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Characterization of Bacterial isolates from Soil for α-Amylase Production

Pushpendra Singh[1]*,Ravindra Singh[1], Paras Gupta[2] and Arvind Kumar Srivastava[2]
1, Department of Biological sciences, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidhyalaya, Chitrakoot, Satna, (MP) - India
2, United Institute of Pharmacy, Naini Allhabad, (UP) - India

Abstract

The major advantage of using microorganisms for the production of amylases is the economical bulk production capacity and the fact that microbes are easy to manipulate to obtain enzymes of desired characteristics. Today a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in extracellular enzymes of which amylases are of particularly significant industrial importance.Total 10 bacterial cultures were isolated from collected soil samples. Among 10 bacterial isolates, 7 isolates showed the amylolytic activity. These 10 isolate was identified according to Bergey's manual of systemic Bacteriology .These isolates related to Bacillus sp.The optimum pH for the growth of all the cultures was observed near pH 7. Submerged fermentation was carried out for the production of α-amylase was observed in the range of 0.047-1.47 U/min/mL. The maximum activity of α-amylase was 1.47 (U/min/mL) after 48 hours was recorded, have great significance.

Key-Words: Characterization, α-Amylase activity, *Bacillus sp*, Amylase prodution

Introduction

α-Amylases (E.C. 3.2.1.1.) are starch-degrading enzymes that catalyze the hydrolysis of internal α-1,4-*O*- -glycosidic bonds in polysaccharides with the retention of α-anomeric configuration in the products (10) . These enzymes account for about 30 % of the world's enzyme production (11). Amylases are among the most important enzymes and are of great significance for biotechnology.α-Amylases have potential application in a wide number of industrial processes such as food, fermentation, textile, paper, detergent, and pharmaceutical industries. However, with the advances in biotechnology, the amylase application has expanded in many fields such as clinical, medicinal and analytical chemistry, as well as their widespread application in starch saccharification and in the textile, food, brewing and distilling industries (12).α-Amylase has been derived from several fungi, yeasts and bacteria.

*** Corresponding Author**
E.mail: pushpendra_singhbiotech@yahoo.co.in

Many Microorganism used in α-Amylases and ß-amylases production including *Bacillus subtilis, B. cereus, B. polmyxa, B.amyloliquefaciens, B. coagulans, B. subtilis, Lactobacillus, Escherichia, Proteus, B. lincheniformis, Bacillus steriothermophilu, Bacillus megaterium, Strepotmyces sp., Pseudomonas sp. etc.* α-Amylases from plant and microbial sources have been employed as food additives. Barely amylases have been used in the brewing industry. Fungal amylases are widely used for the preparation of oriental foods, in spite of the wide distribution of amylases, microbial sources, namely fungal and bacterial amylases are used for the industrial production due to advantages such as cost effectiveness, consistency, less time and space required for production. Among bacteria, *Bacillus sp*. is widely used for thermostable α-amylase production to meet industry. Filamentous filling have been used for the production of amylases for centuries (3-9).

Material and Methods

The given 10 cultures were isolated from soil of Mahatma Gandhi Chitrakoot Gramodaya Vishwavidhyalaya, Chitrakoot, Satna (M.P.). To study the cultural characteristics of the bacterial isolate, the pure cultures of isolates were streaked on the sterile Nutrient agar plate. These plates were incubated in incubator (MAC make) at 37.5 for 24 h. After

incubation time, colony characteristics i.e., size, shape, margin, elevation, pigments production, consistency and texture were noted. Gram staining was performed to investigate morphological properties; microscopy was carried out by compound light microscope (GETNER India make). To observe the influence of pH on the growth of the organisms, organisms were allowed to grow in the nutrient broth medium having different pH (5 to 9) for the production of amylase. The amylase activity was determined the following method of an assay mixture containing, enzyme extract, starch as substrate and DNS as coupling reagent was used. One unit of amylase activity was defined as the number of µ moles of maltose liberated by 1 mL of enzymes solution per minute. All processes were done by standard method [4,5]

Results and Discussion

All 10 cultures were streaked on nutrient agar plate and after 48 h incubation time cultural characteristics were studied. As shown in all plates. Culture No. 4,5,9 showed small colonies, low convex elevation and semi transparent opacity with no pigmentation. All other isolates had shown big colonies with irregular and uneven margin as well raised elevation with moist consistency. No pigment production was observed in any of isolates but all the colonies were opaque. Detail results are noted in Table No: 1.

As shown in Table No: 2. All 10 isolates were studied for their morphological characterization observed under light microscope, all of them were in rod shape and arranged in chain or singly. The cultures No.4,5,9 were found gram's negative while other isolates were gram's positive.

As described in Table No: 3 all the cultures were studied for amylase production in 2% starch containing broth and amylase assay was carried out after 24 and 48 h of incubation time, which clearly show that after 24h culture 9 and 10 has given maximum 0.71 and 0.55 U/min/mL amylase production whereas after 48h culture No.5 was able to produce 1.47 U/ min/mL amylase.

Conclusion

The main objective of our study was to characterize the 10 α-amylase producing cultures and to check their ability enzyme production especially amylase. Morphologically all the isolates were single or in chain arrangement and actively motile during microscopic examination, except cultures No.4,5,9,all others were Gram positive, rod shaped, spore former organisms, Culture No.4,5,9 were Gram negative or Gram variable isolates. All the isolates were able to grow in a wide range of pH (5 to 9), but optimum pH for the growth for the all isolates was near neutral (7.0). Submerged fermentation was carried out for the production of amylase. Culture No.**5** was found to be most promising culture for production of α-amylase. This was a preliminary study for characterization of α-amylase producing isolates.No. 1, 5, 9 and 10 cultures saws many high α-amylase activity under both condition i.e 24 h and 48 h incubation, as compare to other cultures. They shows amylase production significantly high in 48 h i.e nearly double activity in culture No.5 ,the activity increase from 0.48 to 1.47 (U/min/mL) is nearly 3 times higher at 48 h then 24 h.

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Table 1: Culture characteristics of the isolates

Culture No.	Size	Shape	Margin	Elevation	Consistency	Opacity	Pigmenta Tion
1	Big	Irregular	Uneven	Raised	Moist	Opaque	Nil
2	Big	Irregular	Uneven	Raised	Moist	Opaque	Nil
3	Big	Irregular	Uneven	Raised	Moist	Opaque	Nil
4	Small	Round	Uneven	Low convex	Moist	Semi transparent	Nil
5	Small	Round	Uneven	Low convex	Moist	Semi transparent	Nil
6	Big	Round	Uneven	Raised	Moist	Opaque	Nil
7	Big	Round	Uneven	Raised	Moist	Opaque	Nil
8	Big	Round	Uneven	Raised	Moist	Opaque	Nil
9	Small	Round	Uneven	Low convex	Moist	Semi transparent	Nil
10	Big	Irregular	Uneven	Raised	Moist	Opaque	Nil

Table 2: Morphological characteristics of the isolates:

Culture No.	Size	Shape	Arrangement	Gram 's Reaction
1	Big	rod	Single / chain	Gram Positive
2	Big	rod	Single / chain	Gram Positive
3	Small	rod	Single / chain	Gram Positive
4	Intermediate	rod	Single / chain	Gram Negative
5	Intermediate	rod	Single / chain	Gram Negative
6	Big	rod	Single / chain	Gram Positive
7	Big	rod	Single / chain	Gram Positive
8	Big	rod	Single / chain	Gram Positive
9	Intermediate	rod	Single / chain	Gram Negative
10	Big	rod	Single / chain	Gram Positive

Table 3: α-Amylase activities after 24 and 48 h

Culture No.	Incubation Time(hrs.)	Amylase Activity (U/min/mL)	Incubation Time(hrs.)	Amylase Activity (U/min/mL)
1	24	0.41	48	0.92
2	24	0.07	48	0.16
3	24	0.14	48	0.27
4	24	0.03	48	0.047
5	24	0.48	48	1.47
6	24	0.15	48	0.14
7	24	0.15	48	0.22
8	24	0.16	48	0.20
9	24	0.71	48	0.8
10	24	0.55	48	0.91

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