Research Article

Amylase Production from Bacteria Isolated in the Soil Receiving Kitchen Wastes

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ABSTRACT

Enzymes are the most important substances used to day 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. The isolation and manipulation of pure culture of starch degrading microorganisms from soil have a great importance on biotechnology field. In this research the bacterial strain was isolated from soil receiving kitchen wastes where conditions for growing were controlled, the bacteria isolated showed that belong to the genus bacillus. The strain grows well at 37°c and the 2% starch concentration, with PH near neutral. The enzyme activities were observed at 2% starch concentration.

INTRODUCTION

Enzymes are biological molecules that catalyze (i.e., increase the rates of) chemical reactions. (Abe, J., Bergman, F.W., 1988). In enzymatic reactions, the molecules at the beginning of the process are called substrates, are converted into different molecules, called products. Almost all chemical reactions in a biological cell need enzymes in order to occur at rates sufficient for life. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell (Pelczar et al., 1986).

Soil is the habitat of a diverse array of organisms which include both micro flora and fauna. Soil micro organisms 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).

Amylases are produced by a variety of living organisms, ranging from bacteria to plants and humans. Bacteria and fungi secrete amylases to the outside of their cells to carry out extra-cellular digestion. (Cordeiro et al., 2003).When they have broken down the insoluble starch, the soluble end products such as (glucose or maltose) are absorbed into their cells.

Although many microorganisms produce this enzyme, the ones most commonly used for their industrial production are *Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquifaciens* and *Aspergillus niger(*Brook, Elizabeth J., and all 1969).

Amylase is an enzyme that catalyses the breakdown of starch into sugars, different organisms has been studied to produce this enzyme, a wide studied is bacillus subtili was studied to develop different forms of amylase such as *alpha*-amylase, ß-glucanase (gummase) and hemicellulase (Aiba S, Kitai K, and all , 1983).

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).

A very much larger number of enzymes find use in chemical analysis and clinical diagnosis.

Non-microbial sources provide a larger proportion of these, at the present time. (www.lsbu.ac.uk), Microbes are preferred to plants and animals as sources of enzymes because: they are generally cheaper to produce, their enzyme contents are more predictable and controllable, reliable supplies of raw material of constant composition are more easily arranged, and plant and animal tissues contain more potentially harmful materials than microbes, including phenolic compounds (from plants), endogenous enzyme inhibitors and proteases (Kathiresan K, Manivannan S ,2006).

Nowadays with the increasing of technology, enzymes are produced in different industries for their importance and use in daily life, among those enzymes produced industrially amylase belong in due to its role, before industrial production laboratory production must be done in order to get best results and starting materials.

The aim of this study is to isolate starch degrading soil bacteria and to analyze enzymatic activities of the bacteria isolated.

MATERIALS AND METHODS

STARTING MATERIAL

In this research the starting material is soil sample, soil sample was collected aseptically in the area receiving kitchen wastes; Bacillus sp was isolated by using plate technique (Roy RR, Jana S C & Nanda G 1997).

- 1 gram of soil was weighted and added to 99 ml of sterile distilled water (1/100dilution).
- Mix well.
- While still suspended, further dilute the suspension by removing 1 ml with a
- P-1000 micropipete and add it to a test tube containing 9 ml of sterile distilled water(1/10 dilution — 1/1000 total dilution).
- The agar side labeled.
- 50 µl of mixture was removed (a further dilution) and transfered to the center of a starch-agar plate.
- Using sterile technique, dip a cell spreader in alcohol, hold it in a flame briefly and briefly air cool it then by touching it to the outer edge of the agar. Use this to spread the 50 µl sample evenly around the plate.
- Incubate one plate agar-side up in the 37°C incubator.
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MEDIA

Culture media was prepared by using nutrient agar and soluble starch, from the prepared solution.

Medium for bacterial isolation

Beef Extract:	0.3%
Peptone:	0.5%
Agar:	1.5%
Starch hydrolysis	1%

This media general know as nutrient agar with starch hydrolysis.

Medium for bacterial production (g/l)

Bacteriological Peptone	6
$MgSO_4 \cdot 7H_2O$	0.5
KCl	0.5
Starch	1

Identification of Microorganisms

Gram staining was performed to differentiate microorganisms either gram positive or gram negative, catalase test also was performed to check catalase positive and catalase negative.By using iodine the detection of starch degrading microorganisms were established.

Gram Stain Protocol

- “Heat-fix” the slide with the specimen by passing it over a heat source, such as a flame, several times using a forceps.
- Flood the fixed smear with crystal violet solution and allow remaining for 1 Minute.
- Rinse off the crystal violet with distilled or tap water.
- Flood the slide with iodine solution. Allow to remain for one minute.

- Rinse off the iodine solution with distilled or tap water.
- Flood the slide with decolorizer for one to five seconds
- Rinse off the decolorizer with distilled or tap water
- Flood the slide with safranin. Allow to remain for 30 seconds
- Rinse off the safranin with distilled or tap water
- Dry the slide on bibulous paper or absorbent paper and place in an upright position.

Catalase Test Protocol

- Clean a glass slide
- Put a drop of hydrogen peroxide 3%
- Add a isolated colony
- Check for results

Results obtained were useful to characterize isolated microorganism.

Bacterial Amylase Production

The microorganisms obtained were used in order to perform the enzyme activities, by adding loopful bacterial culture into amylase production medium. The medium was sterilized and distribute into 30ml conical flask. conical flask containing amylase production medium with isolated microorganisms were incubated on sheker at room temperature for 48 hours and enzyme extraction was performed.

Enzyme Extraction

By using high speed centrifuge in 20 min at 5000 ppm a bacterial culture was poured into centrifuge tube and the supernatant was decant which is expected to be the crude enzyme extract.

The crude obtained by repeating the same procedure were used for demonstration of enzyme activities.

Demonstration of Enzyme Activity

In this step 1 ml of culture extract "enzyme" were pipettes into a test tube, 1 ml of 1% soluble starch in citrate-phosphate buffer (pH6.5) was added incubation in a water bath at 40 °C for 30 minutes was performed.

Set up a blank consisting of 2mL of the enzyme extract that has been boiled for 20 minutes (boiling inactivates the enzyme), added to the starch solution and treated with the same reagent as the experimental tubes. Stop the reaction by adding 2 mL of *DNS reagent (1.0 g of 3, 5, dinitrosalicyclic acid, 20 mL of NaOH and 30 grams of sodium potassium tartarate in 100 ml) Boil for 5 minutes, cool and add 20 ml of distilled water then determine color intensity at 540 nm.

RESULTS

Out of four petri dishes prepared two of them showed good results than others after incubation period of 48 hours at room temperature. These were selected due to clear zone showed in the centre of petri dishes this shows the ability of starch degrading and those two were used for microorganism identification and biochemical analysis and microscopic observation give results recorded in table one.

Table 1: Morphological and biochemical characteristics of isolated strain

TEST	RESULTS
Grams staining	+
Shape	bacilli
Growth at RT	+
Growth on starch agar	+
Starch hydrolysis	+
Catalase	+
Growth at 0.7 PH	+

Table one show the morphological and biochemical characteristics of isolated strain after all tests.

By applying procedure described the amylase activity was determined by using calorimeter and the results obtained were summarized in figure one.

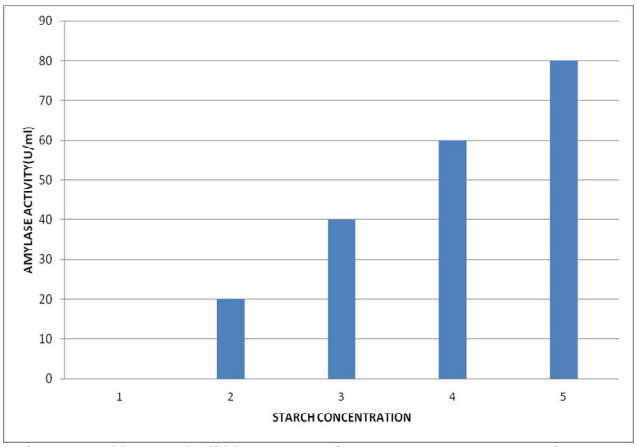

Fig. 1: Effect of different substrate concentration on amylolytic activity of *Bacillus* strain isolated from kitchen waste. Cells were kept for dialysis and collected after 48h forenzyme assay

DISCUSSION

In this research a pure strain of *Bacillus* was isolated from kitchen waste. The kitchen wastes, mostly consists up starchy materials and I found bacteria isolated from such places may have better potential to produce enzyme under adverse condition (Sakai et al., 2001). In the present study we observed 37^{o}C as the optimum growth temperature for the presently reported *Bacillus* strain and higher temperature (50^{o}C) supported less number of colonies. This could be due to the mesophilic nature of the species. As per earlier report of Aiba et al. (1983) high temperature may inactivate the expression of gene responsible for the starch degrading enzyme. Among the physiological parameters, optimum temperature, substrate concentration and pH range are the most important for enzyme production by microbes (Bose and Das, 1996; Gupta et al., 2003).

Table 2: Effect of temperature on amylase activity in entrapped cells exposed at different time intervals. Isolated strain was grown at the optimum growth condition on 2% starch nutrient broth

Temperature (^{O}C)	Time of exposure	
	5 min	10 min
40	10±1.67	10.44±1.25
50	15.55±0.87	24.44±3.65
70	35.54±4.52	36.44±2.98

Enzyme production from microorganism is directly correlated to the time period of incubation (Smitt et al.,1996). The present study observed enhanced enzyme activity with the increase in incubation time. This result is different from some of the recent observation in some *Bacillus* species (Aiyer, 2004), where increase in incubation time decrease enzyme activity. It was also observed that amylase activity by entrapped cells in a dialysis chamber, increased with the increase in temperature. The influence of temperature on amylase production is related to the growth of microbes (Kathiresan and Manivannan, 2006). Saito and Yamamoto (1975) observed similar result in *Bacillus licheniformis.*

In this research different parameters are not examined but could be important for the future use and development of industrial uses.

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