Full Length Research Paper

Ecofriendly management of mixed coconut oil cake waste for lipase production by marine Streptomyces indiaensis and utilization as detergent additive

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The utilization of agro waste for the production of lipase enzyme was one of the ecofriendly methods for the management of waste. From 34 actinomycetes strains screened from sediments of Tiruchendhur coastal areas of Tamil Nadu, India, 26 strains exhibited lipase activity. The marine actinomycete strain MAC 7 was used for the production of extracellular lipase by using mixed coconut oil cake waste as substrate. The strain showed maximum lipase activity at pH 9 and temperature 55°C. The solid state fermentation was carried out for 8 days with 80% moisture content. The lipase extracted from marine actinomycete was highly alkaline and thermophilic in nature. The enzyme was further utilized as a good detergent additive.

Key words: Actinomycete, coconut oil cake, lipase, Streptomyces indiaensis, solid state fermentation, wheat bran.

INTRODUCTION

The aquatic world contains a wide variety of living species and represents greatest potential for discovering new enzymes (Cherif et al., 2007). Microbial enzymes are relatively more stable and active with extraordinary properties (Bull et al., 2000; Ghosh et al., 2005). Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are the enzymes that catalyze the hydrolysis and the synthesis of esters formed from glycerol and long chain fatty acids. Lipases are more useful (Sharma et al., 2001) in food additivites, clinical reagents and detergent additives, medicines and biodegradation of plastics such as polyhydroxyalkanoates (PHA) and polycaprolactone (PCL) (Jaeger et al., 1995; Mochizuki et al., 1995).

The partial decomposition of solid waste produces leachate and affects ground water and land environment. It also causes bad odour and increases chance for pathogens which cause serious diseases to organisms. So, solid wastes are used as sources for the production of novel enzymes like lipase. This is one of the best methods for the management of solid waste in an ecofriendly way. Solid state fermentation (SSF) has more advantages than submerged fermentation (SMF) due to low capital investment, simplification of the fermentation media, absence of complex machinery, reduced energy requirement and improved product recovery, more thermostable (Lonsane et al., 1985; Pandey et al., 1999). Mixed solid substrate fermentation (Benjamin and Pandey, 1998; Imandi and Garapathi, 2007) was a novel process for enhanced lipase production by Candida rugosa and Yarrowia lipolytica. The maximum lipase activity was observed in Bacillus subtilis in solid state fermentation using ground nut oil cake as substrate (Chaturvedi et al., 2010). Thermostable lipases from (Ahmed et al., 2009; Dutta and Ray, 2009; Nawani and Kaur, 2000) many Pseudomonas and Bacillus sp. have

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been isolated and studied. The actinomycete *Streptomyces griseochromogenes* isolated from shrimp pond showed higher lipase activity (Gunalakshmi et al., 2008).

**MATERIALS AND METHODS**

**Isolation and screening of lipase producing actinomycetes from sediment sample**

The marine sediment samples were collected from two different points of Tiruchendur coastal areas of Tamil Nadu, India. The collected samples were transported immediately to the laboratory. The samples were air dried and were incubated at 55°C for 10 min. The samples were serially diluted and spread on actinomycete isolation agar with pH 9 and incubated for 7 to 14 days and the plates were observed for the appearance of actinomycete colonies. The bacterial and fungal contaminations were controlled by the addition of each 20 and 100 mg/L of nystatin and cycloheximide with agar media. The pure actinomycetes cultures were maintained on nutrient agar media. The actinomycetes were grown on tributyrin agar plates and the zone of clearance was observed due to the hydrolysis of tributyrin (Hun et al., 2003; Nair and Kumar, 2007). The actinomycetes that showed the maximum zone of clearance was selected for further analysis.

**Solid state fermentation**

Modified mineral salt solution was used for the inoculation of the strain. The media composition (g/100 ml) was magnesium sulphate 0.05 g, dipotassium hydrogen phosphate 0.1 g, sodium chloride 3 g, ferrous sulphate 0.001 g, manganous chloride 0.001 g, zinc sulphate 0.001 g. It was incubated at 55°C in an incubator shaker at 180 rpm for 24 h. The different wastes with the following combinations were used for the enzyme production: Substrate 1) coconut oil cake (10 g), substrate 2) soymeal + coconut oil cake (5 g + 5 g), substrate 3) coconut oil cake (3.3 g) + soymeal (3.4 g) + wheat bran (3.3 g). They were dried at room temperature to reduce the moisture content and ground to the desired size. Each substrate (1, 2, 3) of 10 g was added with 80 ml of modified (SS) mineral salt solution and sterilized. To the sterilized media, 10 ml of inoculum was added. Each flask with substrates 1, 2 and 3 was incubated at 55°C in an incubator shaker at 180 rpm for 8 days.

**Optimization of different parameters**

The different parameters like pH of the medium ranging from 6 to 11, temperature (35 to 60°C), moisture content (50 to 100%), substrate concentration (5 to 15%) and sodium chloride concentration (1 to 5%) were optimized for enzyme production. All the experiments were carried out in 250 ml Erlenmeyer flask containing 100 ml of medium. It was incubated for 8 days at 55°C in an incubator shaker at 180 rpm.

**Enzyme extraction**

The crude enzyme from the fermented substrate was extracted by using 0.05 m sodium phosphate buffer (pH 9.0). The fermented substrate was mixed with 100 ml of buffer and was kept in the rotary shaker (180 rpm) at 55°C for 1 h. The raw extract was obtained by centrifugation at 10,000 rpm for 15 min at 4°C. The clear supernatant obtained from centrifugation was used to determine the enzyme activity.

**Lipase assay**

Lipase activity was measured by titrimetric method using olive oil emulsion method (Watanabe et al., 1977). The reaction mixture with 5 ml of olive oil emulsion (25 ml olive oil and 75 ml 2% polyvinyl alcohol), 4 ml of 0.2 M tris buffer, 1 ml of 110 mM CaCl₂ and 1 ml enzyme solution was incubated for 30 min at 55°C. The control containing boiled inactivated enzyme (at 100°C for 5 min) was treated similarly. After incubation, the enzyme activity was blocked by 20 ml of acetone ethanol (1:1) mixture and the liberated free fatty acid was titrated against 0.05 M NaOH using phenolphthalein as an indicator. One unit of lipase was defined as the amount of enzyme, which liberates 1 µmol of fatty acid/min under standard assay conditions. The enzyme activity was expressed as IU/ml.

**Partial purification of enzyme**

The crude enzyme was precipitated by the addition of 60% (v/v) volume of chilled acetone and stored overnight at -4°C. It was centrifuged at 10,000 rpm for 10 min. The precipitate was suspended in sodium phosphate buffer and incubated overnight at 4°C. The enzyme was dialyzed against same phosphate buffer. It was loaded on column (2.5 × 17 cm) of DEAE Sephadex A-50 already equilibrated with sodium phosphate buffer. The purified enzyme was collected and stored at 4°C for further use.

**RESULTS AND DISCUSSION**

**Screening of potential isolate for lipase activity**

Out of 26 lipase producing actinomycetes screened from sediments of Tiruchendur coastal areas of Tamil Nadu, India, MAC 7 strain was selected due to larger clear zone formation on Tributyrin agar medium. It was identified as *Streptomyces indicaensis* using 16S rRNA sequencing (Genbank accession number for nucleotide sequence: JQ801298).

**Influence of additives on lipase activity**

Figure 1 showed the moderate increase of enzyme activity from 3rd to 5th day and the highest lipase activity was observed on the 4th day (220.8 ± 0.20 IU/ml) by using mixed waste (coconut oil cake with inducers soy meal and wheat bran) than coconut oil cake and combined oil cake with soy meal. From the 4th day onwards, the lipase yield was decreasing slowly due to the consumption of nutrients by the microbes. The lipase activity (175.6 ± 0.25 IU/ml) observed in mixed coconut oil cake with soymeal was greater than coconut oil cake (141.6 ± 0.15 IU/ml) as substrate in the 4th day. The results indicated that soymeal and wheat bran act as inducers and additives for the lipase production. So, the combination of both with oil cake increased the lipase production. Manoj et al. (2010) reported that the lipase production was more in groundnut oil cake than in coconut oil cake. The lipase activity was highest in mixed waste of bagasse and wheat bran (Imandi and Garapathi, 2007) than wheat bran and bagasse used as substrate.
Optimization of substrate

From Figure 2, it was observed that 10 g of substrate showed maximum lipase production than 5 g (133.3 ± 0.47 IU/ml) and 15 g (95.8 ± 0.30 IU/ml) due to its easier penetration by the microbes. The less lipase production at higher substrate level was due to low mass transfer rate and difficulty in penetration of the organism (Rao et al., 2003). The mixed waste of bagasse and wheat bran (10 g) showed highest lipase activity (Imandi and Garapathi, 2007).

Effect of pH, temperature and incubation time on enzyme production

The maximum lipase activity was observed in pH 9 (Figure 3) and at a temperature of 55°C (Figure 4). Gunalakshmi et al. (2008) observed highest activity for marine actinomycete strain at 55°C and pH 8. pH is an important parameter required for the growth of microbes in the respective media. The results indicated that there is a strong influence of alkaline pH on lipase production. Figure 3 showed that the alkaline nature of marine actinomycete was due to its maximum activity at pH 9. It also exhibited higher enzyme activity on pH 10 (204.6 ± 0.15 IU/ml) and 11 (191.6 ± 0.32 IU/ml). It showed the least value for pH 6 (66.6 ± 0.20 IU/ml). It exhibited maximum activity at higher temperature in the range of 55°C. The higher enzyme activity was also observed in the temperatures 50°C (200.9 ± 0.85 IU/ml) and 60°C (191.7± 0.17 IU/ml). The results show the stability of the enzyme was from pH 8 to 11 and at temperatures ranging from 45 to 60°C. So the marine actinomycete preferred more alkaline and thermal conditions for maximum enzyme production.

Effect of moisture content on lipase activity

The lipase activity was maximum at 80% moisture content. The maximum lipase activity was observed at 80% moisture content in Y. lipolytica (Imandi and Garapathi, 2007). At 70% moisture content, the activity was slightly higher (175.5 ± 0.50 IU/ml) than at 50% moisture content. From Figure 5, it was observed that the optimum moisture content for lipase production was 80%. The enzyme activity was high in favourable moisture
conditions of 70 to 80%. It was lowest at 50% (50.7 ± 0.25 IU/ml). Low moisture contents lead to the reduction of solubility of nutrients and higher moisture contents lead to decrease in the porosity due to the stickiness of media
(Lonsane et al., 1985).

Sodium chloride tolerance

The marine actinomycete showed highest activity at 3% sodium chloride concentration (Figure 6). The salt concentration of 4% also showed an increased activity of 195.7 ± 0.43 IU/ml. It showed the sodium chloride tolerance level from 2 to 4%. It showed the least value for 1% sodium chloride concentration (137.5 ± 0.55 IU/ml). So, this organism preferred alkaline pH condition for maximum growth and highest lipase production. The maximum lipase activity was observed at 4% sodium chloride concentration (Gunalakshmi et al., 2008).

Application of lipase as detergent additive

The partially purified lipase enzyme was used as an additive in the detergent industry. Four multistained (grease, oil, mud and pickle) white pieces of cloth (5 cm × 5 cm) were taken in four flasks with 100 ml of water each. One flask was used as control. Detergent (Surf Excel-5 mg/ml) was put in the second flask. Enzyme (1 ml) was put in the third flask while the fourth flask contained both enzyme and detergent. All the flasks were incubated at 55°C for 30 min and the observations were recorded before and after incubation. After incubation, the pieces of cloth were rinsed with water and dried. The enzyme extract with the combination of detergent removed the stain successfully. It showed the effective utilization of enzyme extract as a powerful detergent additive.

Conclusions

The results presented in the Figures 1 to 6 show that mixed waste with inducers had the highest lipase production than single waste used as substrate. The addition of mixture of soy meal and wheat bran induced the lipase production. The marine actinomycete S. indaensis preferred alkaline conditions (e.g. pH 9, higher temperatures 55°C) for maximum lipase activity. So this marine actinomycete was used for enhanced production of lipase enzyme and utilization as additive in detergent industries. In the solid state, fermentation mixed waste (with inducers) used as substrate showed an efficient ecofriendly management of waste and it reduced the environmental pollution.

REFERENCES


Figure 6. Effect of sodium chloride on lipase activity.


