Cloning, expression and characterization of a novel esterase from a South China Sea sediment metagenome*

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Abstract Lipolytic enzymes, including esterases and lipases, represent a group of hydrolases that catalyze the cleavage and formation of ester bonds. A novel esterase gene, scsEst01, was cloned from a South China Sea sediment metagenome. The scsEst01 gene consisted of 921 bp encoding 307 amino acid residues. The predicted amino acid sequence shared less than 90% identity with other lipolytic enzymes in the NCBI non-redundant protein database. ScsEst01 was successfully co-expressed in Escherichia coli BL21 (DE3) with chaperones (dnaK-dnaJ-grpE) to prevent the formation of inclusion bodies. The recombinant protein was purified on an immobilized metal ion affinity column containing chelating Sepharose charged with Ni2+. The enzyme was characterized using p-nitrophenol butyrate as a substrate. ScsEst01 had the highest lipolytic activity at 35°C and pH 8.0, indicative of a meso-thermophilic alkaline esterase. ScsEst01 was thermostable at 20°C. The lipolytic activity of scsEst01 was strongly increased by Fe2+, Mn2+ and 1% Tween 80 or Tween 20.

Keyword: marine sediment; metagenome; lipolytic; expression; esterase characterization

1 INTRODUCTION

Lipolytic enzymes, including esterase (EC 3.1.1.1) and lipase (EC 3.1.1.3), are a group of hydrolases that catalyze the cleavage and formation of ester bonds. Most lipolytic enzymes have wide substrate tolerance, high stability in organic solvents, and no specific cofactor requirement, which are characteristics that make lipolytic enzymes easy to catalyze. Lipolytic enzymes consequently have potential applications in laundry, food engineering, pharmaceutical and biodiesel production (Bornscheuer, 2002).

Lipolytic enzymes are typically derived from animals, plants and microorganisms. Nevertheless, fewer than 1% of marine microorganisms can be cultivated in the laboratory by conventional methods (Aman et al., 1995). Metagenomics is widely used for functional gene screening of uncultured microorganisms (Handelsman et al., 1998; Schmeisser et al., 2007). Lipolytic enzymes have been identified using metagenomic libraries from pond water (Ranjan et al., 2005), soils (Lämmlle et al., 2007), activated sludge (Roh and Villatte, 2008), plant rhizosphere soil (Lee et al., 2010) sheep rumen (Bayer et al., 2010) and various marine environments such as tidal flat sediments (Lee et al., 2006), seawater (Chu et al.,...
2008), intertidal flat sediments (Kim et al., 2009) and deep-sea sediments (Park et al., 2007; Jeon et al., 2009; Hu et al., 2010; Fu et al., 2011; Jiang et al., 2012).

Nearly 71% of the earth’s surface is covered by ocean. The marine environment is extremely diverse, with features such as cold seeps, hydrothermal vents, abyssal plains, oceanic trenches, seamounts, oceanic volcanoes and deep-sea whale falls, as well as varied conditions of pressure, salinity, temperature, nutrient composition and light. Marine microorganisms are a huge untapped source of lipolytic enzymes, especially esterase, with extreme marine environments thus constituting a vast pool of novel lipolytic biocatalysts (Kennedy et al., 2008). As a consequence of this diverse marine environment, various lipolytic enzymes have distinct characteristics, such as thermostability, low temperature activity and heavy metal tolerance.

In our study, we designed primers to directly screen the esterase genes from the metagenomic DNA of South China Sea deep-sea sediments. The esterase gene scsEst01 was cloned and co-expressed in *Escherichia coli* BL21 (DE3) with the chaperone dnaK-dnaJ-grpE. Our results reveal that scsEst01 is a novel meso-thermophilic alkaline esterase.

### 2 MATERIAL AND METHOD

#### 2.1 Metagenomic DNA extraction and esterase gene cloning

Deep-sea sediments were collected from the northern portion (114°46′46.684′′E, 19°11′11.674′′N) of the South China Sea at a water depth of 1 394 m during the cruise aboard the R/V Ke Xue Yi Hao. This sampling area is generally considered to be a methane-enriched area. Metagenomic DNA was extracted from 0.5 g sediment samples using a PowerSoil DNA Isolation kit (MoBio, West Carlsbad, CA, USA) following the manufacturer’s instructions.

An esterase gene was amplified using metagenomic DNA as a template with the following primers: 5′-GG-AAATTCCATATGGCCAGCCGCAAGCACTTTC-3′ (*Nde* I site underlined) and 5′-CCCAGCCTTTATGGCTGCTGGCCGCTTCCGGGA-3′ (*Hind* III site underlined). The amplified esterase gene was ligated into a pMD18-T vector (TaKaRa, Dalian, China) and transformed into *E. coli* Top10. The pMD18-T-scsEst01 positive clone was sequenced at Shanghai Majorbio Bio-pharm Technology Co. (Shanghai, China).

#### 2.2 scsEst01 sequence analysis

The scsEst01 gene was annotated by a BLASTX search of the NCBI non-redundant protein sequence (nr) database. Multiple alignments between amino acid sequences of lipolytic enzymes were performed using CLUSTAL X (Thompson et al., 1997), with conserved blocks across the aligned sequences visualized with DNAAMAN software. A phylogenetic tree was constructed by the neighbor-joining method using MEGA v4.0 software (Tamura et al., 2007).

#### 2.3 Overexpression with chaperones and purification of scsEst01

The scsEst01 gene was digested by *Nde* I and *Hind* III (MBI Fermentas, Ottawa, Canada) from pMD18-T-scEst01 and cloned into a pET-28a expression vector (Novagen, Billerica, MA, USA) digested with the same enzymes. The pET-28a-scsEst01 recombinant plasmid was transformed into *E. coli* Transenta (DE3) chemically competent cells. After induction with 0.5 mmol/L isopropyl-β-D-thiogalactoside (IPTG) at different temperatures (16, 25, and 28°C) for 8–14 h, the target protein was expressed. Under each of these induction conditions, however, the recombinant protein was generated as an inactive inclusion body.

Chaperone plasmid sets (Takara) pTf16, pKJE7, pG-Tf2, pGro7 and pG-KJE8 combined individually with pET-28a-scsEst01 were co-transformed into *E. coli* BL21 (DE3). The transformants were inoculated in Luria-Bertani (LB) medium with 20 μg/mL chloramphenicol, 50 μg/mL kanamycin, 0.5 mg/mL L-arabinose and/or 5 ng/mL tetracycline at 37°C, and then induced for 8 h with 0.5 mmol/L IPTG at 25°C. The crude lysates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

The chaperone plasmid set pKJE7 (coding dnaK-dnaJ-grpE chaperones) with pET-28a-scsEst01 was selected for medium-scale fermentation. Recombinant *E. coli* cells were cultured in 2.5 L of LB medium containing 20 μg/mL chloramphenicol, 50 μg/mL kanamycin and 0.5 mg/mL L-arabinose at 37°C with 200 r/min shaking. When the OD_600 reached 0.5, the cultures were induced with 0.5 mmol/L IPTG overnight at 25°C. Cells were harvested by centrifugation at 8 000×g for 20 min at 4°C.