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Full Length Research Paper

Thermostable α-amylase production by *Bacillus firmus* CAS 7 using potato peel as a substrate

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Thermostable alkaline α-amylase producing bacterium *Bacillus firmus* CAS 7 strain isolated from marine sediment of Parangipettai coast grew maximally in both shake flasks. Potato peel was found to be a superior substrate for the production of α-amylase at 35°C, pH 7.5 and 1.0% of substrate concentrations. Under optimal conditions, *B. firmus* produced 676 U/ml of α-amylase which was optimally active at 50°C and pH 9.0.

Key words: α-Amylase, potato peel substrate, *Bacillus firmus* CAS 7, thermostable, marine.

INTRODUCTION

Amylase is one among such enzymes that are very much important in the field of biotechnology. It constitutes a class of industrial enzymes having approximately 25% of the enzyme market (Rao et al., 1998). Amylase enzymes are distributed in all the animal, plant and microbial kingdoms. However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors (Pandey et al., 2000).

The potato is grown and consumed all over the world, and a large number of processed food industries market potato-based products. Although, potato peel does not pose serious disposal and environmental problems, meaningful utilization of this nutrient-rich waste has not drawn much attention. Earlier, Banana waste has also been used as a substrate for α-amylase production (Krishana and Chandrasekaran, 1996; Kokab et al., 2003) and many studies has been carried out on amylase production using agriculture waste materials (Nimkar et al., 2010; Singh et al., 2010).

During the course of this study, potato peel on an average was found to contain adequate amounts of nutrients, such as carbohydrates and protein, to support microbial growth. This study reports the ability of the strain *Bacillus firmus* CAS 7 to produce α-amylase on potato peel and the optimization of the various parameters for the enzyme production.

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MATERIALS AND METHODS

B. firmus was isolated from marine sediments of Parangipettai coast, India (Lat. 11° 24 N; Long. 79° 46 E) for protease production. Interestingly, the strain showed promising results while screening amylase production with potato peel as substrate.

Preparation of substrate

Potato peel was dried oven at 70°C for 24 h, ground well and sieved through standard-mesh sieves to obtain powder and stored in polyethylene bags at room temperature (25 ± 5°C). For amylase production, the cultures were grown in mineral salt medium which contained 1.10 g $Na_2HPO_4 \cdot 2H_2O$, 0.61 g $NaHPO_4 \cdot 2H_2O$, 0.30 g KCl, 0.01 g $MgSO_4 \cdot 7H_2O$ and 100 ml distilled water at pH 7.0. Potato peel substrate added to the medium served as the source of carbon. This medium was used for optimizing the various process parameters.

Optimization of culture conditions

B. firmus CAS 7 was subjected to different culture conditions to derive the optimum conditions for amylase production. Growth and amylase production were estimated at selected temperatures (30, 35, 40, 45 and 50°C), substrate (potato peel) concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%) and pH (5.0, 6.0, 7.0, 8.0 and 9.0). All the experiments were carried out in 500 ml Erlenmeyer flask containing 100 ml of starch medium (0.5% peptone, 0.3% yeast extract, 1% substrate concentration, 0.3% NaCl, 0.1% K2HPO4 and 0.02% $MgSo_4.H_2O$). Sterile medium was inoculated with 2.0% of inoculum containing 2.15 9 106 cells ml^{-1}. Inoculated flasks were maintained in water bath shaker at 150 rpm for 48 h.

Extraction and purification of amylase enzyme

Culture broth was centrifuged at 6,000 rpm for 30 min and enzyme in the culture broth was precipitated with 60% ammonium sulphate saturation. The precipitate was dialysed against 20 mM potassium phosphate buffer for 12 h at 4°C. Further purification was carried out in ion exchange chromatography (DEAE-Sephadex). The dialysed protein was applied to a DEAE-Sephadex A-50 column (20 mm diameter × 60 mm long), pre-equilibrated with 20 mM potassium phosphate buffer (pH 7.0). After washing the column with three volume of equilibration buffer, bound proteins were eluted stepwise using phosphate buffers of increasing molarity and decreasing pH values at room temperature (approx. 25°C). The flow rate was adjusted to 24 ml h^{-1} and fractions (1 ml each) were collected. The fractions showing high α-amylase activity were pooled and concentrated in lyophilizer. Amount of protein content was estimated during different levels of purification by the method of Lowry et al. (1951).

Enzyme assay

Amylase enzyme activity was estimated by reducing sugar method of Miller (1959) using 3,5-dinitrosalicylic acid (DNS). The assay mixture containing 250 µL of 50 mM Tris/HCl buffer (pH 7.5), 250 µL of 1% soluble starch (substrate), and 500 µL of appropriately diluted enzyme solution and the mixture was incubated at 50°C for 10 min. The reaction was stopped by adding 3 ml of DNS reagent and maintained in boiling water for 3 min and 1 ml of Rochelle salt solution was added finally. OD of the reaction mixture was measured at 540 nm. OD values were plotted in a standard graph prepared with different concentration of D-glucose. One unit of enzymatic activity was defined as the amount of enzyme required to produce 1 µM of glucose/min under the assay condition.

RESULTS AND DISCUSSION

Many micro-organisms especially several species belonging to *Bacillus* are known to produce variety of extracellular enzymes and they have a wide range of industrial applications (Annamalai et al., 2011). The present study reported the optimization of culture conditions for amylase production by *B. firmus* CAS 7, which showed optimum growth and enzyme production at 50°C (Figure 1a and b), pH 9.0 (Figure c and d), and1.0% of substrate concentration (Figure e and f). Four different temperatures of 30, 40, 50 and 60°C were chosen to determine the optimum temperature for enzyme production, which was found to be 50°C with 676 U/ml while a higher temperature of 45°C has been used for a *Bacillus* species, which resulted in enzyme production of 10 U/ml (Ramesh and Lonsane, 1987).

The amylase production was high during the stationary phase (48 h) of growth of the organism, whereas maximum bacterial growth occurred at 42 h (Figure 2). On comparison of the data obtained for α-amylase production on potato peel was found to produce higher levels of α-amylase. Thus, *B. firmus* was found to produce 676 units/ml of α-amylase in 48 h on potato peel. Moreover, Qian et al. (1992) reported α-amylase production by *Bacillus subtilis* on pure wheat bran with enzyme activity of 1720 U/g dry bacterial bran at

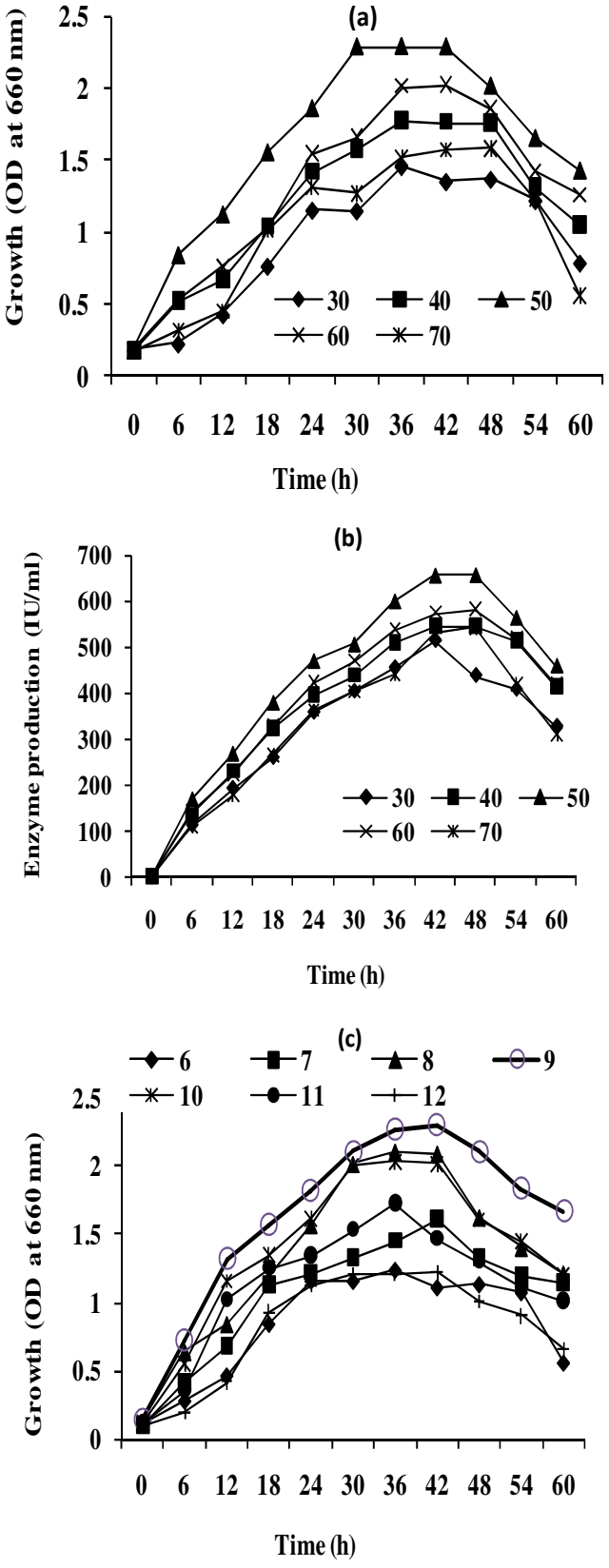

Figure 1. Growth (left panels) and enzyme production (right panels) by B. *firmus* a selected temperature (a, b), pH (c, d) and substrate concentration (e, f).

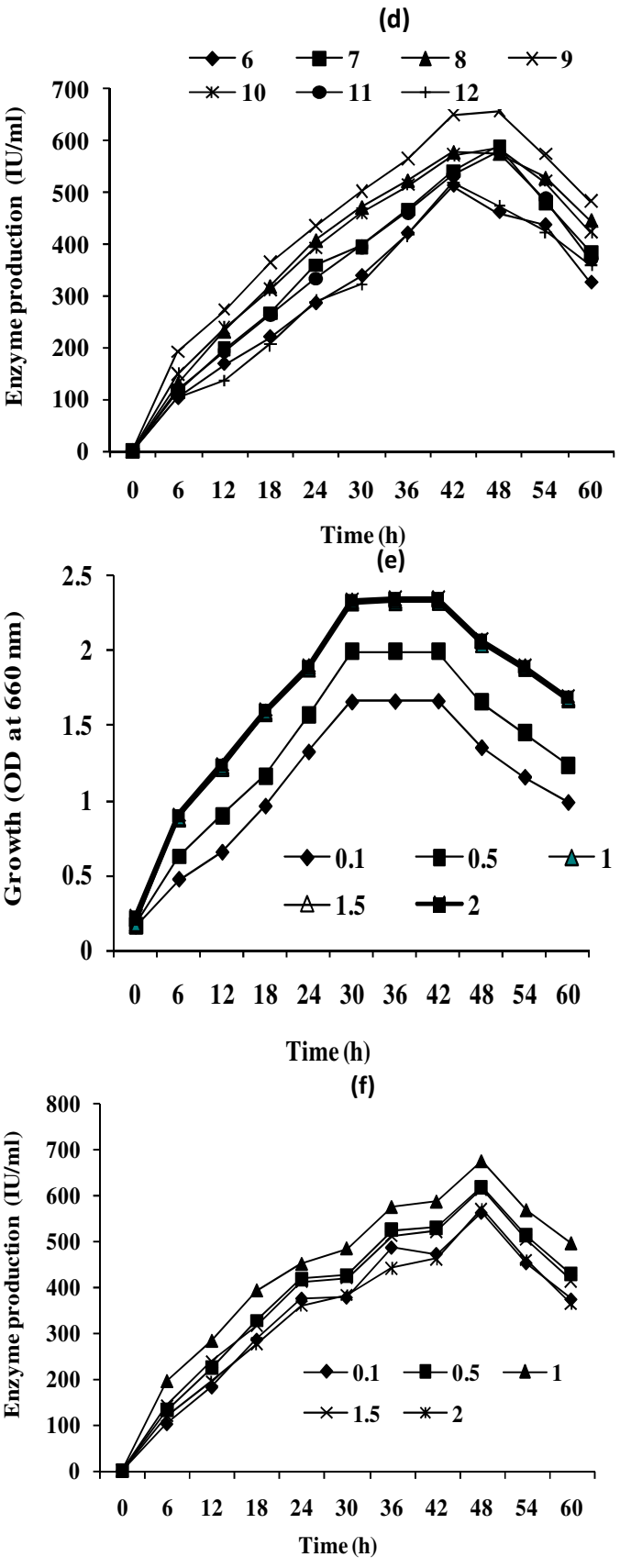

Figure 1. Contd

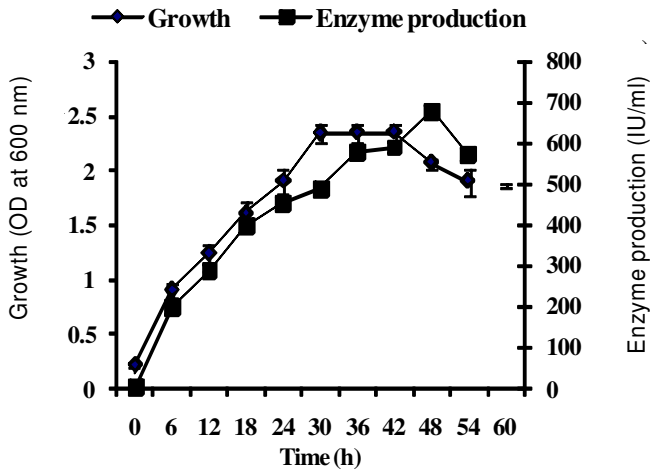

Figure 2. Growth Vs enzyme production by *B. firmus* CAS 7.

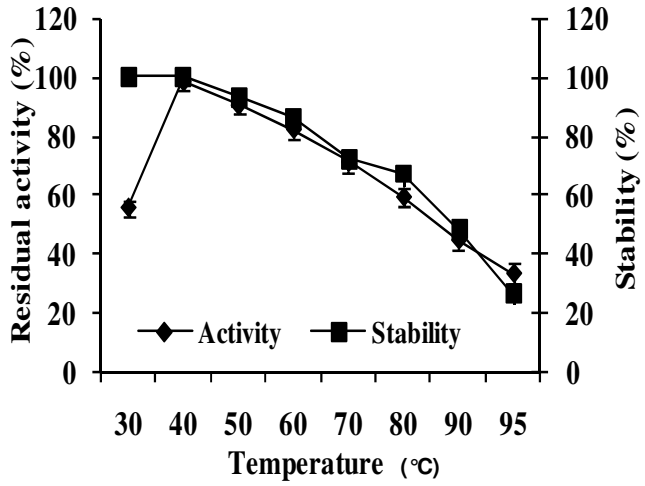

Figure 3. Effect of temperature on activity and stability of α-amylase from *B. firmus* CAS 7.

laboratory scale.

Optimum temperature and pH of amylase enzyme

That the α-amylase activity of our strain of *B. firmus* CAS 7 is sustained even at such high temperature as 50°C (Figure 3) and pH 9.0 (Figure 4) and retained up to pH 11.0 is new and has immediate relevance to industrial application. It is comparatively less and the enzyme has still wider ranges of tolerance than the previously reported for amylases (Balton et al., 1997; Lin et al., 1998; Stamford et al., 2001; Das et al., 2004). Many of the amylases reported were thermostable but not alkaline active, whereas some of them were alkaline active but not thermostable. But the amylase from our present study was thermostable (50°C) and also alkaline active (pH

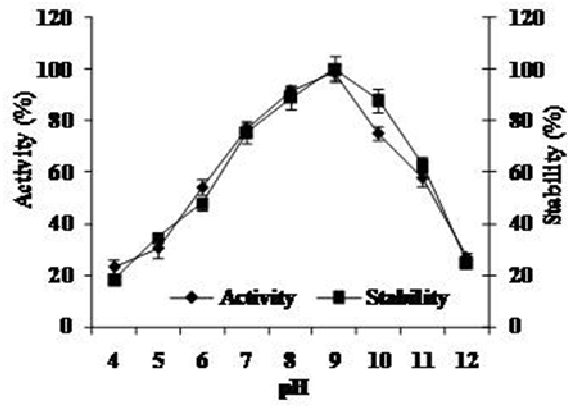

Figure 4. Effect of pH on activity and stability of α- amylase from *B. firmus* CAS 7.

11.0) which is significant in industries dealing with amylases. Thermostable α-amylase find a number of applications in commercial processes, such as thinning and liquefaction of starch, which takes place above 100°C (Sharp et al., 1989).

For the determination of the optimum pH, the amylase was tested over a pH range of 3 to 11 at 50°C. The amylase was 100% stable at pH 9.0 and it retains more than 60% even at pH 11.0. It is comparatively high and the enzyme of our strain B. firmus CAS 7 has still wider ranges of tolerance than the previously reported for amylases (Stamford et al., 2001; Das et al., 2004; Oyeleke and Oduwole, 2009). *Bacillus* species producing alkaline amylases have been often reported (Horikoshi, 1971). Though these enzymes have their optimum pH in neutral to alkaline range, they are not thermostable and are susceptible to heat above 40°C. While the enzyme α-amylase of *Bacillus stearothermophilus* has been reported to be thermostable, it did not act in the alkaline range (Pfueller and Elliot, 1969).

Conclusion

From these promising results, it can be conclude that this enzyme have their own merits in terms of higher enzyme activity or higher temperature and pH tolerance. Their performance on potato peel, a novel and inexpensive substrate was highly satisfactory and promises practical application.

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