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Studies on Effect of Carbon Sources on Amylase Obtained From Isolated Strains of Different Soil

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Abstract

In the presence study an attempt has been made by isolate α-amylase producing strain from different soil sources and mass culturing for production of large amount of biomass. The soil samples were collected from different locations. The collected soil samples were aseptically transferred into the starch agar plates. Amylase production was confirmed by flooding the plates with grams iodine solutions. The bacterial amylase production has been enhanced by transferring nutrient broth bacterial culture into the amylase production media containing various carbon sources such as starch, sucrose, lactose and maltose in different concentration. Amongst all carbon sources maximum activities has been shown in maltose 0.2 % and 0.1 % shown enzyme activity 1368 IU and 1272 IU. The maximum was also shown by sucrose 0.1 % of 1488 IU.

Keywords: Amylase, starch agar plates and carbon sources.

Introduction

Starch degrading amylotic enzymes are of great significance in biotechnological applications ranging from food, fermentation, textile to paper industries. The amylases can be derived from several sources such as plants, animals and microbes. The microbial amylases meet industrial demands. An amylase is an enzyme that breaks starch down into sugar. Amylase is present in human saliva, where it begins the chemical process of digestion. Foods that contain much starch but little sugar, such as rice and potato, taste slightly sweet as they are chewed because amylase turns some of their starch in the mouth. The pancreas also makes amylase (alpha amylase) to breakdown dietary starch into di and tri saccharides which are converted by other enzymes to glucose to supply bacteria also produce amylase. Diastase, amylase was the first enzyme to be discovered and isolated by anselne payen in 1833. (1-4) The major advantage of using microorganisms for production of amylases is in economical bulk production capacity and

microbes are also easy to manipulate to obtain enzymes of derived characteristics. However enzymes from fungal and bacterial sources have dominated applications in industrial sectors (5). In view of its importance in industrial application, in the present study an attempt has been made to isolate α-amylase producing strain from different soil sources and mass culturing for production of large amount of biomass. Inoculation of culture in different carbon sources (maltose, starch, lactose & sucrose) and to study the effect of carbon sources upon enzyme activity.

Results and Discussion

The media optimization is an important aspect to be considered in the development of fermentation technology. However, there are only a few reports concerning the optimization of media composition especially for bacterial and fungal strains in amylase production. Among chemical parameters, carbon source and concentration of carbon source of the growth medium plays a very important role by inducing enzyme secretion. Amylase is an inducible enzyme and is generally induced in the presence of carbon sources such as starch, its hydrolytic product, or maltose. Still the role of glucose in production of amylase is controversial. Xylose has been reported to strongly repress amylase production. Although the carbon source supports good growth in *A.nidulans*. The enzyme activity of the strain isolated from soil by various carbon sources and concentration of various carbon sources has been shown in Table 3 & Fig. 1 and 2. Most report available on the induction of amylase in different strains of bacterial special suggests that the general inducer molecule is maltose which increases many fold enzyme activity. It was also true in our study maltose 0.2% and 0.1% shown enzyme activity shows 1368 IU and 1272 IU. The maximum activity was also shown by sucrose 0.1% of 1488 IU. The nature of composition of media for optimal production of amylase by bacterial strain has been developed with study.

Table 3: Enzyme Activity

Carbon source	gm	mg of carbon source released	Enzyme activity IU/ml
Maltose	0.05	0.122	1.464
	0.10	0.202	2.424
	0.15	0.134	1.608
	0.20	0.190	2.250
Sucrose	0.05	0.073	0.876
	0.10	0.235	2.820
	0.15	0.062	0.744
	0.20	0.125	1.5
Lactose	0.05	0.194	2.328
	0.10	0.094	0.9
	0.15	0.167	2.004
	0.20	0.209	2.508
Starch	0.05	0.119	1.428
	0.10	0.090	1.08
	0.15	0.099	1.188
	0.20	0.087	1.044

Fig. 1: Screening of Amylase producing bacteria by Crowd Plate Technique.

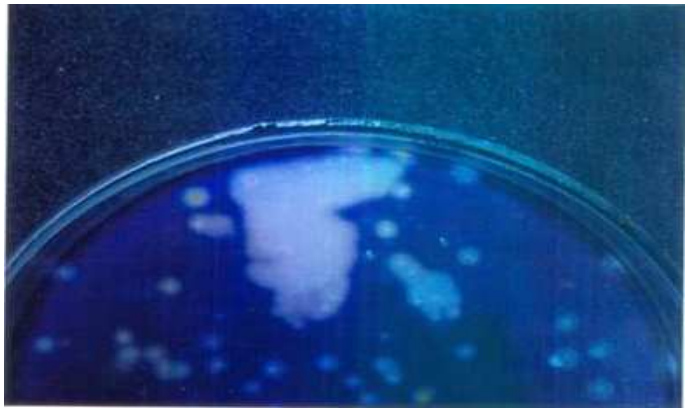
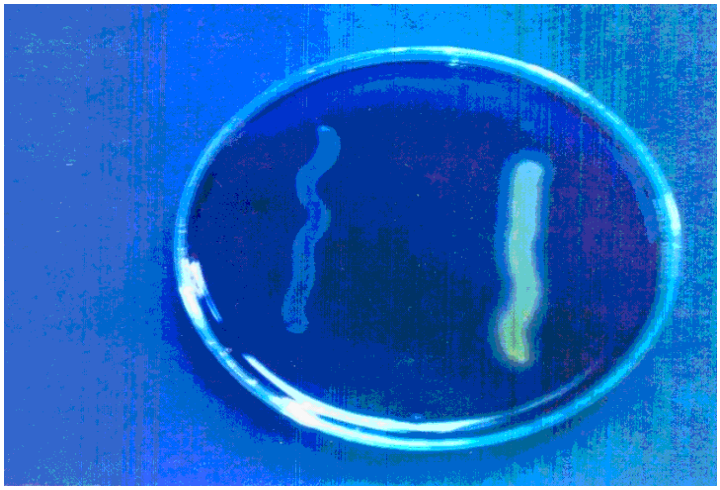


Fig. 2: Screening of Amylase producing bacteria by Streak Plate Technique



Materials and Methods

Bacteriological peptone, beef extract, agar, soluble starch, sodium Potassium tartarate, 3,5-Dinitro salicyclic acid, mallose & all the remaining ingredients used were obtained from S.D.fine chemicals

Isolation of α-amylase producing starch from soil samples:
The soil contains a rich deposit of both bacteria and fungi which produces amylases. The soil samples were collected from different locations, using hand towel into a "Ziploc" bag. The collected soil samples sprinkled aseptically into the starch agar plates. All the plates were incubated in an incubator for 36 hours at 37° C (6-9). After the incubation period, the amylase production was confirmed on starch agar plates by flooding the plates with grams iodine solution**.** Initially, the plates acquired deep blue color. After few seconds, the deep blue colour around some bacterial colonies which confirm extracellular secretion from the bacterial strain (10).

Table No.1: Composition of starch agar media

Ingredients	For 1000 ml
Peptone	5 gm
Beef extract	3 gm
Soluble starch	2 gm
Agar	15 gm
Water	1000 ml

Sub culturing

Using a sterile loope, the amylase producing colonies were scrapped from starch agar and inoculated in a 250 ml conical flask containing 100 ml of nutrient broth, the inoculam was incubated in an incubator at 37° C for 48 hours. After the period of incubation, the culture was transferred in a nutrient agar slant for preservation.

Bacterial amylase production (6)

The bacterial amylase production has been enhanced by transferring a 2ml of nutrient broth bacterial culture into the amylase production media containing various carbon sources such as starch, sucrose, lactose and maltose in different concentration.

Table No.2: Media composition (Bacterial amylase production)

Ingredients	For 1000 ml
Bacteriological peptone	6 gm
$MgSO_4$, $7H_2O$	0.5 gm
KCl	0.5 gm
Carbon source	1 gm

Carbon source: starch, maltose, sucrose, lactose.

Procedure

Fifty ml of amylase production was prepared in 16 conical flask with 4 type of carbon sources in 4 different concentration (0.05 gm, 0.1 gm, 0.15 gm & 0.2 gm). The media was prepared by dissolving all ingredients in 50 ml distilled water and sterilized by auto claving at 121°C for 15 minutes.

Transferring bacterial culture in production media

From the nutrient broth culture, 2 ml has been transferred to the amylase production medias and incubated for 48 hours at 37° C in an incubator.

Extraction of enzyme from bacterial

Two ml of production media culture was transferred into centrifuge tubes and spinned for 20 minutes at 5000 rpm. After 20 minutes, the supernatant portion was decanted, which is the crude enzyme extract.

Demonstration of enzyme activity

One ml of crude enzyme extract from all conical pipetted out into a test tube. To this 1 ml of 1% soluble starch solution was added. The solution was mixed by swirling and incubated for exactly 3 minutes at 20° C. then colour reagent solution, [Solution 1) 12 gms of Sodium potassium tartarate was dissolved in 8 ml of 2M NaOH, by direct heating and constant stirring. Solution 2) 0.1 m of 3,5-dinitro salicylic acid solution was dissolved in 20 ml of deionized water by direct heating and constant stirring. Solution 1 was added slowly with constant stirring to solution 2. The solution was dissolved to 40 ml with deionized water. The solution should be stored in an amber coloured bottle at room temperature] 1 ml was added to all test tubes. The test tubes were capped and placed in a boiling water bath for exactly 15 minutes and cooled on ice to room temperature. Finally add 9 ml of deionized water, mixed well by inversion and determine the colour intensity at 540 nm, using suitable spectrophotometer against blank. The results were tabulated.

Blank solution

One ml of 1% soluble starch solution, one ml of distilled water and one ml of color reagent solution heated for 15 minutes, cooled on ice to room temperature. Then 9 ml of deionized water was added.

Determination of enzyme activity

$$\text{Units/ml enzyme} = \frac{(\mu g \text{ of maltose realeased})\ (\delta f)}{1}$$

δf – dilution factor
1 = volume (in ml of enzyme used)

Conclusion

In the present study an attempt has been made by isolate amylase producing strain from six samples and studied the impact of various carbon sources on enzyme activity. Amongst all carbon sources maximum activity has been shown by sucrose 0.1%, maltose 0.2% and 0.1%. As an extension of this work, impact of various nitrogen sources, media, pH, speed of rotation (rpm) on enzyme activity can be carried out in future.

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