PREFACE

Metagenomics, a molecular method based on direct isolation and analysis of nucleic acids, proteins and lipids from environmental samples, reveals structural and functional information about microbial communities. Microbial lipases are industrially important and have gained attention due to their stability, selectivity and broad substrate specificity. Bacteria, yeast and fungi are potential sources of lipases. The industrial demand for new sources of lipases, with different catalytic characteristics, stimulates the isolation and selection of new strains. Lipase-producing bacteria have been found in different habitats such as industrial wastes, vegetable oil processing factories, dairy plants and soil contaminated with oil and oil seeds among others. Among the various kinds of bacteria, *Bacillus* group exhibited interesting properties that make them potential candidates for biotechnological applications. Lipases are classified into eight families, I to VIII, based on their amino acid sequences. It is a widely accepted fact that more than 99% of microorganisms present in soil samples are not readily culturable and therefore not accessible for biotechnology or basic research. The genomes of these uncultured species encode a largely untapped reservoir of novel enzymes and metabolic capabilities. As such more than 99% of microorganisms have unique and potentially very useful abilities such as waste degradation, enzyme production and synthesis of compounds that could find use as drugs or antibiotics.

The present investigation was undertaken to explore the lipase-producing bacterial gene(s) in bakery soil. Metagenomic DNA (mgDNA) was extracted using Porteous et al. method with some modifications. The purity and cloneability of extracted mgDNA was confirmed by polymerase chain reaction (PCR) and restriction digestion. The isolated and purified soil mgDNA was partially digested with Sau3AI, and the DNA fragments 0.5–3.0 kb in size were ligated into *Bam*HI digested pET-32a vector. The vector was transferred to *Escherichia coli* BL21(DE3) pLysS competent cells. The recombinant clones were screened, and a positive clone carrying the recombinant plasmid KBS-plip1 exhibited lipolytic activity. The positive clone was confirmed with the band shift of the recombinant

plasmid and DNA sequencing. The sequencing of the 996-bp insert DNA in the clone KBS-plip1 revealed an open reading frame (ORF) of 891 bp encoding the protein with 296 amino acids which showed 99% sequence homology with the DNA of the lipase/esterase-producing unculturable bacteria, KC182797. The phylogenetic tree was constructed using neighbour joining method. Subsequently, the nucleotide sequence was deposited in the BanKit of NCBI under the accession number KF743145.

Multiple sequence alignment of amino acids revealed 23–55% homology with the unculturable bacterial lipases. KBS-plip1 was classified as family IV or hormone-sensitive lipase (HSL) family. The HSL family conserved HGGG motif (amino acid 23–26) was found upstream of the active-site motif in KBS-plip1. Hormone-sensitive lipase contains the lipase conserved catalytic consensus pentapeptide GDSAG. Catalytic triad DPM (amino acids 145–147) and HVF (amino acids 228–230) are also the conserved sequences of HSL family and present in the KBS-plip1 amino acid sequence. The cloned lipase gene overexpressed in the presence of 0.5 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG). The size of recombinant protein, fused with N- terminal His- tag, was found to be ~40.86 kDa including the insert sequence encoding 296 amino acids (33867.4 Da) and pET-32a vector backbone sequence contributing 55 amino acids (6988.52 Da). The modelled enzyme is a folded dimmer and consists of nine α-strand attachment with seven β-helices. The Ramachandran plot validated the modelled structure of KBS-plip1. This is significant as it revealed a good quality protein as high percentage (>95%) of amino acid residues are in the favoured region.

Biochemical characterization of the KBS-plip1 lipase revealed maximum activity at a concentration of 1.0 $\mu g \cdot ml^{-1}$ and in the presence of 1.0% tributyrin, beyondthis the enzyme activity became saturated. The kinetic study of the KBS-plip1 lipase revealed V_{max} and K_m values to be 227 U/ml and 0.806 mg/ml, respectively. The purified protein exhibited a specific activity 96.32 U/mg with 2.23 purification fold. For maximum activity of the enzyme, a temperature of 37°C and pH 7.5, presence of the divalent cations Ca^{2+}, Mn^{2+}, Zn^{2+} and Fe^{2+} were essential whereas EDTA inhibited the catalytic activity; but CTAB and gum arabic enhanced the activity. NaCl at a concentration of 1.5 M increased the enzyme activity by 2.5 fold. The organic solvents such as ethanol, 1-propanol, acetone, acetonitrile, glycerol and DMSO enhanced the activity by 3.5 fold. The enzyme was stable at 37°C up to 100 min, but at 45°C and beyond (55°C)

the enzyme activity decreased drastically with half-life of 60 and 20 min, respectively.

The thermal stability of the crude lipases isolated from the culturable soil bacterial strains KBS-101, KB2F, KBS-103, KBS-105 and KBS-107 retained 50% catalytic activity at 60°C up to 80 min. The enzyme demonstrated industrial application in laundry detergent formulation. On the basis of bacterial growth kinetics, lipase production yield, detergent stability and thermal stability of the lipases isolated from the bacterial strains KBS-101, KB2F, KBS-103, KBS-105 and KBS-107 were characterized. The 16S rDNA isolated from these bacterial isolates were amplified and sequenced. Phylogenetic and sequence homology study for the sequences of the bacterial strain KBS-101 showed 97% 16S rRNA sequence homology with *Bacillus* methylotrophicus strain (HQ844459) and represented to be its closest phylogenetic neighbour. *Bacillus tequilensis* strain (HQ234273) showed 99% sequence homology with KB2F, strain KBS-103 showed 99% sequence homology with the unculturable strain HQ764973. The strain KBS-105 showed 100% sequence homology with *Bacillus badius* strain GQ497939, KBS-10797% with *Enterobacter* sp. FJ440555.

An inoculum size of 2.0% was effective in producing maximum lipase by all the bacterial strains. The strains KBS-101 and KBS-105 possessed higher accumulation of lipase on supplementing the media with glucose as the carbon source, KB2F revealed the maximum accumulation in the presence of lactose, KBS-103 and KBS-107 in the presence of galactose. The maximum enzyme activity was shown by the strains KBS-101 and KB2F in the presence of beef extract, whereas KBS-103 and KBS-107 in the presence of yeast extract. The strain KBS-105 possessed higher accumulation of lipase when the medium was supplemented with peptone. The maximum substrate specificity for olive oil was revealed by the lipases isolated from the strains KBS-101, KBS-103 and KBS-105, whereas KB2F and KBS-107 showed for tributyrin. Maximum lipase accumulation and cell biomass was obtained in the case of KBS-101 and KBS-103 after 24 h, strains KB2F, KBS-105 and KBS-107 showed the maximum accumulation after 48 h. The optimum pH for maximum lipase accumulation by the bacterial strains KBS-101 and KBS-105 was 8.5, and for KB2F and KBS-103 it was 7.5 and KBS-107 at 9.0. The optimum temperature for maximum lipase accumulation from the bacterial strains KBS-101, KBS-103, KBS-105 and KBS-107 was 37°C and for KB2F it was 45°C. The agitation rate of 200 rpm revealed

maximum lipase accumulation by all five bacterial strains. Out of divalent cations used, Ca^{2+} enhanced the enzyme activity in the case of strains KBS-10 and KBS-103; Fe^{2+} in the case of KB2F and KBS-107; Co^{2+} in the case of KBS-105 and Mg^{2+} in the case of KBS-107.

The culture supernatant containing crude lipases extracted from the bacterial strains exhibited antimicrobial activity against the bacterial species *E. coli* (MTCC 40), *Bacillus subtilis* (MTCC 619), *Staphylococcus aureus* (MTCC 737), *Klebsiella pneumoniae* (MTCC 109) and *Pseudomonas aeruginosa*; and two fungal species Candida *albicans* (3017) and *Fusarium oxysporum* (MTCC 284). The culture supernatant containing crude lipases from the bacterial strain KBS-101 showed antibacterial activity against all the bacterial strains; KBS-103 showed activity against *E. coli* (MTCC 40), *B. subtilis* (MTCC 619) and *S. aureus* (MTCC 737); whereas KBS-105 showed activity against *E. coli* (MTCC 40). The culture supernatant containing crude lipases of KBS-101 and KBS-105 exhibited the antifungal activity against *F. oxysporum* (MTCC 284).

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