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Isolation, characterization and partial purification of α-amylase from a marine bacillus NH-25

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Abstract: Total 399 marine strains were isolated from the sea water sample and screened for thermostable amylase production. Out of these 52 were to have amylogenic activity. Among them 2 isolates were able to grow and produce amylase at 55°C. Strain NH-25 tolerates 30% salt, a wide pH range (4-8) and retained 64% activity at 50°C after 60 minutes.

Keywords: α-amylase, marine microorganism, thermotolerant.
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INTRODUCTION

α-Amylase (E.C.3.2.1.1.) are starch degrading enzymes that hydrolyze internal α-1,4,- glycosidic linkages in starch and produce α-anomeric configured products[1-3] . They belong to glycoside hydrolase group of enzymes (GH13 family)[4]. They are of immense implication as they are one of the most important enzymes having approximately 30% of the world enzyme production[5]. They have impending applications in industrial processes like food, pharmaceuticals, detergents, starch conversion, fuel alcohol production, textile and paper [6].

There has been a lot of research on amylase from plants[7,8] from animals[9,10] and terrestrial microorganisms [11-13] but less work was done for marine amylases, especially from microorganisms in deep sea environment.

The deep sea environment has engrossed much interest because of 71% of the earth's surface is covered by sea water with an average depth of 3800m, as niches of robial life and microbial enzyme recourses with substantial exploitation potential[14].
In our recent study, samples were isolated from Arabian Sea and their diversity for amylases were studied.

MATERIALS AND METHODS

Screening of marine bacteria

For the primary screening of marine bacterial samples from different locations of Arabian Sea were 2216E marine agar medium containing 5 g/l peptone, 1 g/l yeast extract, 0.01 g/l ferric phosphate, 20 g/l agar 750ml aged sea water 250ml distilled water and stored at 4°C.

Selection of amylogenic strains

Marine collection of strains was tested using 2216E supplemented with 1% soluble starch.

Effect of media on growth and enzyme production

Six different media were tested for this purpose i.e. 2216E, 2216E supplemented with 1% soluble starch, nutrient agar, nutrient agar supplemented with 1% soluble starch, Luria Bertani agar, Luria Bertani agar supplemented with 1% soluble starch.

Effect of heat on growth and enzyme production

Farther than 52 strains were incubated on LB agar supplemented with 1% soluble starch at 40°C, 45°C, 50°C, 55°C and 60°C.

Culture conditions

NH-25 was incubated with 1% starter culture in LSB supplemented with 1% starch for 72 hrs. at 37°C and 160 rpm in orbital shaker. The cell-free supernatant containing α-amylase was harvested by centrifugation at 6000 rpm for 10 min at 4°C and used as the crude enzyme solution for further testing and purification.

Gram's Staining

Slides were prepared to check the morphology and purity of the culture.

Enzyme concentration

Cell free broth was concentrated 5X on rotary evaporator (Buchi) using water bath at 37°C and chiller at 0°C.

Protein estimation

Protein was determined by Coomassie blue protein assay using BSA as a standard (Bradford, 1976).

Enzyme assay

Amylase activity was assayed by Bernfeld method using dinitrosalicylic acid reagent (Bernfeld, 1955). One unit of enzyme activity corresponds to a release of 1mg maltose in 3 minutes at 20°C.

pH profile of enzyme

20µg of protein from concentrated cell free broth diluted up to 1ml with various buffers was reacted with 1ml of 1% soluble starch at 20°C for 3 mins. Reducing sugars released were determined by Bernfeld assay.

Heat stability of enzyme

Theromostability of the crude enzyme was monitored by measuring the residual activity after heating the preparation at 50°C in a water bath for 60 mins. 50µl aliquots (20µg protein) were removed at 15 minutes interval and mixed with the substrate to measure the residual activity.

Salt tolerance

Salt tolerance of the enzyme was determined by measuring the enzyme activity in presence of 1-5 M NaCl. 50µl aliquots were assayed for residual activity.

Enzyme purification

Concentrated cell free broth was fractionated by 35% ammonium sulfate. Precipitates were removed by centrifugation at 6,000 rpm. The supernatant was treated with 85% ammonium sulfate were collected by centrifugation at 12,000 rpm (Beckman, Avanti J301,17, 418g) and solubilized in 25mM Tris HCl buffer (pH 8.0) containing 2.5mM calcium acetate.

RESULTS AND DISCUSSION

On analysis of the gathered strain of marine origin, 52 strains showed clear zone of starch hydrolysis on agar plates (Figure 1).

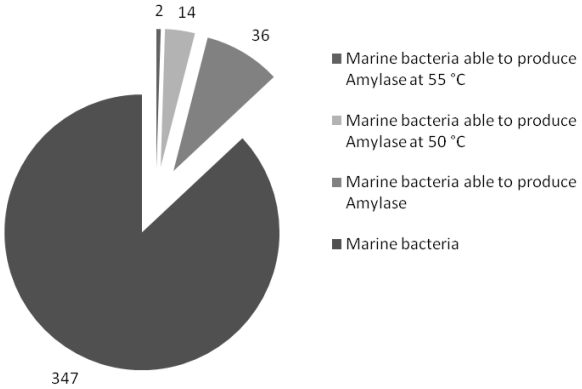

Figure 1: Screening of marine, thermotolerant, amylogenic bacteria.

Amongst the six different media, Luria Starch agar supplemented with 1% starch was found to be the better ones. Further screening of the enzyme in the extended pH range of 3-8; which dropped steeply on the alkaline side. The drop of the enzyme activity on acidic side was gradual with 64% activity observed at pH 3.0 (Figure 2).

Optimum temperature for enzyme activity was 37°C. the enzyme is thermotolerant as indicates by retention of 64% activity after 60 minutes exposure to 50°C (Figure 3).

The amylase is also salt tolerant as it retained 87% residual activity in the presence of 5 M NaCl with starch as a substrate (Figure 4). During partial purification specific activity of the enzyme was increased from 24.35U/mg to 68.78U/mg.

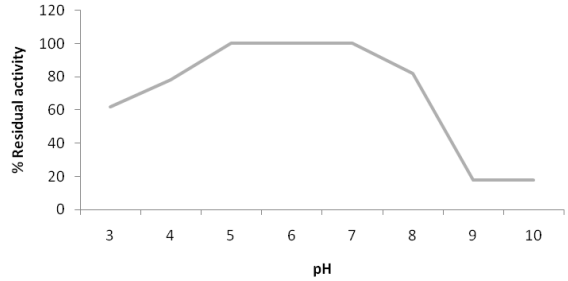

Figure 2: Effect of pH on enzyme activity.

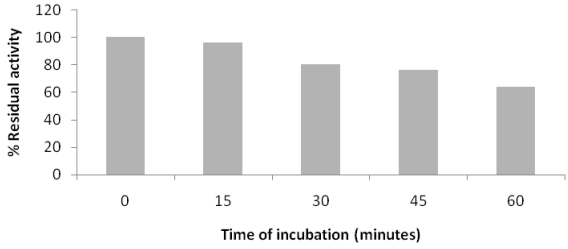

Figure 3: Effect of temperature on enzyme activity.

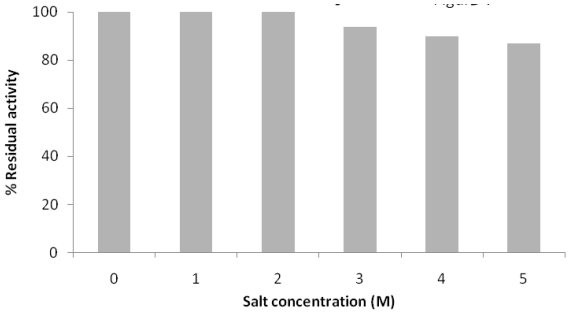

Figure 4: Effect of salt on enzyme activity.

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