ISOLATION AND CHARACTERIZATION OF STARCH DEGRADING BACTERIA FROM GARDEN SOIL, GANPAT UNIVERSITY, GUJARAT, INDIA

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Abstract

Background: *Enzymes are the most important substances used in so many areas either in research, medicine or commonly in industries. Starch degrading bacteria are mostly important in food, texture, fermentation and paper industries. Amylase production from bacteria is economical because the enzyme production rate is higher in bacteria as compared to other microorganism. The isolation and manipulation of pure culture of starch degrading microorganisms from soil have a great importance on biotechnology as well as microbiology field.*

Methods: *Present study involves the isolation by dilution method, characterization and identification by bergey's menual of systematic bacteriology. Enzyme assays by DNS a method. Optimization of enzymes at different parameters.*

Results: *In this research the bacterial four strains A1, A2, A3 and A4 were isolated from soil at area of college campus Ganpat University, Gujarat, India. Isolated bacteria have great amylase activity at 37˚Ctemperature, the highest hydrolysis zone of starch using with concentration of 1.5% amylase activity at different pH in highest growth of bacteria at pH 6.7 and amylase activity at different time duration at 48hrs with high activity 23.5U/ml in isolate A4 by DNS a method.*

Conclusions: *In the presence study, the bacteria were isolated from the garden soil from the college campus. This area also consisting of starchy materials we found starch degrading bacteria have capacity to grow in physical condition pH, temperature and concentration of starch. As per morphological character, colony character and biochemical testing isolated bacteria similar to genus bacillus.*

Key Words: *Soil sample, Amylase enzyme, Bacillus spp., Starch degrading, DNS method.*

INTRODUCTION

Amylases can be obtained from several sources such as plant, animal and microbes (Kathiresan and Manivannan, 2006). Starch degrading amylolytic enzymes are most important in the biotechnology industries with huge application in food, fermentation, textile and paper (Pandey et al., 2000).

Amylolytic enzymes are widely distributed in bacteria and fungi. They are categorized in to exo-acting, endo-acting and debranching enzymes. Among the amylases, β-amylase is exo-acting whereas α- amylase is endo-acting enzyme. Unusual bacterial amylases are found in acidophilic, alkalophilic and thermoacidophilic bacteria (Boyer and Ingle, 1972). There are various reports on starch degrading microorganisms from different sources and respective amylase activity (Aiba et al., 1983; Tonkova et al., 1993; Kathiresan and Manivannan, 2006).

Soil is the habitat of a diverse array of organisms which include both micro flora and fauna. Soil microorganisms play a very important role in soil fertility not only because of their ability to carry out biochemical transformation but also due to their importance as a source and sink of mineral nutrients (Jenkinson and Ladd, 1981).

This is done in soil general because of exo enzymes that those microbe release in the environment and degrades the soil components (Saito N, Yamamoto K (1975). Soil receiving the garden is one of the rich sources of microorganisms. In this starch degrading microorganism isolated sources plant. Biologically active enzymes may be extracted from any living organism. A very wide range of sources are used for commercial enzyme production from *Actinoplanes* to *Zymomonas*, from spinach to snake venom. Of the hundred or so enzymes being used industrially, over a half is from fungi and yeast and over a third are from bacteria with the remainder divided between animal (8%) and plant (4%) sources. (Ryan SM, Fitzgerald and all 2006). The present investigation dealt with isolation of a bacterial strain, from soil samples collected from college campus Ganpat University, Kherva, Gujarat, India and physiological and biochemical features were determined. Its amylolytic activity under different physiological conditions was correlated with the growth of three isolates. In the present study, we report the isolation and characterization of bacteria.

MATERIALS AND METHODS

Sample collection: Soil samples were collected from garden area of college campus, Ganpat University, Gujarat, India, by sterile spatula depth of 9-10 inch near the plant transferred in to sterile zip bag.

Isolation of starch degrading bacteria: Soil samples were collected from different sites of garden sources. Serial dilution was made and was plated on starch containing nutrient agar plate. 0.1ml of the diluted sample was spreading on plate. Then the plates were kept for incubation at 37°C for overnight.

Screening for Amylase Activity (Starch Iodine Test): Bacteria were isolated by serial dilution and streak plate methods. The aliquots (0.1 ml) were plated in triplicates on Nutrient Agar (NA) medium [(w/v) 0.5% peptone; 0.3% beef extract; 0.5% NaCl; 1.5% agar, pH 7] and incubated at 30±2°C for 72 h. The nutrient agar plates containing 2% starch (Starch Agar plates) were inoculated with test bacterial isolates and incubated at 30±2°C and plates were flooded with Gram's iodine (Gram's iodine- 250 mg iodine crystals added to 2.5gm potassium iodide solution, and 125ml of water, stored at room temperature) to produce a deep blue colored starch-iodine complex. In the zone of hydrolysis no blue colour forms, which is the basis of the detection and screening of an amylolytic strain. The colonies which were showing zone of clearance on starch agar plates were maintained on to nutrient agar slants.

Morphological and Biochemical Characteristics: Gram staining, motility, indole production, methyl red, Vogues Proskauer's, citrate utilization, triple sugar iron, nitrate reduction, catalase, oxidase, gelatin liquefaction, urease, hydrolysis of casein, hydrolysis of starch were carried out.

Amylase production: Amylase was produced by using complex medium containing starch 1.0%, yeast extract 0.04%, (NH4)2HPO4 0.4%, KCl 0.1% and MgSO47H2O 0.05%, and semi-synthetic medium containing peptone 0.4%, (NH4)2HPO4 0.4% and KCl 1.0%(Kim, T.U, 1995).

Cultural characterization: The isolates were observed under the microscope to obtain the colony morphology i.e. colour, shape, size, nature of colony and pigmentation (Dipali Parmar2012 & Quang D, 2000)

Enzyme assay for amylase enzyme: A suitable volume of isolated culture broth incubated for 48 hr was centrifuged at 5000 rpm for 20 min at 4°C. Supernatant was recovered. Amylase was determined by spectrophotometric method. 1.0ml of crude enzyme into a test tube and 1ml of 1% soluble starch in sodium phosphate buffer (pH 7) was added in test tube. The test tubes were covered and incubate at 35°Cfor 10 minutes. (Miller GL, 1959).Then 2.0 ml DNS reagent was added in each tube to stop the reaction and kept in boiling water bath for 10 minutes. After cooling at room temperature, final volume was made to10ml with distilled water. And the absorbance was read at 540 nm by spectrophotometer. A unit of amylase activity was defined as the amount of amylase required to catalyze the liberation of reducing sugar equivalent to 1 mol of D glucose per minute under the assay conditions.

CHARACTERISTICS OF AMYLASE

Determination of pH: 1% Starch was used as a substrate. Substrate solution was prepared in sodium phosphate buffer at pH 5, 6, 7 and 8 in different test tubes. 1 ml each of crude enzyme solution was added into buffer tubes. Then the mixture was incubated at 35°C for 10 min, reactions were terminated by adding 2 ml DNS reagent and the mixture was incubated in boiling water for 10 min. After cooling at room temperature, final volume was made to 10ml with distilled water and the activity of enzymes was determined by taking the absorbance at 540nm.

Determination of optimum temperature: 1 ml of substrate was taken into six different test tubes and 1 ml of phosphate buffer pH 7 was added in each test tubes. Tubes were marked with different temperature (at 30, 35, 40°C). 1 ml of crude enzyme solution was added in each tube. Then tubes were incubated at specific temperature for 10 minutes. Reactions were terminated by adding 2 ml DNS reagent and the mixture incubated in boiling water for 10 min .After cooling at room temperature, final volume was made to 10ml with distilled water and the activity of enzymes were determined by taking the absorbance at 540nm.

Determination of optimum time: Similarly to observe the effect of different time duration such as 24, 48,72hrs on amylase activity.

Determination of Concentration of Starch: Similarly to observe the effect of different substrate concentration on amylase activity, the dialyzed aliquot was added to different starch concentrations (0.5, 1.0, 1.5, and 2%) and the activity was observed following the method of (Miller, 1959).

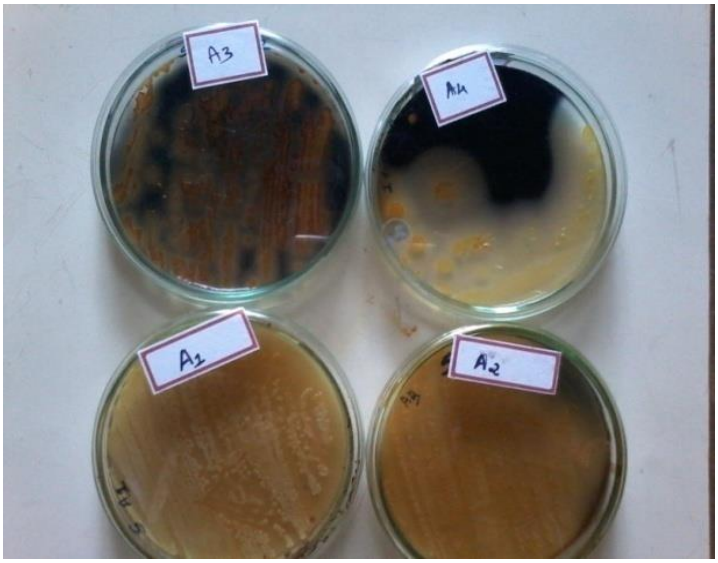
RESULTS AND DISCUSSION

Observation of Staining

Microscopic Observation	A1	A2	A3	A4
Shape	Spheres	long rods	long rods	long rods
Arrangement	single ,double and in clusters	single ,pairs, chain	single ,pairs, chain	single, pairs, chain
Gram reaction	Gram positive	Gram positive	Gram positive	Gram positive
Spore staining	Non spore former	Spore former	Spore former	Spore former

Cultural Characteristics

Characteristics	A1	A2	A3	A4
Size	medium	Medium	Large	Large
Shape	punctiform	Irregular	Irregular	Round
Margin	entire	Entire	Entire	Entire
Elevation	raised	low convex	low convex	Flat
Consistency	Butyrous	Butyrous	Moist	Butyrous
Texture	smooth	Smooth	Rough	Rough
Opacity	opaque	Opaque	Opaque	Opaque
Odor	earthy	Earthy	Earthy	Earthy
Pigmentation	crymy white	crymy white	White	White



Biochemical Characterization: Isolates ware found to have the ability to hydrolyze Starch and gelatin, Oxidase positive, Catalase positive, Nitrate reduction positive, Urease test and VP test positive. Whereas, indole and citrate test were negative.

Observation of Biochemical Tests

No	Media	Test	Observation			
			A1	A2	A3	A4
1	Starch agar plate	Starch Hydrolysis	+	+	+	+
5	GPB broth	Methyl Red test	+	-	-	-
		Voges –Proskauer test	+	+	+	+
6	Simmons citrate agar slant	Citrate utilization	-	-	-	-

7	Urea Broth	Urea hydrolysis	+	+	+	+
9	1% peptone	Indole production	-	+	-	-
10	2% peptone	H2S Production	+	+	+	+
11	Glucose	Carbohydrate fermentation	+	+	+	+
12	Lactose	Carbohydrate fermentation	-	+	-	+
	Maltose	Carbohydrate fermentation	+	+	-	+
13	Sucrose	Carbohydrate fermentation	+	+	+	+
15	Xylose	Carbohydrate fermentation	-	+	+	-
16	Peptone Nitrate broth	Nitrate reduction	+	+	+	+
		Ammonia production	+	+	+	+
18	Nutrient agar	Oxidase	+	+	+	+
19	Nutrient agar	Catalase	+	+	+	+

Determination of Different Parameter

Parameter	**A1**	**A2**	**A3**	**A4**
Temperature (37˚C)	+	+	+	+
pH 7.6	+	+	+	+
Starch concentration (1.5%)	+	+	+	+

Bacterial isolates were tested for production of amylase by the starch hydrolysis test. On the basis of the area of clearance, characterization, and high amount of amylase activity determined by different parameter such as pH, Temperature, Time and Concentration of starch.

CONCLUSION

In the presence study, the bacteria were isolated from the garden soil from the college campus. This area also consisting of starchy materials we found starch degrading bacteria have capacity to grow in physical condition pH, temperature and concentration of starch. We have isolate, characterized and identify bacteria on the basis of their biochemical characterization as well as production of amylase.

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How to Cite this Article: Patel G. Isolation and Characterization of Starch Degrading Bacteria from Garden Soil, Ganpat University, Gujarat, India. Indian J Microbiol Res 2015;2(2):111-114.