JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2011. Vol. **5**(2), p. 925-928

Molecular and Biochemical Studies on Lipase Producing Thermophiles from Taptapani Hot Water Spring, Orissa, India

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(Received: 12 January 2011; accepted: 21 February 2011)

Three lipase producing thermophilic bacteria (AK-P1, AK-P2 and AK-P3) were isolated from the Taptapani hot water spring in Orissa, India. The crude extra cellular lipases from cell-free culture supernatant were reacted in an olive oil mixture and their lipolytic activities were compared. Identification of the bacteria was carried out using biochemical tests, 16SrRNA sequencing and sequences submitted to NCBI GenBank. Strain AK-P2 exhibited the highest lipolytic activity of 5.5 U/ml was identified as Brevibacillus spp. The lipolytic activities of strains AK-P1 and AK-P 3 were 3.5 U/ml and 4.5 U/ml respectively. Strains AK-P1 and AK-P 3 were identified as Acinetobacter sp. and Porphyrobacter sp. The GenBank accession numbers of the 16S rRNA gene sequences determined in this study for the strains AK-P1, AK-P2 and AK-P3 are HM359120, HM359119 and HM359118 respectively.

Key words: Lipase, Thermophilic, GenBank, 16SrRNA**.**

Thermophiles are a group of microbes adapted to thrive in geothermally extreme habitats and are of good source of thermostable enzymes (Brock 1978, 1985). The investigation and application of thermostable enzymes from thermophilic microorganisms have received immense attention from various industries. The modern concept of the nature of the thermostability of the enzymes from the thermophilic microorganisms can be reduced to two basic ideas. Firstly, the enhanced thermal stability of enzymes in thermophilic cells could be due to their molecular structure being different from that of their counter part mesophilic proteins. Secondly, the increased stability of the enzymes could be due to their supramolecular association with other components of the cell (lipids, polysaccharides or proteins). In order to survive at high temperatures, thermophilic prokaryotes (Archaea and Eubacteria) adopt different strategies like GC-rich codons, the ratio of charged amino acids compared to uncharged amino acids, ionic interactions, amino acid preferences and their distribution, post-translational modifications, and solute accumulation (Trivedi etal. 2006). Most of the industrial microbial lipases derived from fungi and mesophilic bacteria (Sharma, 2001). Bacteria produce different classes of lipolytic enzyme, including carboxylesterases (EC 3.1.1.1), which hydrolyse small ester containing molecules at least partly soluble in water, true lipases (EC 3.1.1.3), which display maximal activity towards water insoluble long-chain triglycerides, and various types of phospholipase (Arpigny and Jaeger 1999).

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More than 50 lipases have been identified, purified and characterized to date which originate from natural sources such as animals, plants and microorganisms (native or genetically engineered). A large number of beneficial thermophiles which produced lipases with good thermal stabilities have been found in diverse habitats (Wang et al. 1995). Their thermal stabilities particularly in the absence of sufficient amounts of water increase their popularity (Nirupama et al. 1998).The enormous potential of microbial lipases arises from the facts that they are (1) quite stable and active in organic solvents, (2) do not require cofactors, (3) exhibit a high degree of enhantio- and regioselectivity, and (4) possess a wide range of substrate specificity for the conversion of various unnatural substrates (Nakatani et al. 1992; Jager & Reetz 1998). Therefore screening and identification of thermostable lipolytic bacteria will act as a meaningful addition to the database on bacterial lipase research.

MATERIAL AND METHODS

All isolates were initially evaluated by conventional tests i.e. Gram stain, growth and morphometric characteristics on thermus agar ATCC 697 medium, growth at 42°C, catalase, oxidase, motility, indole production, gelatin liquefaction, oxidative fermentative carbohydrate utilization, decarboxylation of lysine, urease activity. Additional tests included phenylalanine deamination, nitrate reduction, citrate utilization, H_2S production, hydrolysis of starch and growth in the presence of 6.5 % sodium chloride.

The cultures AK-P1, AK-P2 and AK-P3 isolated from Taptapani hot water spring (19° 30' 0N lat and 84° 24' 0E long) of Ganjam District, Orissa, India were maintained on thermus agar and stored at 4°C.Screening of lipase producers was carried out using tributyrin agar plate according to Lawrence 1967. Each culture was streaked onto the tributyrin agar plate and incubated at 48°C for 2 days. The lipase producing bacteria were identified by the presence of surrounding clear hydrolytic zones. The identified bacterial lipase producers were cultivated in production medium (50 ml) in triplicate (one control and two replicates /sample). After incubation at 48°C for 36h the cultures were centrifuged at 8000 rpm at 4°C for 15 min. The crude lipase solution was obtained by filtering through a milipore 0.22 ìm filter membrane and lipolytic activity was measured by universal titrimetric method (Cardenas et al. 2001; Godfrey et al. 1996). Each reading was taken in triplicate and the activity was calculated as amount of enzyme required liberating one micromole equivalent fatty acid per mL/min.

DNA preparation and PCR amplification

Genomic DNA was extracted from the isolates using Chromous Genomic DNA isolation kit (RKT09). Each genomic DNA used as template was amplified by PCR with the aid of 16SrDNA primers (16S Forward Primer: 5'-AGAGTRTGATCMTYGCTWAC-3' 16S Reverse Primer: 5'-CGYTAMCTTWTTACGRCT-3').

16S rRNA sequencing and data analysis

16S rRNA sequence analysis was performed using the ABI 3130 genetic analyzer and Big Dye Terminator version 3.1 cycle sequencing kit. The three 16SrRNA sequences were aligned and compared with other 16SrRNA genes in the GenBank by using the NCBI Basic Local alignment search tools BLAST n program.

RESULTS AND DISCUSSION

Lipolytic activities of three lipase producing bacteria are shown in figure 1. All the three cultures showed good lipolytic activities. Strain AK-P2 exhibited the highest lipolytic activity of 5.5 U/ml followed by strains AK-P1 and AK-P 3 with an activity of 3.5 U/ml and 4.5 U/ml respectively (Figure1).

The enzyme has shown very good stability up to 65°C for 20 min. Further it decreased considerably at 70°C followed by a sharp decline in enzyme activity after 70°C. The exposure of the enzyme at 75°C for 20 min decreases the enzyme activity to 26% of the total activity (Figure 2).

More than 1400bp of the 16S rRNA genes of strains AK-P1, AK-P2 and AK-P3 were sequenced. Analysis of the 16S rRNA sequences confirmed the strain AK-P1 was most similar to Acinetobacter sp. 01B0 NCBI accession no.GQ178044 (100%). The strain AK-P2 was found to be most similar to Brevibacillus sp. Borstelensis AR9 NCBI accession no. EF139656 (99.9%) and the strain AK-P3 shares closest

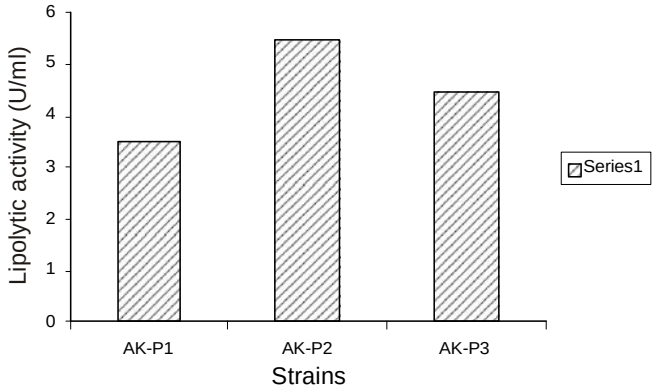

Fig. 1. Lipolytic activities of lipase-producing bacteria isolated from Taptapani hot water spring

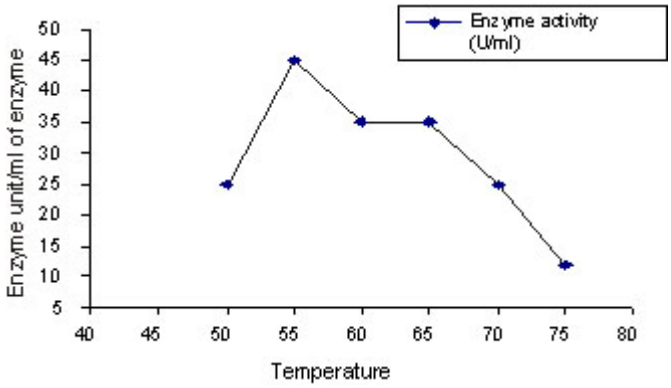

Fig. 2. Thermal stability of crude lipase produced by AK-P2

homology with Porphyrobacter cryptus(T) ALC-2 NCBI accession no. AF465834 (99.2%).All the 16SrRNA sequences of the present investigation have been successfully submitted to NCBI with the accession no.HM359120, HM359119 and HM359118 and are available for the research utilization. These findings are of their own type from this hot water spring.

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