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Determination of alpha-amylase activity of Streptomyces spp isolated from Bangladeshi soils

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Abstract

Streptomyces, the largest genus of actinobacteria, is a group of bacteria which is most commonly isolated from soil. In this study, total 24 indigenous Streptomyces spp. were isolated from soil samples collected from different parts of Bangladesh Of them, two species designated as S-31 and S-109 were studied to determine their alpha-amylase activity. The α-amylase activity of S-31 and S-109 was 2.26 unit/ml and 2.67 unit/ml .Based on this study, it can be concluded that both species of Streptomyces were good candidate for amylase production.

Keywords: Streptomyces, α-amylase, Soil

Introduction

Streptomyces is the well-known genus of actinomycetes which is represented in nature by the largest number of species and varieties. Soil is the main source of actinomycetes[12]. Although this group of bacteria can be found in aquatic habitats, they are mainly transient in nature. The intense screening of soil for Streptomyces species from the 1940s onward led to the discovery of many new chemical compounds including antibiotics and other bioactive substances. As interest in a particular product intensified so did the interest in the identity of the producing microorganisms.

The genus Streptomyces is now a days receiving attention for its ability to produce different industrially important enzymes such as amylases, pullulanase and glucosyltransferase, isoamylase, α-glucosdases etc. These enzymes from Streptomyces are being successfully used to produce many sugar syrups such as glucose syrup, maltose syrup, various trisaccharide syrup and some rare sugars such as palatinose, panose etc [4].

There were various reports about α –amylase production from Streptomyces.α-Amylase from Streptomyces hygroscopicus converts starch to maltose in 75% yield although the α-amylase of Streptomyces aureofaciens gives a product with 50% yield of maltose[3].α-Amylase of Streptomyces praecox was noteworthy for its characteristic action pattern. An α-amylase from Streptomyces praecox was purified and its characteristic action, the conversion of maltotriose (G3) without appreciable formation of glucose (G1), was investigated by Suganuma et al (1980). Now-a-days, many researchers of α-amylase from Streptomyces involves transferring of gene responsible for enzyme production to fast growing cells for better and earlier production of the enzymes[1,2,5,9,13].

In Bangladesh,α -Amylase is used for the production of high glucose and maltose syrups that are used in food and pharmaceuticals. Garment and textile industries also use huge amount of amylases for the removal of starch from cloths. But unfortunately there is no industry for amylase production in this country. So amylases are imported from other countries which is very costly. Moreover, Purificatiion and determination of activity of α- amylase from indigenous Streptomyces spp. has not yet been reported so far in Bangladesh.

Research is continuing for the production and purification of α-amylase under shake culture conditions to initiate and develop a biotechnological process for the commercial production of α-amylase in Bangladesh. The present piece of work was undertaken with a view to determine the activity of α-amylase produced from selected Streptomyces spp. The research finding will help to develop new dimension of the exploring microbial biotechnology that will enrich the economy of Bangladesh.

Materials and Methods

Screening of Streptomyces spp. for production of enzymes

The organisms (S-31 and S-109) were maintained as working culture in Oatmeal agar medium[10] and kept at -20°C in glycerol broth medium to preserve them for considerably long time[15].

Media used

These organisms were grown to produce extracellular a-amylase. Primarily two types of media were used for this experimental procedure, seed culture medium and fermentation medium[6].

Preparation of Seed culture

Approximately 50 ml of seed culture medium was taken in each 250-ml conical flasks. The test organisms were inoculated in the seed culture medium with the aid of pre- sterilized inoculating loops. Each organism was inoculated in two flasks and the remaining one flask was used as control. All the flasks were incubated in a rotary shaker incubator (Gallenkamp, England) at 37°C for 48 hr at 170 rpm.

Fermentation

5 ml of each seed culture was transferred aseptically to 30-ml of fermentation medium in 250-ml conical flasks. All the flasks were incubated in a rotary shaker incubator at 37°C for 72 hr at 170 rpm.

Separation of cells from broth

Cells were separated by refrigerated centrifugation (at 10000 rpm for 10 min.) and the supernatant was collected. The clear supernatants were used for the determination of enzyme activities.

Enzyme assay

α- Amylase activity was determined using 1% starch dissolved in distilled water as substrate. The reaction mixture containing 0.8-ml substrate and 0.2-ml enzyme solution was incubated at 45°C for 25 min. and the reaction was stopped by adding 3 ml DNS reagent. All enzyme samples were assayed in duplicate with boiled enzyme blanks for each determination. The enzyme activity was expressed as µ-mole/min, which corresponded to µ-mole of glucose equivalent released per minute under the assay conditions.

$$\text{Enzyme } (\alpha\text{-amylase}) \text{ activity} = \frac{\text{Product concentration} \times 1000 \times \text{Dilution factor}}{\text{Molecular weight of glucose} \times \text{incubation time}}$$

Determination of glucose Standard curve

Procedure: Seven test tubes were taken, six of them were labelled 1 to 6 for different glucose concentration and one was labelled blank. 1.8 ml of substrate was taken in each tube and 0.2 ml of different dilutions (0.5mg/ml, 1mg/ml, 1.5mg/ml,2mg/ml,2.5mg/ml and 3mg/ml) of glucose was added in 6 test tubes and 0.2 ml of buffer was taken in the blank tube.3 ml DNS reagent was added to each tube and mixed thoroughly by vortex. Then the tube was immersed in boiling water bath for 15 minutes and were cooled under running tap water.6ml distilled water was added to all test tubes. Using the blank as reference, absorbance was taken and a graph was plotted for values of glucose concentration against corresponding absorbance.

Enzyme (α-amylase) activity assay

Procedure: One blank tube containing 0.8 ml of substrate and 0.2 ml of buffer, enzyme blank tube containing 0.8 ml of substrate and enzyme sample tube containing 0.8 ml substrate and 0.2 ml of enzyme sample were taken. Each tube was vortexed well. The tubes were then incubated for 5 minutes at 50ºC. 03 ml of DNS was added to the tubes and 0.2 ml of enzyme was added to enzyme blank tube. All the tubes were kept at 100ºC for 15 minutes. Then 6 ml distilled water was added to all test tubes. The absorbance was observed at 540 nm. The concentration of sugar resulting from enzyme action was found from the standard curve.

Results

The corresponding absorbance for different concentration of glucose were recorded (Table1).The value of glucose concentration against corresponding absorbance was plotted in a graph (Figure-1).The actual optical density was calculated(Table-2). From the calculation (figure-2) it was found that the enzyme (α-amylase) activity of S-31 and S-109 was 2.26 unit/ml and 2.67 unit/ml respectively

Discussion

Most of the life saving drugs has been isolated from various Streptomyces species under the family Streptomycetaceae [14], but Streptomyces spp. have not been yet studied extensively for industrially important enzymes. As these organisms use a wide range of organic compounds as sole source of carbon, energy and growth, they can be an important source of carbohydrate hydrolyzing enzymes. There are relatively few reports of industrially potent enzymes from Streptomyces spp. α-amylase from microorganisms especially from Streptomyces spp. has not yet been well reported from Bangladesh.Starch is the predominant carbohydrate in nature. It can be converted to fermentable sugars by α-amylase. α-amylase is widely used in various industries; in starch processing, brewing, paper manufacture and pharmacy[7] . The liquefaction and saccharification of starch are achieved with amylase preparation derived from bacteria, fungi and plants [16].

An attempt was taken to screen the industrially important Streptomyces spp. from soil samples collected from different areas of Bangladesh. In the present study, we have studied the α-amylase activity of both species and both S-31 and S-109 shows the activity and was candidate for α-amylase production. Of them, S- 109 produces higher α-amylase activity and was selected as the most potent Streptomyces spp. for α-amylase production.

The research findings opened the door for further study with the Streptomyces spp. in terms of immobilization of the α-amylase and transfer of the gene for the enzyme to fast growing bacterial cells for better and earlier production of the enzymes.

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Table 1: OD for glucose standard curve (at 540 nm).

conc	Blank	0.5mg/ml	1.0mg/ml	1.5mg/ml	2.0mg/ml	2.5mg/ml	3.0mg/ml
OD	0.000	0.178	0.381	0.452	0.626	0.781	0.964

Table 2: OD for enzyme assay at 540 nm)

organisms	Blank	Enzyme blank	Enzyme sample
OD for S-31	0.000	1.375	1.629
OD for S-109	0.000	1.314	1.614

Fig:1 Glucose standard curve for Enzyme assay

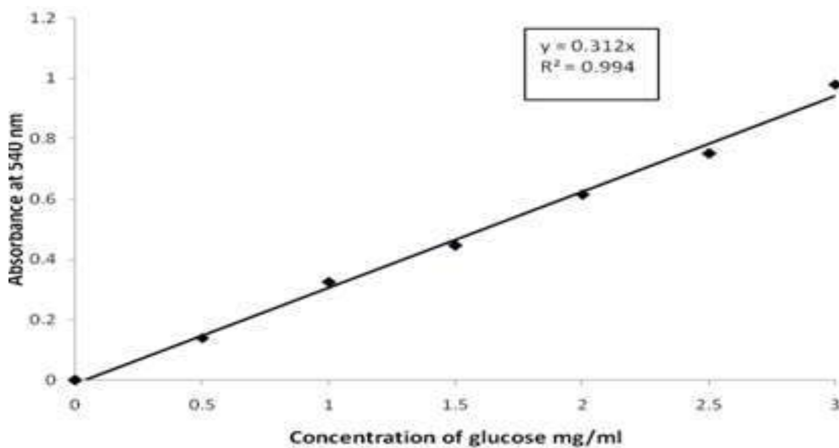

Figure :2 Calculation of enzyme activity

Actual OD for S-31 = Enzyme sample – Enzyme blank
= 1.629 – 1.375
= 0.254 nm
Here, Y= 0.312X or X= Y÷ 0.312 = 0.254÷ 0.312 = 0.814

$$\text{So, Enzyme activity} = \frac{\text{Product concentration} \times 1000 \times \text{Dilution factor}}{\text{Molecular weight of glucose} \times \text{incubation time}}$$

$$= \frac{0.814 \times 1000 \times 12.5}{180 \times 25}$$

= 2.26

So, Enzyme activity of S-31 was 2.26 Unit/ml

Actual OD for S-109 = Enzyme sample – Enzyme blank
= 1.614 – 1.314
= 0.300 nm
Here, Y= 0.312X or X= Y÷ 0.312 = 0.300 ÷ 0.312 = 0.962

$$\text{So, Enzyme activity} = \frac{\text{Product concentration} \times 1000 \times \text{Dilution factor}}{\text{Molecular weight of glucose} \times \text{incubation time}}$$

$$= \frac{0.962 \times 1000 \times 12.5}{180 \times 25}$$

= 2.67

So, Enzyme activity of S-109 was 2.67 Unit/ml