Using α–Amylase from *Bacillus subtilis* as a Catalyst for the Production of Sweet Potato Biofuels.

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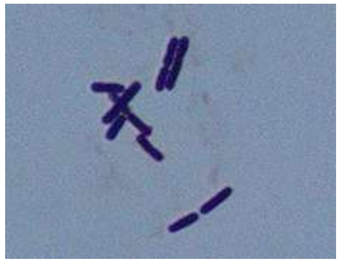
Abstract

Bacillus subtilis is an aerobic, Gram-positive, endospore-forming bacterium that has the ability to produce and secrete the hydrolyzing carbohydrate enzyme, α-amylase. α–Amylase is an enzyme that is used in various industries to rapidly degrade complex polysaccharides (e.g. starches) into smaller oligosaccharides. α–Amylase from *B. subtilis* is currently being investigated as a potential source for producing fermentable sugars from the remnants of sweet potatoes. These fermentable sugars will be bioconverted into ethanol as a source for biofuels. *B. subtilis* was cultured in a 2 L Sartorius-stedim Biostat ® A+ Plus fermentation system. An optical density probe was used to measure bacterial growth within the culture. α–Amylase activity was assayed throughout the culturing period to determine the time-frame of maximum α–amylase production. Enzyme production was stimulated 5-fold and higher by the addition of high concentrations of starches.

Introduction

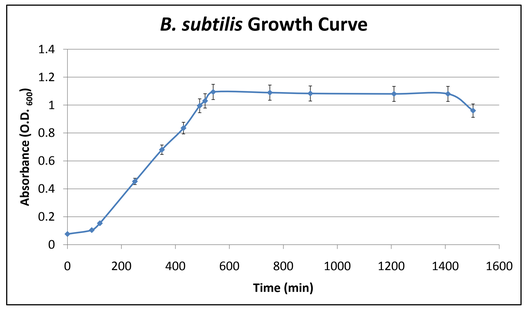
Bacillus subtilis is a Gram positive, endospore forming bacteria that is a common soil inhabitant (Micrograph 1). This bacteria is known to produce and secrete various compounds and substances such as carbohydrolases. The carbohydrolase that is being investigated is that of α–amylase. α–Amylase is the enzyme, α–1,4-glucan-4-glucanohydrolase that catalyzes the internal cleavage of α–D-(1-4) glucosidic bonds in starch and other complex polysaccharides into smaller oligosaccharides. These enzymes are among the most important enzymes in the food, detergent and textile industries. The biofuel industry is another industry that may also benefit from the use of these enzymes. The biofuel industry may utilize these enzymes to initiate the conversion of starchy materials into fermentable sugars for bioethanol production. The main goal of this project is to utilize the ruminants of sweet potatoes as a feedstock to produce biofuels using α–amylase from *B. subtilis*. The reason being is that sweet potatoes have a high starch content of about 25% of it dry weight.



Micrograph 1. Gram stain showing Gram-positive rods

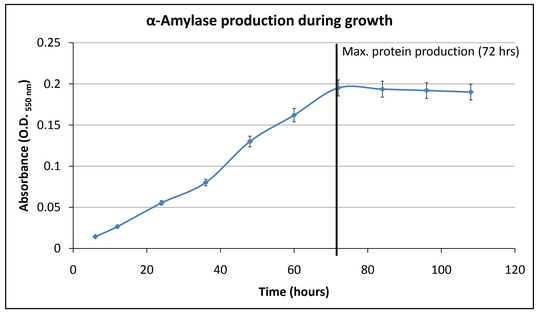
Methodology

1) *Bacillus subtilis* was cultured on nutrient agar (NA) plates and isolated colonies were obtained.
2) Nutrient agar plates were supplemented with 1.0% (w/v) soluble starch and 1.0% (w/v) sweet potato.
3) Colonies of *B. subtilis* from the NA plates were use to inoculate the supplemented plates and allowed to incubate overnight at 37°C to check for starch hydrolysis. Also, the same colony was also subcultured on fresh NA plates.
4) Plates were flooded with Lugol's iodine and allowed to sit for 5 minutes and rinsed for 5 minutes. Halos surrounding bacterial colonies on both plates were noted and recorded.
5) An isolated colony of *B. subtilis* from the subcultured NA plate was then inoculated into a 50 ml flask of nutrient broth (NB) and incubated at 37°C overnight in a shaker water bath with agitation of 150 rpm.
6) 1 ml of the overnight culture was used to inoculated 100 ml of NB to determine the growth curve of *B. subtilis*.
7) Absorbance readings (O.D. 580) and time (min) was taken beginning with inoculation (t = 0 min) and every 20 min for the first hour then every 30 min after the first hour and graphed (Graph 1)
8) From the subcultured NA plate, *B. subtilis* was upscaled from 5 ml to 100 ml then to the 2 liter Sartorius-stedim Biostat ® A+ Plus fermentation vessel. Fermentation parameters were set as follows: temp = 37°C, agitation = 150 rpm, and pO_2 = 60% (Figure 1).
9) 500 ml of the *B. subtilis* cultured in the 2 liter vessel was aseptically transferred to the 10 liter B+ fermentation system for the determination α–amylase production and activity (Figure 2).
10) During growth in the 10 liter vessel, culture samples were obtained and centrifuged to obtain the culture supernatant for testing.
11) Assays were performed on the supernatant to determine α–amylase production and activity. These assays were the 3,5-dinitrosalicylic acid (DNS) assay[1] and the starch-iodine method by Xiao[2]. Measurements were recorded and plotted (Graphs 3 and 4).
12) A Biuret protein assays was also performed to aid in the determination of protein concentration within the culture supernatant during cultivation (Graph 2).

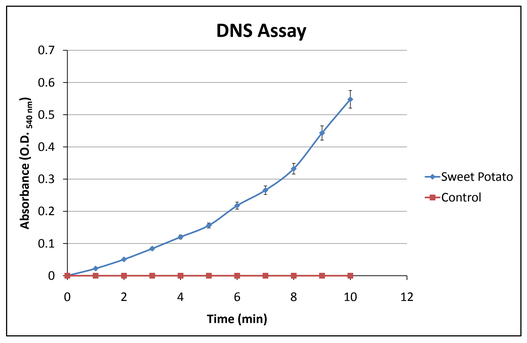


Graph 1. Growth curve of *B. subtilis*

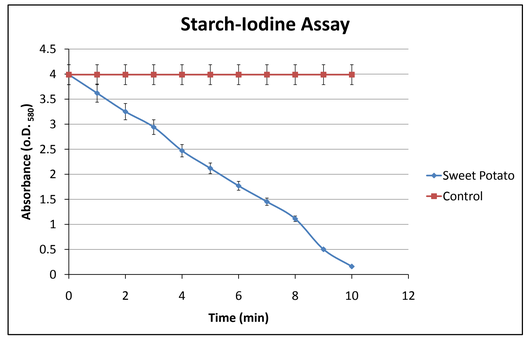
Data Analysis



Graph 2. Enzyme production during growth period using the biuret method



Graph 3. Enzymatic production of reducing sugars



Graph 4. Complex degradation by enzyme

Figure 1. 2 liter Biostat A+ fermenter

Figure 2. 10 liter Biostat B+ fermenter

Discussion

The Sartorius-stedim Biotechnology Laboratory will continue its research on alpha amylase production from *B. subtilis*. Through the more precise assay, the starch-iodine method, we were able to obtain an enzyme activity of 3.41 units ml^{-1}. It is determined that the maximum activity is directly proportional to concentration and therefore have determined that maximum production occurs after 72 hours of culturing within the Biostat B+ fermentation system. Purification of alpha-amylase is currently being researched along with enzyme identification within the culture media.

References

1) P. Bernfeld, *Methods Enzymol*. **1** (1955) 149
2) Xiao, Z. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. *Anal. Biochem*. 2007. **1**:362 (**1**):154.

Acknowledgements

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