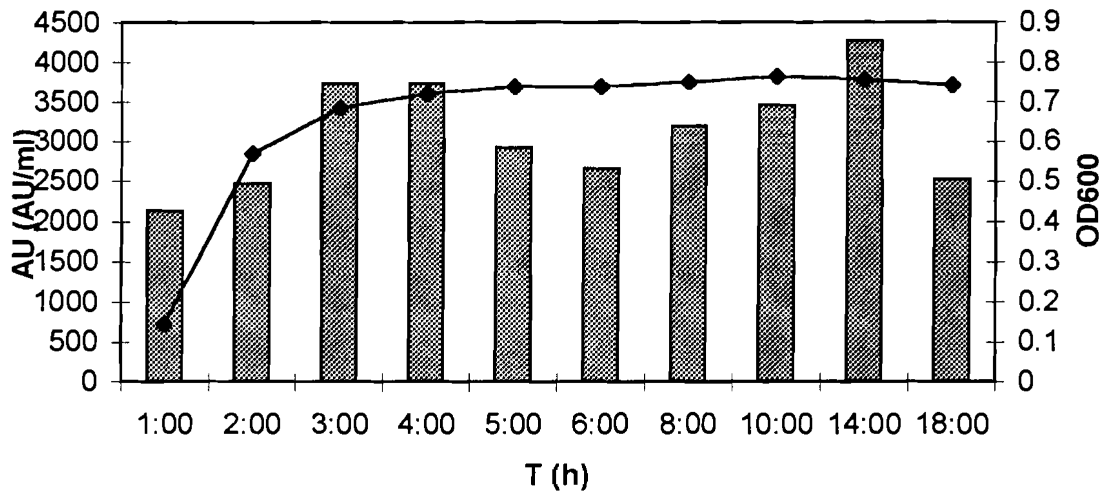

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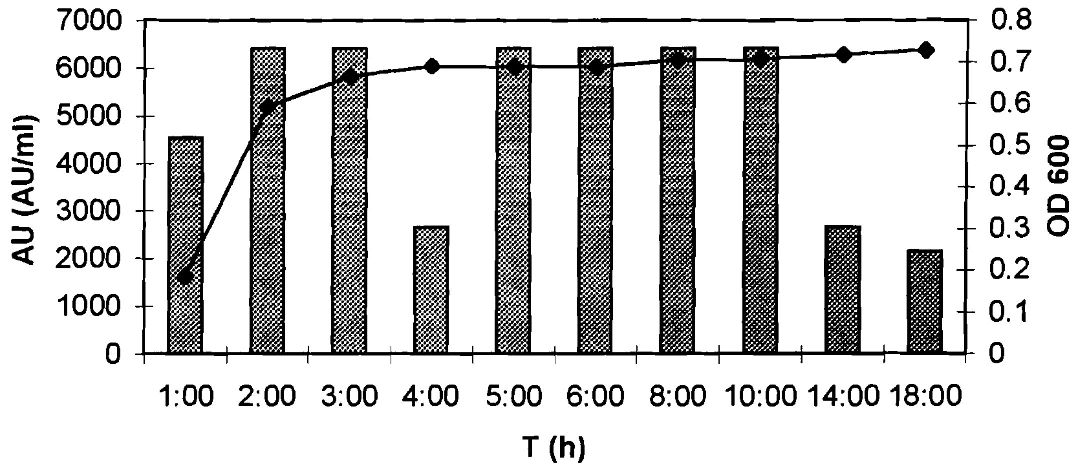
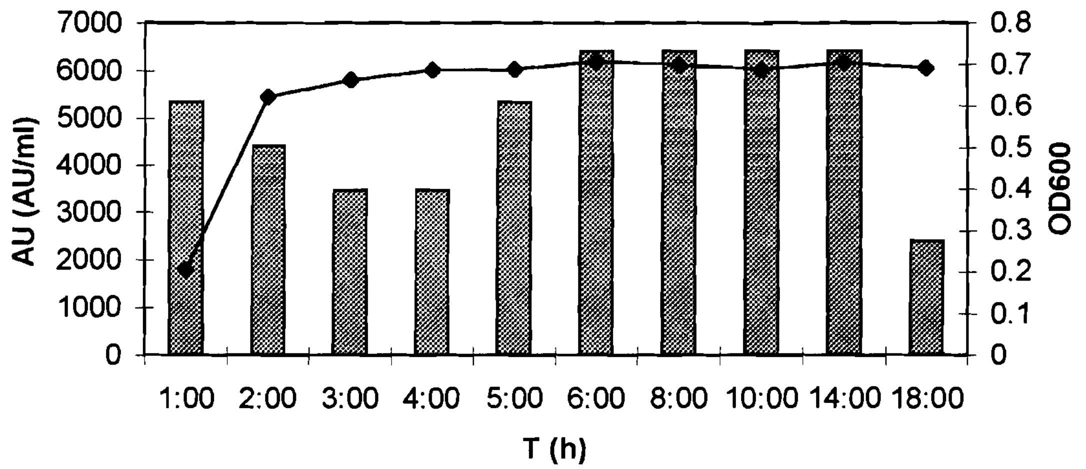


D.



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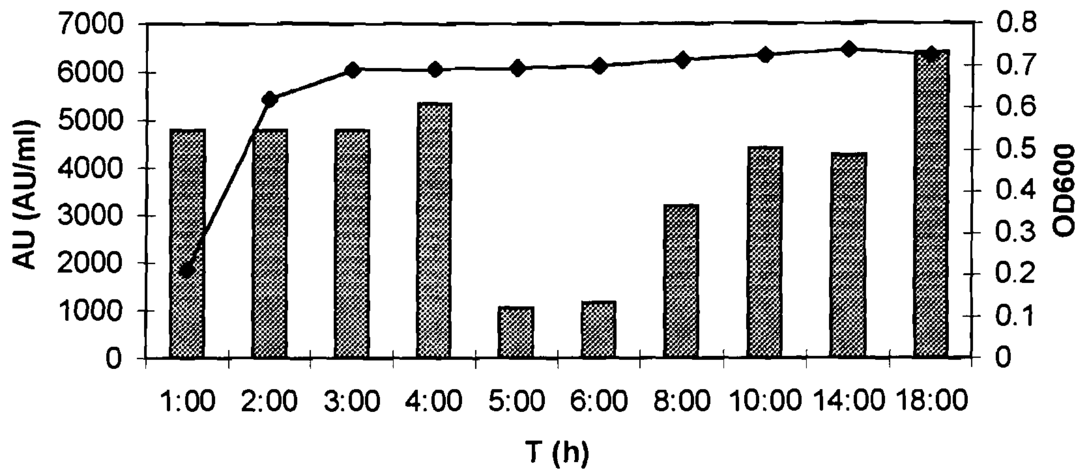

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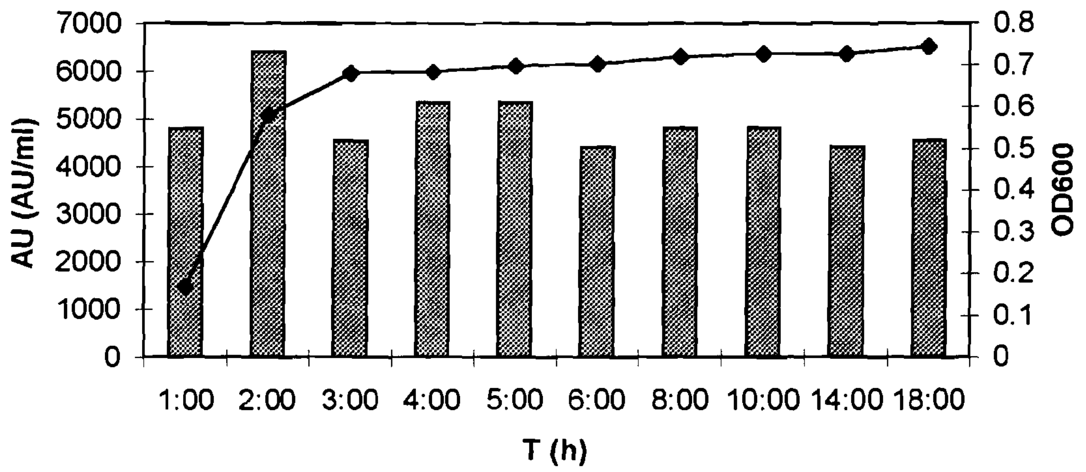

TABLE 3. Effect of 0.2% sugar concentration on amylase activity

Organism	Media[a]						
	TSB	TSB-fru	TSB-gal	TSB-glu	TSB-lac	TSB-mal	TSB-suc
Bacillus coagulans	+	+	+	+	+	+	+
Bacillus megaterium	+	+	+	+	+	+	+
Bacillus polymyxa	+	+	+	+	+	+	+
Bacillus sphaericus	+	+	+	+	+	+	+
Bacillus subtilis	+	+	+	+	v	+	+
Bacillus thuringiensis	+	+	+	+	+	+	+

[a] Organisms were grown in typtic soy broth (TSB) media alone or TSB supplemented with various sugars at a final concentration of 0.2%; fru, fructose; gal, galactose; glu, glucose; lac, lactose; mal, maltose; suc, sucrose. A (+) sign indicates 100% amylase activity and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Cultures were shaken at 250 rpm.
Data are the result of three experiments.

TABLE 4. Effect of 1.0% sugar concentration on amylase activity

Organism	Media[a]						
	TSB	TSB-fru	TSB-gal	TSB-glu	TSB-lac	TSB-mal	TSB-suc
Bacillus coagulans	+	-	+	-	+	-	+
Bacillus megaterium	+	-	+	+	v	v	+
Bacillus polymyxa	+	+	v	+	+	+	+
Bacillus sphaericus	+	v	+	+	+	+	+
Bacillus subtilis	+	-	+	-	v	-	+
Bacillus thuringiensis	+	+	+	+	v	+	v

[a] Organisms were grown in tryptic soy broth (TSB) media alone or TSB supplemented with various sugars at a final concentration of 1.0%; fru, fructose; gal, galactose; glu, glucose; lac, lactose; mal, maltose; suc, sucrose. A (+) sign indicates 100% amylase activity, a (-) indicates an absence of amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile TSB was used as a negative control which tested (-) for amylase activity. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 5. Effect of 2.0% sugar concentration on amylase activity

Organism	Media[a]						
	TSB	TSB-fru	TSB-gal	TSB-glu	TSB-lac	TSB-mal	TSB-suc
Bacillus coagulans	+	-	+	-	+	-	+
Bacillus megaterium	+	-	+	-	v	v	+
Bacillus polymyxa	+	+	v	+	+	+	+
Bacillus sphaericus	+	+	+	+	+	+	+
Bacillus subtilis	+	-	+	-	v	-	+
Bacillus thuringiensis	+	+	+	+	+	+	+

[a] Organisms were grown in typtic soy broth (TSB) media alone or TSB supplemented with various sugars at a final concentration of 2.0%; fru, fructose; gal, galactose; glu, glucose; lac, lactose; mal, maltose; suc, sucrose. A (+) sign indicates 100% amylase activity, a (-) indicates an absence of amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 6. Effect of temperature on amylase activity

Organism	Temperature[a]					
	Control	4° C	30° C	37° C	50° C	100° C
Bacillus coagulans	+	+	+	+	v	-
Bacillus megaterium	+	+	+	+	+	-
Bacillus polymyxa	+	+	+	+	-	-
Bacillus sphaericus	+	+	+	+	v	-
Bacillus subtilis	+	+	+	+	-	-
Bacillus thuringiensis	+	+	+	+	-	-

[a] Organisms were grown in tryptic soy broth (TSB) media at 37° C with 250 rpm of continuous shaking. A (+) sign indicates 100% amylase activity, a (-) indicates an absence of amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile TSB was used as a negative control which tested (-) for amylase activity. Data are the result of three experiments.

TABLE 7. Effect of varying salt concentrations on amylase activity

Organism	Salt concentration[a]			
	1.00 M	0.75 M	0.50 M	0.25 M
Bacillus coagulans	+	+	+	+
Bacillu megaterium	v	v	+	+
Bacillus polymyxa	v	v	v	v
Baciilus sphaericus	v	+	+	+
Bacillus subtilis	v	v	v	v
Bacillus thuringiensis	v	v	v	v

[a] Organisms were propagated in tryptic soy broth at 37° C with 250 rpm shaking. A (+) sign indicates 100% amylase activity and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Data are the result of three experiments.

TABLE 8. Effect of propagation in Luria-Bertani media at 37° C with shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	+	+	+	v	v	v
Bacillus megaterium	+	+	+	+	+	+	+	-
Bacillus polymyxa	+	+	+	+	+	+	v	v
Bacillus sphaericus	+	+	+	+	+	+	v	v
Bacillus subtilis	+	+	+	+	+	+	v	v
Bacillus thuringiensis	+	+	+	+	+	+	v	v

[a] A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity as determined using the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile Luria-Bertani media was used as a negative control which tested (-) for amylase activity. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 9. Effect of propagation in minimal media at 37° C with shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	+	+	+	+	+	v
Bacillus megaterium	+	+	+	+	v	v	-	-
Bacillus polymyxa	+	+	+	+	v	v	v	v
Bacillus sphaericus	+	+	+	+	+	+	+	-
Bacillus subtilis	+	+	+	+	+	+	-	-
Bacillus thuringiensis	+	+	+	+	+	+	+	-

[a] A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity as determined using the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile minimal media was used as a negative control which tested (-) for amylase activity. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 10. Effect of propagation in 2XYT media at 37° C with shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	+	+	+	+	+	+
Bacillus megaterium	+	+	+	+	+	+	v	v
Bacillus polymyxa	+	+	+	+	+	+	v	v
Bacillus sphaericus	+	+	+	+	+	+	+	v
Bacillus subtilis	+	+	v	v	v	v	v	v
Bacillus thuringiensis	+	+	+	+	+	+	+	+

[a] A (+) sign indicates 100% amylase activity and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile 2XYT media was used as a negative control which tested (-) for amylase activity. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 11. Summary of amylase activity from *Bacillus* species grown at 37° C with shaking

Organism	Activity units (AU)/ml[a]				
	BHI	LB	MM	TSB	2XYT
Bacillus coagulans	6400	2667	4267	6400	6400
Bacillus megaterium	6400	3200	1200	6400	3733
Bacillus polymyxa	4800	4800	2533	6400	4800
Bacillus sphaericus	6400	3200	3200	6400	5333
Baciilus subtilis	6400	4800	1600	6400	2700
Bacillus thuringiensis	6400	4800	3200	6400	6400

[a] Organisms were propagated at 37° C with shaking at 250 rpm in the following media: BHI, brain heart infusion; LB, Luria-Bertani; MM, minimal media; TSB, tryptic soy broth; and 2XYT. Activity units were calculated as described in the Materials and Methods. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 12. Effect of propagation in brain heart infusion media at 37° C without shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	v	v	v	v	-	-
Bacillus megaterium	+	+	+	+	+	+	v	v
Bacillus polymyxa	v	v	v	v	v	v	v	v
Bacillus sphaericus	+	+	+	+	v	v	v	v
Bacillus subtilis	v	v	-	-	-	-	-	-
Bacillus thuringiensis	v	v	v	v	v	v	-	-

[a] A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile brain heart infusion media was used as a negative control which tested (-) for amylase activity. Data are the result of three experiments.

TABLE 13. Effect of propagation in Luria-Bertani media at 37° C without shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	v	v	v	v	v	v	v	v
Bacillus megaterium	v	v	v	v	v	v	v	v
Bacillus polymyxa	+	+	+	v	v	v	v	v
Bacillus sphaericus	v	v	v	v	v	v	v	v
Bacillus subtilis	+	+	+	v	v	v	v	v
Bacillus thuringiensis	+	+	+	+	+	+	+	v

[a] Organisms were grown in Luria-Bertani (LB) media at 37° C without 250 rpm of continuous shaking. A (+) sign indicates 100% amylase activity and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile LB media was used as a negative control which tested (-) for amylase activity. Data are the result of three experiments.

TABLE 14. Effect of propagation in minimal media at 37° C without shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	v	v	v	v	v	v
Bacillus megaterium	+	v	v	v	v	v	v	-
Bacillus polymyxa	+	+	+	v	v	-	-	-
Bacillus sphaericus	v	v	-	-	-	-	-	-
Bacillus subtilis	v	v	v	v	v	-	-	-
Bacillus thuringiensis	+	v	v	v	v	v	v	-

[a] Organisms were grown in minimal media (MM) at 37° C without 250 rpm of continuous shaking. A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile MM was used as a negative control which tested (-) for amylase activity. Data are the result of three experiments.

TABLE 15. Effect of propagation in tryptic soy broth at 37° C without shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	+	+	v	v	v	v
Bacillus megaterium	+	+	+	+	+	+	+	v
Bacillus polymyxa	v	v	v	v	v	v	v	v
Bacillus sphaericus	+	+	+	v	v	v	v	v
Bacillus subtilis	+	+	v	-	-	-	-	-
Bacillus thuringiensis	+	+	+	v	v	v	v	v

[a] Organisms were grown in tryptic soy broth (TSB) at 37° C without 250 rpm of continuous shaking. A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile TSB was used as a negative control which tested (-) for amylase activity. Data are the result of three experiments.

TABLE 16. Effect of propagation in 2XYT media at 37° C without shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	+	v	v	v	v	-
Bacillus megaterium	+	+	+	+	+	+	v	v
Bacillus polymyxa	+	+	+	+	+	+	+	v
Bacillus sphaericus	+	+	+	+	+	v	v	v
Bacillus subtilis	+	v	v	-	-	-	-	-
Bacillus thuringiensis	v	v	v	v	v	v	v	v

[a] Organisms were grown in 2XYT media at 37° C without 250 rpm of continuous shaking. A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile 2XYT media was used as a negative control which tested (-) for amylase activity. Data are the result of three experiments.

TABLE 17. Summary of amylase activity from *Bacillus* species grown at 37° C without shaking

Organism	Activity units (AU)/ml[a]				
	BHI	LB	MM	TSB	2XYT
Bacillus coagulans	633	6400	2233	2533	1267
Bacillu megaterium	4800	6400	1133	5333	3733
Bacillus polymyxa	4800	2733	600	3300	5333
Baciilus sphaericus	2533	6400	2167	1667	3467
Bacillus subtilis	100	1667	800	133	200
Bacillus thuringiensis	1600	4267	1067	3267	6400

[a] Organisms were propagated at 37° C without shaking at 250 rpm in the following media: BHI, brain heart infusion; LB, Luria-Bertani; MM, minimal media; TSB, tryptic soy broth; and 2XYT. Activity units were calculated as described in the Materials and Methods. Data are the result of three experiments.

TABLE 18. Effect of propagation in tryptic soy broth at 30° C with shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	+	+	+	+	+	+
Bacillus megaterium	+	v	v	v	v	v	-	-
Bacillus polymyxa	+	+	+	+	+	+	+	+
Bacillus sphaericus	+	+	+	+	+	+	+	+
Bacillus subtilis	+	+	+	+	+	+	+	+
Bacillus thuringiensis	+	+	+	+	+	+	+	+

[a] A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity as determined by using the amylase assay describe Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile tryptic soy broth was used as a negative control which tested (-) for amylase activity. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 19. Effect of propagation in Luria-Bertani media at 30° C with shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	v	v	-	-	-	-	-
Bacillus megaterium	+	v	v	v	v	v	v	v
Bacillus polymyxa	+	v	v	v	v	v	v	v
Bacillus sphaericus	+	+	+	+	+	+	+	+
Bacillus subtilis	+	v	v	v	-	-	-	-
Bacillus thuringiensis	+	v	-	-	-	-	-	-

[a] A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile Luria-Bertani media was used as a negative control which tested (-) for amylase activity. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 20. Effect of propagation in minimal media at 30° C with shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	+	+	v	v	v	v
Bacillus megaterium	v	v	v	v	v	v	v	v
Bacillus polymyxa	v	v	v	v	v	v	v	v
Bacillus sphaericus	+	+	+	+	+	v	v	v
Bacillus subtilis	+	v	v	v	v	v	-	-
Bacillus thuringiensis	v	v	v	-	-	-	-	-

[a] A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile minimal media was used as a negative control which tested (-) for amylase activity. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 21. Effect of propagation in 2XYT media at 30° C with shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	+	+	+	+	+	v
Bacillus megaterium	+	+	+	+	+	+	+	+
Bacillus polymyxa	+	+	+	+	+	+	+	v
Bacillus sphaericus	v	v	v	v	v	v	v	v
Bacillus subtilis	+	+	+	+	+	+	+	+
Bacillus thuringiensis	v	v	v	v	v	v	v	v

[a] A (+) sign indicates 100% amylase activity and a (v) indicates variable activity as determined by the amylase assay describe in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile 2XYT media was used as a negative control which tested (-) for amylase activity. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 22. Summary of amylase activity from *Bacillus* species grown at 30° C with shaking

Organism	Activity units (AU) unit/ml[a]				
	BHI	LB	MM	TSB	2XYT
Bacillus coagulans	6400	200	2400	6400	4267
Bacillu megaterium	6400	2167	3400	800	6400
Bacillus polymyxa	6400	2167	6400	6400	5333
Baciilus sphaericus	6400	6400	3400	6400	6400
Bacillus subtilis	6400	100	533	6400	6400
Bacillus thuringiensis	6400	67	150	6400	6400

[a] Organisms were propagated at 30° C with shaking at 250 rpm in the following media: BHI, brain heart infusion; LB, Luria-Bertani; MM, minimal media; TSB, tryptic soy broth, and 2XYT. Activity units were calculated as described in the Materials and Methods. Data are the result of three experiments.

TABLE 23. Effect of propagation in brain heart infusion media at 42° C with shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	+	+	v	v	v	v
Bacillus megaterium	+	+	v	v	v	v	v	v
Bacillus polymyxa	+	+	+	+	+	+	+	v
Bacillus sphaericus	+	+	+	+	+	+	+	v
Bacillus subtilis	+	+	+	+	+	+	-	-
Bacillus thuringiensis	+	+	+	+	+	+	+	+

[a] A (+) sign indicates 100% amylase activity, a (-) no indicates amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the materials and methods. Variable activity is defined as 33% - 66% positive. Sterile brain heart infusion media was used as a negative control which tested (-) for amylase activity. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 24. Effect of propagation in Luria-Bertani media at 42° C with shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	+	+	v	v	-	-
Bacillus megaterium	+	+	+	+	v	v	v	-
Bacillus polymyxa	+	+	+	+	+	+	v	-
Bacillus sphaericus	+	+	+	+	v	v	v	v
Bacillus subtilis	+	+	+	+	-	-	-	-
Bacillus thuringiensis	+	+	+	+	+	+	+	+

[a] A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile Luria-Bertani media was used as a negative control which tested (-) for amylase activity. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 25. Effect of propagation in minimal media at 42° C with shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	+	v	v	v	v	-
Bacillus megaterium	+	+	+	+	v	v	-	-
Bacillus polymyxa	-	-	-	-	-	-	-	-
Bacillus sphaericus	v	v	-	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-	-	-	-
Bacillus thuringiensis	+	+	+	-	-	-	-	-

[a] A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile minimal media was used as a negative control which tested (-) for amylase activity. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 26. Effect of propagation in tryptic soy broth at 42° C with shaking on amylase activity

Organism	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	+	+	+	+	+	+
Bacillus megaterium	+	+	+	+	+	+	+	v
Bacillus polymyxa	+	+	+	v	v	v	v	v
Bacillus sphaericus	+	+	+	+	+	+	v	v
Bacillus subtilis	+	+	+	+	+	+	v	v
Bacillus thuringiensis	+	+	+	+	+	+	+	+

[a] A (+) sign indicates 100% amylase activity and a (v) indicates variable activity as determined by the amylase activity described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile tryptic soy broth was used as a negative control which tested (-) for amylase activity. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 27. Effect of propagation in 2XYT media at 42° C with shaking on amylase activity

Organism	Dilution factor on supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Bacillus coagulans	+	+	+	+	+	v	v	-
Bacillus megaterium	+	+	+	+	+	+	+	+
Bacillus polymyxa	+	+	+	+	+	+	v	-
Bacillus sphaericus	+	+	+	+	+	v	v	v
Bacillus subtilis	+	+	+	+	+	v	v	-
Bacillus thuringiensis	+	+	+	+	+	+	+	+

[a] A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity as determined by the amylase assay described in the Materials and Methods. Variable activity is defined as 33% - 66% positive. Sterile 2XYT media was used as a negative control which tested (-) for amylase activity. Cultures were shaken at 250 rpm. Data are the result of three experiments.

TABLE 28. Summary of amylase activity from *Bacillus* species grown at 42° C with shaking

Organism	Activity units (AU)/ml[a]				
	BHI	LB	MM	TSB	2XYT
Bacillus coagulans	4267	933	1667	6400	1600
Bacillu megaterium	4300	1333	933	5333	6400
Bacillus polymyxa	5333	2400	0	3267	1600
Baciilus sphaericus	5333	2800	100	4800	4533
Bacillus subtilis	1600	400	0	3733	2400
Bacillus thuringiensis	6400	6400	200	6400	6400

[a] Organisms were propagated at 42° C with shaking at 250 rpm in the following media BHI, brain heart infusion; LB, Luria-Bertani; MM, minimal media; TSB, tryptic soy broth; and 2XYT. Data are the result of three experiments.

TABLE 29. Ammonium sulfate fractionation of amylase from *Bacillus thuringiensis*

Percent of ammonium sulfate	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
0	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+
40	+	+	+	+	+	+	+	+
50	+	+	+	+	+	+	+	+
60	+	+	+	+	+	+	+	+
70	+	+	+	+	+	+	v	v
80	+	+	+	+	+	+	v	v

[a] Organisms were propagated in tryptic soy broth (TSB) at 37° C with 250 rpm shaking. Variable amounts of solid ammonium sulfate were added to supernatants to precipitate amylase followed by detection using the amylase assay as described in the Materials and Methods. A (+) sign indicates 100% amylase activity and a (v) indicates variable activity. Variable activity is defined as 33% - 66% positive. Sterile TSB was used as a negative control which tested (-) for amylase activity. Data are the result of three experiments.

TABLE 30. Ammonium sulfate fractionation of amylase from *Bacillus megaterium*

Percent of ammonium sulfate	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
0	+	+	+	+	+	v	v	v
20	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+
40	+	+	+	+	v	v	v	v
50	+	+	+	v	v	v	v	v
60	+	+	+	+	+	+	+	+
70	+	+	+	+	v	v	v	v
80	+	+	+	+	v	v	v	v

[a] Organisms were propagated in tryptic soy broth (TSB) media at 37° C with 250 rpm shaking. Variable amounts of solid ammonium sulfate were added to supernatants to precipitate amylase followed by detection using the amylase assay as described in the Materials and Methods. A (+) sign indicates 100% amylase activity and a (v) indicates variable activity. Variable activity is defined as 33% - 66% positive. Sterile TSB was used as a negative control which tested (-) for amylase activity. Data are the result of three experiments.

TABLE 31. Ammonium sulfate fractionation of amylase from *Bacillus subtilis*

Percent of ammonium sulfate	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
0	+	+	+	+	+	+	+	+
20	+	+	+	+	+	v	v	v
30	+	+	+	+	+	+	+	+
40	+	+	+	+	+	+	+	+
50	+	+	+	v	v	v	v	v
60	+	+	+	v	v	v	v	v
70	v	v	v	v	v	v	v	v
80	v	v	v	v	v	v	v	v

[a] Organisms were propagated in tryptic soy broth (TSB) at 37° C with 250 rpm shaking. Variable amounts of solid ammonium sulfate were added to supernatants to precipitate amylase followed by detection using the amylase assay as described in the Materials and Methods. A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity. Variable activity is defined as 33% - 66% positive. Sterile TSB was used as a negative control which tested (-) for amylase activity. Data are the result of three experiments.

TABLE 32. Ammonium sulfate fractionation of amylase from *Bacillus coagulans*

Percent of ammonium sulfate	Dilution factor of supernatant[a]							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
0	+	+	+	+	+	+	+	+
20	+	+	v	v	v	v	v	v
30	v	v	v	v	v	v	v	v
40	v	v	v	v	v	v	v	v
50	v	v	v	v	v	v	v	v
60	v	v	v	v	v	v	v	v
70	v	-	-	-	-	-	-	-
80	v	-	-	-	-	-	-	-

[a] Organisms were propagated in tryptic soy broth (TSB) at 37° C with 250 rpm shaking. Variable amounts of solid ammonium sulfate were added to supernatants to precipitate amylase followed by detection using the amylase assay as described in the Materials and Methods. A (+) sign indicates 100% amylase activity, a (-) indicates no amylase activity, and a (v) indicates variable activity. Variable activity is defined as 33% - 66% positive. Sterile TSB was used as a negative control which tested (-) for amylase activity. Data are the result of three experiments.

TABLE 33. Summary of ammonium sulfate fractionation of amylase from *Bacillus* species

Organism	Activity units (AU/ml)[a]							
	0	20	30	40	50	60	70	80
Bacillus coagulans	6400	2300	2267	2133	2400	800	0	0
Bacillus megaterium	4533	6400	6400	4400	3267	6400	2533	933
Bacillus polymyxa	6400	0	0	0	0	0	0	0
Baciilus sphaericus	6400	0	0	0	0	0	0	0
Bacillus subtilis	6400	4533	6400	6400	3267	3267	167	33
Bacillus thuringiensis	6400	6400	6400	6400	6400	6400	4800	4800

[a] Organisms were propagated in tryptic soy broth (TSB) at 37° C with shaking at 250 rpm. Variable amounts of solid ammonium sulfate (0%, 20%, 30%, 40%, 50%, 60%, 70%, and 80%) were added to supernatants to precipitate amylase followed by detection using the amylase assay as described in the Materials and Methods. Activity units were calculated as described in the Materials and Methods. Data are the result of three experiments.

DISCUSSION

Understanding conditions necessary for microbial growth allows prediction of how quickly microorganisms will grow in various situations. In correlation with optimal growth are optimal levels of intracellular and extracellular enzyme production. Enzymes serve as catalysts for specific chemical reactions and the growth of an organism depends on the optimal functioning of its enzymes (27, 32). Environmental conditions, such as pH, temperature, and ionic strength play key roles in the expression and activity of enzymes (32).

Amylase plays a critical role in the food and biotechnological industries (1). Although amylase can be purified from many sources, microbial sources have received the most attention due to their low cost production (24). Commercially, production levels of amylase are variable at best and industry is constantly exploiting optional methods of expression and purification (20, 33). A common microbial source is found in the genus *Bacillus*. Most *Bacillus* species produce amylase in order to utilize starch available in the environment. Thus, the objective of this study was to evaluate the effects of different growing environments on the expression of amylase by various *Bacillus* species. Additionally, the stability of crude amylase preparations was investigated.

There is a clear correlation between the conditions found in the environment occupied by a microbe and the properties of its enzyme (33). To investigate this phenomenon, experiments were designed to analyze the effects of different sugars in the growth media on amylase production by different *Bacillus* species. For example, starch and maltose are known amylase inducers in different organisms, including *Bacillus* sp. WN11 (17) and *B. stearothermophilus* (31, 34). Data obtained in this study

indicated maltose induced amylase expression in *B. polymyxa, B. sphaericus,* and *B. thuringiensis* (Tables 3 - 5). Previous studies have also noted a negative effect on amylase expression in the presence of glucose (3, 8). As illustrated in Tables 3 - 5, *Bacillus subtilis* amylase expression was repressed by 1.0% and 2.0% glucose, but not 0.2% glucose, which has been noted by other researchers (8). These data can likely be explained by strain variation. The remaining *Bacillus* species exhibited variable responses to the presence of different sugars. Accountability for these data may be the theory of enzyme repression, although no direct experimental evidence has been obtained. In this model, the sugars would be serving as corepressors and, in combination with repressors, would be bound to the operator region of the DNA, thereby preventing transcription. This model has been used accurately to describe repressible synthesis in alpha amylase produced by *B. lichenformis* 584 in the presence of 0.5% glucose (30), *Bacillus* sp. TS-23 grown in medium containing glucose or fructose (16), *Bacillus subtilis* alpha amylase formation (3), and alpha amylase formation in the presence of fructose in *B. stearothermophilus* (34). Furthermore, it has been reported that the synthesis of carbohydrate-degrading enzymes in most species of the genus *Bacillus* is subject to catabolite repression by readily metabolizable substrates such as glucose and fructose (16).

The nature and amount of carbon and nitrogen sources in culture media also affects microbial growth and enzyme production (8, 16, 17, 31). For example, peptone, polypeptone, and meat extract are required as organic nitrogen sources for high yields of amylase in *Bacillus* sp. WN11 and beta amylase from *Bacillus polymyxa* (17, 24). Compared to these nitrogen sources, amylase production was lower in media containing

yeast extract in *Bacillus* sp. WN11 and *B. sterothermophilus* (17). Furthermore, it has been reported that carbon sources greatly affect the production of thermophilic amylases (16). Thus, the richness of the media plays a vital role in supplying the building blocks of proteins such as amylase. To investigate the influence of media on amylase production, the *Bacillus* species were propagated in media of varying richness and at different temperatures and oxygen availability. Of the five media evaluated, BHI, LB, TSB, and 2XYT are rich in peptones and other nutrients whereas MM provides minimal nutrients. Furthermore, 2XYT and LB contain moderate amounts of yeast extract in contrast to the other media. Temperature and dissolved oxygen also play vital roles. Most organisms grow faster at 37° C versus 30° C and the more aerated a culture is, the faster it grows (17). In this study, growth in MM resulted in minimal amounts of amylase expression (Tables 11, 17, 22, 28). Propagation in BHI and TSB provided optimal amylase activity in all growth conditions for the *Bacillus* species tested, except in non-aerated cultures at 37° C (Tables 11, 17, 22, 28). *Bacillus* species propagated in 2XYT and LB showed lower yields of amylase activity and in some cases more activity in non-aerated cultures (Tables 11, 17, 22, 28).

When comparing growth conditions with shaking for most *Bacillus* species tested, less amylase activity was observed at 30° C vs. 37° C (Tables 11, 17, 22, 28). However, 42° C resulted in less amylase production than at 37° C (Tables 11, 17, 22, 28). It has been shown that temperature may indirectly affect the level of cell-free amylase released into the culture medium by inducing changes in the cell membrane and cell wall, which can affect the release of extracellular enzymes to the culture medium (17). Since all proteins are required to maintain a proper tertiary structure for enzymatic activity, the

elevation of the growth temperature to 42° C may have provided a less than optimal environment for proper protein folding (10).

In addition to playing a vital role in the production of enzymes, the composition and concentration of the media also affect the growth stage at which enzymes are produced in organisms (31). In *B. cereus*, it has been reported that media composition affects amylase production as well as sporulation (29, 31). In this study, growth curves were generated to determine the relationship between growth and amylase production for all *Bacillus* species. Figures 2A - 2F illustrate that amylase was produced at different times throughout the 18 h growth curve and at variable levels. These data were somewhat surprising, but other investigators have documented similar results. In one study, *B. subtilis* strain N did not produce alpha amylase in the logarithmic phase of growth, but produced large amounts of enzyme after the culture entered the stationary phase of growth (34). In a related study different strains of *B. subtilis* showed increases in alpha amylase with cell mass and leveled off when the stationary growth phase was reached (34). In an investigation involving *B. stearothermophilus*, it was determined that optimal amylase production occurred during the logarithmic phase of growth and was inversely proportional to the rate of growth (32). However, alpha amylase synthesis from *B. subtilis* strain GM96101 occurred throughout cell growth (11). Thus, these studies confirm the variable pattern of amylase production. To further help explain the variation in amylase production observed in our study, spore stains were performed on samples obtained from the growth curve of *B. polymyxa*. Spores were identified starting at 8 h and continuing through 18 h (data not shown). These data demonstrated individual cells

were not at the same stage of growth and this variability likely accounted for the sporadic production of amylase activity throughout the growth curve.

One of the first steps performed in most protein purification protocols is ammonium sulfate fractionation to precipitate the protein of interest. Previous studies have determined that the majority of alpha amylase activity in *B. licheniformis* CUMC305 was found in the 30 to 65% ammonium sulfate fraction (15). In this study, data obtained for *B. megaterium*, *B. subtilis*, and *B. thuringiensis* correlate with these findings (Tables 29 – 31). *B. coagulans* exhibited little activity while *B. polymyxa* and *B. sphaericus* amylase activity was inhibited upon fractionation with ammonium sulfate (Tables 33). Although no direct experimental evidence has been obtained, an explanation for the *B. polymyxa* and *B. sphaericus* data could be attributed to lack of stabilization of the protein's tertiary structure. However, one advantage of ammonium sulfate fractionation over other techniques is stabilization of the tertiary structure (1). The lack of activity could also be due to heavy metal contamination. Ammonium sulfate contains small amounts of heavy metals, which have been documented to be detrimental to sensitive enzymes (1). Indeed, metal ions are known to affect amylase production (31). Whatever the reason, it is clear that the enzymatic activity of amylase from *B. polymyxa* and *B. sphaericus* exhibit greater sensitivity to ammonium sulfate than the remaining *Bacillus* species tested.

To investigate the stability of crude amylase preparations from the different *Bacillus* species, experiments were designed to evaluate the effect of different salt concentrations and temperature conditions. A previous report demonstrated that amylase activity is upregulated in *B. licheniformis* CUMC305 by low concentrations of NaCl (15).

In this study, however, upregulation of amylase activity in response to NaCl concentrations was not observed, but rather a variable response depending on the organism examined (Table 7). The effect of temperature on amylase activity has been extensively investigated in *Bacillus* sp. TS-23 (16) and WN11 (11), *B. lichenformis* (17), *B. subtilis* (12), and *B. stearothermophilus* (17). These studies concluded that optimal amylase production and stability occurred at the optimum growth temperature of the organism (16, 17). For example, *Bacillus* sp. TS-23 amylase synthesis occurred at temperatures between 42 and 60 ^{0}C with an optimum of 55 ^{0}C (16). This investigation examined the stability of crude amylase preparations over a range of temperatures. All *Bacillus* species evaluated exhibited amylase activity at 4 ^{0}C, 30 ^{0}C, and 37 ^{0}C and an absence of activity at 100 ^{0}C (Table 6). No attempt was made in this study to correlate these temperatures with optimum growth levels.

In summary, this study illustrates that different growing environments affect the expression of amylase from *Bacillus* species. In order to optimize yields, various environmental factors in addition to strain variation of the producing organism need to be considered. The numerous uses for amylase will likely expand if better yields are obtained. Experiments performed in this study are the first step in this expansion. During the course of these experiments, it became clear that some of the methodology performed could be used in introductory biology laboratories as a teaching tool. Specifically, the observation that growth of various *Bacillus* species in media containing different carbohydrates affects amylase expression could be used to illustrate the theory of genetic regulation. Thus, Appendix A contains a manuscript submitted for publication describing this laboratory exercise.

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APPENDIX A

Publication Copy of paper:

Genetic Regulation of Amylase Expression in *Bacillus*

ABSTRACT

Bacterial growth is dependent on the precise control of its numerous biochemical reactions. These reactions in turn are dependent upon specific enzymes coded for by the cell's DNA. Since many enzymes are not needed in a constant supply, cells have evolved ways to regulate the expression of the DNA encoding these enzymes to conserve biosynthetic energy requirements, a process referred to as genetic regulation. For example, organisms have the ability to use a wide array of compounds as energy sources provided they possess the genes. However, uptake and utilization of a particular energy source is dependent on activating the gene(s) for the synthesis of the specific enzymes. Rather than keeping a constant supply of enzymes ready at all times for all energy sources, organisms control the expression of the DNA encoding a particular enzyme and express it only when needed. In this exercise, students will be exposed to the theory of genetic regulation by examining the ability of bacteria from the genus *Bacillus* to utilize starch as an energy source. By presenting the organism with different sugar sources, genetic control of the gene encoding amylase, an enzyme which degrades starch, can be examined. This procedure is easy to perform, inexpensive, and provides a way to demonstrate various scientific principles in a single bacterial experiment.

INTRODUCTION

Bacterial growth requires a source of energy in order to assemble the various constituents comprising the cell. The energy supply is derived from the controlled break down of various organic substrates present in the external environment, such as polysaccharides, lipids, and proteins. Polysaccharides, for example, are broken down into smaller monosaccharide molecules that can enter the cell and be used as a source of energy, a process referred to as catabolism. For example, catabolic pathways that degrade the polysaccharide starch ultimately produce the rise of bread dough. This is the direct result of the bread yeast generating CO_2. The aroma of rising bread is due to the ethanol produced in the same catabolic breakdown of the carbohydrate glucose that is the final monosaccharide derived from starch. In contrast, anabolic (biosynthetic) pathways use the monomeric building blocks derived from catabolism which are not used in energy yielding biochemical reactions to assemble the various polymers of a cell.

Some members of the genus *Bacillus*, which are classified as heterotrophic, Gram-positive rods, are able to break down starch into its monomeric constituents and utilize them as an energy source (1). To perform this task, these organisms secrete an enzyme designated amylase, which hydrolyzes starch into its glucose monomers. Glucose can then be transported into the cell and converted catabolically into energy. Organisms unable to produce amylase cannot utilize starch as an energy source.

The chromosome is often referred to as the blueprint of the cell, as it contains the instructions (genes) for assembling the numerous molecules of the cell. Some genes are constitutively expressed (always on) because their products are essential for cellular survival. These types of genes are oftentimes referred to as housekeeping genes. The majority of genes, however, are not expressed at all times because a cell rarely needs their products. Expressing or not expressing a given gene is accomplished through gene regulation and is used by an organism to conserve energy (4). If all genes in an organism

were expressed at all times, the energy demand would be so great that it probably could not be met, and the cell could not compete with more efficient organisms for survival.

In this experiment, students will examine the ability of *Bacillus subtilis, Bacillus polymyxa,* and *Bacillus thuringiensis* to genetically regulate amylase production. Growth of these organisms in bacteriological media containing various sugars allows genetic regulation of the amylase gene to be examined. Thus, classroom discussions centering on bacterial growth, enzyme activity, metabolism, and gene regulation can be emphasized with this simple procedure.

PROCEDURE

Table 1 lists materials needed to perform this exercise.

1. Pure cultures of *Bacillus subtilis, Bacillus polymyxa,* and *Bacillus thuringiensis* are aseptically inoculated into 3 ml of tryptic soy broth with and without various sugars (final concentration 2%) in 15 ml sterile disposable conical tubes.
2. Incubate the inoculated cultures at room temperature for approximately16 hrs.
3. Place the conical tubes in a table top centrifuge and centrifuge for 10 min at 4,000 rpm to pellet the bacterial cells.
4. Using a vacuum aspirator, create 3-4 mm sized wells in a starch agar plate. Place one drop (~100 µl) of culture supernatant into the wells using a disposable Pasteur pipette and let the plate incubate overnight (~16 h) at room temperature.
5. After the incubation period, flood the plate with Gram's iodine. A clear halo forming almost immediately around the well indicates starch was digested by amylase.

RESULTS AND DISCUSSION

Bacteria are very efficient organisms. A general rule of thumb is that they do only what it takes to survive. For cell growth, organisms must precisely control the hundreds to thousands of chemical reactions within the cell. This control can be achieved both at the transcriptional or translational levels in theory, but is much more commonplace at the level of transcription in bacteria. For example, when a sugar is provided to a growing bacterial culture, it represents an easily obtained food source. Upon entering the cell the sugar can function as an inducer, stimulating the production of mRNA for a particular set of genes. Conversely, if it functions as a corepressor, inhibition of mRNA synthesis will occur. Inducers and corepressors carry out their function by combining with specific proteins present in the cell called repressors. Repressor proteins either positively or negatively regulate transcription by binding to operator regions in the DNA. Operators may be thought of as street intersections with traffic lights, controlling the flow of mRNA production through green or red lights signals. Figure 1 illustrates how a repressor protein carries out its regulatory function in both enzyme induction and enzyme repression. In general, inducers regulate catabolic pathways and corepressors regulate biosynthetic pathways.

In natural settings, sugar molecules in the form of hexoses are among the most commonly used carbon sources (3). Rarely, however, are individual hexoses abundant in the environment and carbon sources are generally present as large macromolecules or polysaccharides. To be used as an energy source, organisms secrete enzymes to degrade the polysaccharides down into smaller, more useable forms. For example, starch and cellulose have similar structures, but they differ in how the individual glucose units are attached (3). The type of covalent bond that holds these polysaccharides together is referred to as a glycosidic bond, and this bond can exist in two orientations, alpha (α) and beta (β). Starch is composed of alpha glycosidic bonds and is more digestible than

cellulose, which is composed of beta glycosidic bonds. Consequently, few bacteria can digest cellulose whereas starch digestion by bacteria is much more common. Starch is hydrolyzed by the enzyme amylase, which breaks the alpha glycosidic bonds. Table 2 lists some of the more common polysaccharides found in nature that can be utilized by microorganisms.

The ability of various *Bacillus* species to regulate expression of the enzyme amylase was investigated. By simply growing the organisms in the presence of different sugars, amylase gene expression was examined. Table 3 illustrates how amylase production in different *Bacillus* species was effected by growth in the presence of various sugars. Figure 2 demonstrates the appearance of amylase activity assayed on a starch plate. *Bacillus polymyxa* and *Bacillus subtilis* demonstrated identical amylase patterns, suggesting similar modes of regulation. Amylase production in *Bacillus thuringiensis* produced a different pattern and appears to be regulated by a distinct mechanism.

The regulation of amylase activity can be explained by the theory behind enzyme repression (Figure 1) and the structures of the sugars used. Glucose, galactose, and fructose are monosaccharides whereas maltose (glucose + glucose) and sucrose (glucose + fructose) are disaccharides. In *Bacillus polymyxa* and *Bacillus subtilis* the gene coding for amylase activity is turned off (enzyme repression) when glucose or fructose is present, either in the form of a monosaccharide or disaccharide. Galactose, however, can not function as a corepressor and the system remains on. Thus, a cell that is given an easily obtained sugar source such as glucose or fructose does not need to waste valuable energy in the biosynthesis of amylase, so the gene for amylase is turned off (no mRNA synthesis). Amylase expression in *Bacillus thuringiensis* appears to be constitutive, as amylase production was never repressed, regardless of the sugar used. In all these organisms, cells were propagated in tryptic soy broth, a chemically undefined media. To rule out the possibility that a componet of this undefined media was inducing amylase

gene expression, cells were also grown in minimal media (2) with the same results (data not shown).

ADDITIONAL EXERCISES

Many deviations from the experiment outlined above can be performed. Other sugars, salts, etc. may be added at varying concentrations to see if they have an effect on the regulation of the amylase gene. Tryptic soy broth, used in this experiment, is a relatively inexpensive medium and is commonly used in bacteriological settings. Other media and growth temperatures, however, may be used as *Bacillus* species grow under a wide array of conditions. After supernatants are obtained, the pH can be adjusted to different values using hydrochloric acid or sodium hydroxide and amylase activity assayed. This will allow a demonstration on how enzymes are effected at different pH values. Supernatants can also be subjected to different temperatures for different lengths of time to demonstrate temperature stability of the enzyme. Furthermore, this experiment could be made more quantitative by measuring the zones of clearing among different isolates. Soil samples obtained from diverse settings could be inoculated into bacteriological media and supernatants tested for amylase activity. In general, any variation that can be thought of can be tested since the ability to detect amylase activity is easy and straightforward.

SUPPLIES NEEDED AND COST SAVING MEASURES

Considerable effort was expended to make this experiment feasible in a wide array of laboratory settings. Since junior college and high school laboratories may not be as well equipped as a four-year institution, variations are presented. Table 1 provides a listing of materials and equipment needed for the experiment outlined. This procedure is relatively inexpensive and several alternatives are available to cut costs even further. Whenever bacteria are used, an autoclave is required to sterilize bacteriological media and properly dispose of contaminated bacterial cultures. Alternatives to an autoclave

include a portable steam pressure sterilizer (Fisher, AA-70-1696; $285.00) or a home canning pressure cooker that can reach 15 pounds per square inch at 121°C. We have also been able to sterilize media sufficient for this experiment by bringing it to a boil for 10 min using a hot plate. Melted and cooled (45°C) agar (20 ml) is poured into an empty Petri dish and allowed to solidify. Prepared media are also available, reasonably priced, and eliminate the need for autoclaving. Prepared disinfectants can be purchased for clean up of bacterial cultures, but a good alternative is a 20% chlorox solution.

Upon arrival, bacterial cultures need to be transferred to nutritive media using aseptic techniques. Carolina Biological Supply Company offers various organisms at reasonable prices. For example, various *Bacillus* cultures purchased through Carolina Biological Supply Company are under $10.00. Most diagnostic laboratories are also willing to send out routinely cultured organisms free of charge. Additionally, the worldwide web is a good source of information regarding bacterial culture suppliers. A vacuum aspirator can easily be constructed using a side-arm flask, rubber tubing, Pasteur pipette, and an air supply. Gram's iodine can be purchased from Fisher Scientific or can be made using ethanol (10 ml), iodine (1 g) potassium iodide (2 g), and distilled water (300 ml). Add the ethanol and iodine to a container and the potassium iodide and distilled water in a separate container. The two solutions are then mixed together in a brown bottle or a clear bottle wrapped in aluminum foil.

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Table 1. Material list [c].

Supplies	Catalog Number	Price
Bacterial cultures	various suppliers	\$0.00 - \$10.00
Conical tubes(15 ml) [b]	14-95-9-49B	\$134.20; 125 tubes
Ethanol [b]	A406F-1GAL	\$61.37; 1 gal
D-fructose [b]	L-95-500	\$52.12; 500 g
D-galactose [b]	G1-100	\$53.11;100 g
Glucose [b]	BP-656	\$48.22; 500 g
Gram's iodine [b]	LC14900-1	\$13.32; 500 ml
Iodine [b]	I35-100	\$79.96; 100g
Inoculating loop [a]	AA-70-3060	\$1.75
D(+)-maltose [b]	BP684-500	\$47.22; 500 g
Petri dishes [a]	AA-74-1250	\$4.45; 20-100x15 mm
Potassium iodide [b]	P412-500	\$148.42; 100 g
Starch agar [b]	AA-79-06200	\$31.70; 100 g
D-sucrose [b]	BP220-1	\$26.83; 1 kg
Tryptic soy agar [b]	DF0369-15-8	\$18.30; 100 g
Tryptic soy broth [b]	DF0370-15-5	\$11.50; 100g

[a] Carolina Biological Supply Company (1999).

[b] Fisher Scientific (1999).

[c] This is a comprehensive list of materials and not all items listed are needed to perform this experiment.

Table 2. Commonly used polysaccharides in heterotrophic metabolism.

Polysaccharide	Polymer Composition	Source	Degradative Enzyme
Starch	Glucose	Plants	Amylase
Cellulose	Glucose	Plants	Cellulase
Glycogen	Glucose	Animals	Amylase
Pectin	Galacturonic Acid	Plants	Pectinase
Chitin	N-Acetylglucosamine	Fungi	Chitanase

Table 3. Genetic regulation of amylase activity.

Organism	Media[a]					
		Monosaccharides			Disaccharides	
	TSB	TSB-gal	TSB-fru	TSB-glu	TSB-mal	TSB-suc
Bacillus polymyxa	+	+	-	-	-	-
Bacillus subtilis	+	+	-	-	-	-
Bacillus thuringenisis	+	+	+	+	+	+

[a] Tryptic soy broth (TSB) was used alone or supplemented with various sugars at a final concentration of 2%: gal = galactose; fru = fructose; glu = glucose; mal = maltose; suc = sucrose. A (+) sign indicates amylase activity whereas a (-) sign indicates no amylase activity. Sterile TSB was used as a negative control which tested (-) for amylase activity.

Figure Legends

Figure 1. Starch plate showing a comparision between positive amylase activity (well 1) and the absence of activity (well 2).

Figure 2. Regulation of mRNA synthesis. O, operator; P, promoter.
(A) Enzyme Induction. When an inducer is present, it binds to a repressor and the repressor-inducer complex cannot bind to the operator. This allows RNA polymerase to bind to the promoter and start transcription. When an inducer is absent, the repressor binds to the operator preventing RNA polymerase binding to the promoter and hence, no mRNA synthesis occurs.
(B) Enzyme Repression. When a corepressor is present, it interacts with a repressor and the resulting complex binds to the operator, preventing RNA polymerase from binding to the promoter. Transcription is turned off. When a corepressor is absent, the repressor cannot bind to the operator, allowing RNA polymerase to bind to the promoter and transcription may ensue. Some genes are not inducible or repressible and are synthesized continuously. Genes that are always turned on are referred to as constitutive.

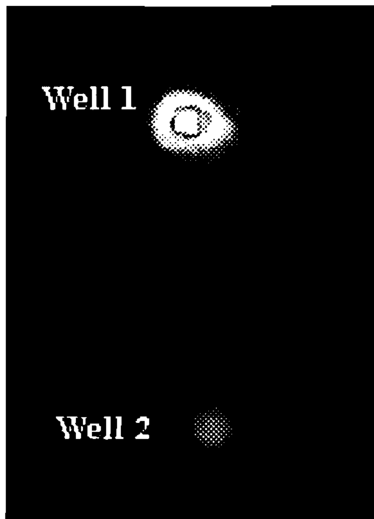

FIG. 1.

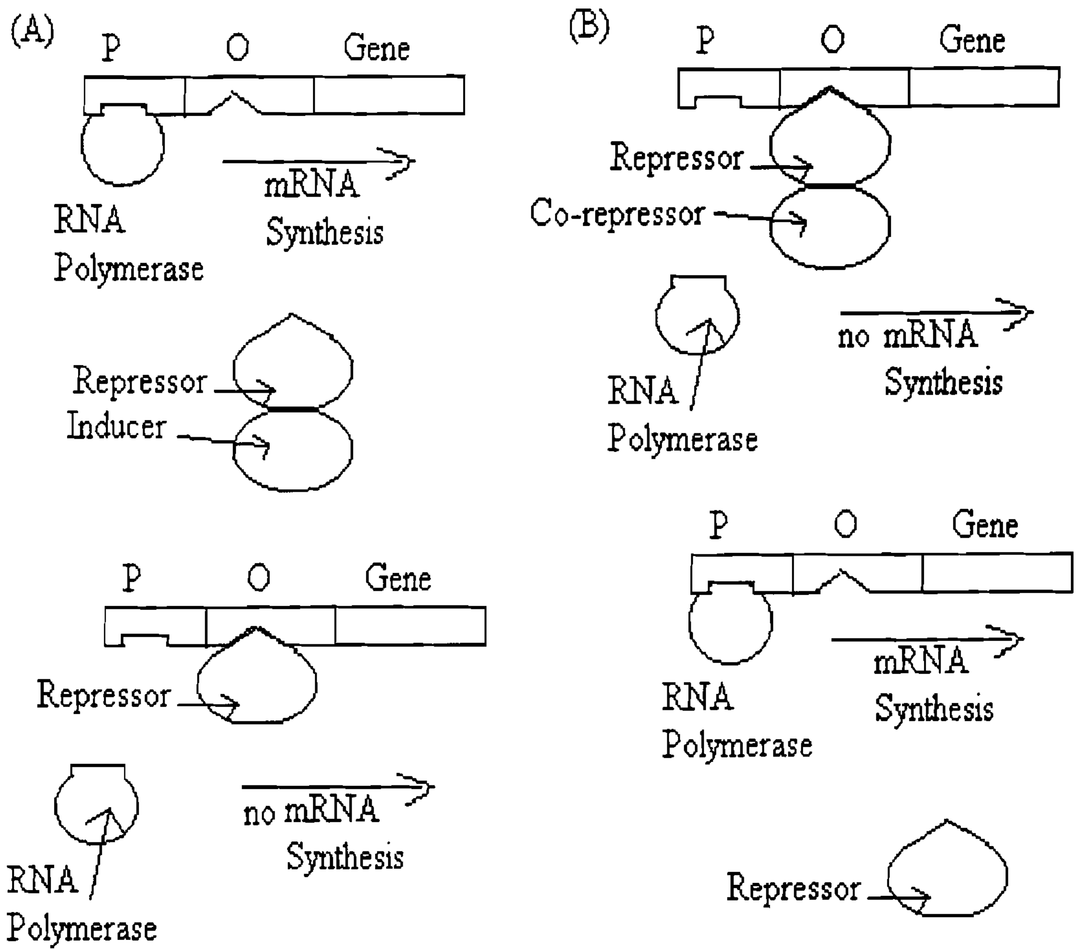

FIG. 2.

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