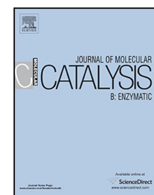
Journal of Molecular Catalysis B: Enzymatic 95 (2013) 1–8

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb



Isolation and characterization of a novel organic solvent-tolerant and halotolerant esterase from a soil metagenomic library

Sidi Wang [a], Kui Wang [b], Liang Li [a], Yuhuan Liu [a,*]

[a] School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, People's Republic of China
[b] Asiapac (Dongguan) Bio-technology Co., Ltd., Dongguan 523808, People's Republic of China

ARTICLE INFO

Article history:
Received 11 November 2012
Received in revised form 17 May 2013
Accepted 17 May 2013
Available online 30 May 2013

Keywords:
Lipolytic enzymes
Metagenomic library
New lipolytic enzyme family
Moderately thermostable
Halotolerant

ABSTRACT

Soil metagenome conceals a great variety of unexploited genes for industrially important enzymes. To identify novel genes conferring lipolytic activity, one metagenomic library comprising of 200,000 transformants were constructed. Among the 48,000 clones screened, 19 clones which exhibited lipolytic activity were obtained. After sequence analysis, 19 different lipolytic genes were identified. One of these genes, designated as *estWSD*, consisted of 1152 nucleotides, encoding a 383-amino-acid protein. Multiple sequence alignment and phylogenetic analysis indicated that EstWSD and its closest homologues may constitute a new family of bacterial lipolytic enzymes. The best substrate for the purified EstWSD among the ρ-nitrophenol esters examined was ρ-nitrophenol butyrate. Recombinant EstWSD displayed a pH optimum of 7.0 and a temperature optimum of 50 °C. This enzyme retained 52% of maximal activity after incubation at 50 °C for 3 h. Furthermore, EstWSD also exhibited salt tolerance with over 51% of its initial activity in the presence of up to 4.5 M NaCl for 1 h. In particular, this enzyme showed remarkable stability in 15% and 30% dimethylsulfoxide, ρ-xylene, hexane, heptane, and octane even after incubation for 72 h. To our knowledge, it is the first report to find a novel esterase belonging to a new lipolytic family and possessing such variety of excellent features. All these characteristics suggest that EstWSD may be a potential candidate for application in industrial processes.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Lipolytic enzymes such as esterase (EC 3.1.1.1) and lipase (EC 3.1.1.3) are extensively distributed in plants, animals and microorganisms, they can catalyze the hydrolysis, synthesis or transesterification of ester bonds, varying in the ability to hydrolyze substrates with different chain length [1,2]. It is generally accepted that esterases hydrolyze short-chain esters (acyl chain length of <10), while lipases act on water-insoluble (acyl chain length of >10) triglycerides [3]. During the past decades, lipolytic enzymes have been drawing worldwide attention due to their great potential in industrial applications, including oleochemical industry, production of biodegradable polymers, detergent industry, food flavoring, waste treatment, oil biodegradation, biodiesel production and so on [1,2]. Most of these reactions proceed in non-aqueous environments, and one main problem existing in the application of lipolytic enzymes is that they are less stable and active in the presence of organic solvents [4,5]. Therefore, lipolytic enzymes with organic solvent-tolerance are in great demands and have attracted worldwide attention from scientists and industrialists [5,6]. To date, most of the microbial lipolytic enzymes, including organic solvent-tolerant lipolytic enzymes, were obtained by traditional cultivation techniques [2], but more than 99% of microorganisms in the environment are not readily culturable [7]. This yet uncultured microorganisms, adapted to different environments, potentially conceal an enormous treasure of untapped genes for biotechnologically important biocatalysts [8]. As a cultivation-independent approach, metagenomics serves as an effective tool for studying the whole microbial community within varieties of environments, including both cultured and uncultured microorganisms [8,9]. Since its introduction, a significant number of biotechnologically important enzyme-coding genes have been identified [8,10–15].

Soil microorganisms have been the most valuable source of natural products, providing industrially important antibiotics and biocatalysts. The microbial diversity in soil surpasses that of other environments and is far greater than that of eukaryotic organisms [16]. The quantity of microbes existing in 1 g of soil may up to 10 billion and they may possibly represent thousands of different species [17]. In this study, we constructed a metagenomic library from Turpan Basin soil and screened for genes encoding lipolytic activity. 19 new lipolytic genes were obtained by using activity-based screening. A novel esterase, EstWSD, shows no significant similarity to any known enzymes except hypothetical proteins. Based on

* Corresponding author. Tel.: +86 20 84113712; fax: +86 20 84036215.
E-mail addresses: wangsd0801@126.com (S. Wang), uk0829@126.com (K. Wang), liliangsemail@163.com (L. Li), lsslyh@mail.sysu.edu.cn (Y. Liu).

1381-1177/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.molcatb.2013.05.015

multiple sequence alignment and phylogenetic analysis, we can draw the conclusion that EstWSD and its closest homologs may constitute a new family of lipolytic enzymes. The target gene (*estWSD*) was subcloned and expressed in *E. coli* BL21(DE3). The recombinant enzyme was purified to homogeneity and its biochemical properties were partially characterized.

2. Experimental

2.1. Bacterial strains, plasmids, and materials

E. coli strains DH5α and BL21(DE3) (Novagen, Madison, WI) were used as host strains for gene cloning and protein expression, respectively. The pUC118 (TaKaRa, Dalian, China) was employed to construct metagenomic library. pET-32a(+) (Novagen, Madison, WI) was used to express the target protein. Restriction enzymes, T4 DNA ligase and DNA polymerase were purchased from Takara (Dalian, China). ρ-Nitrophenol (ρ-NP) esters were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and reagents were of analytical grade and were obtained from commercial sources, unless indicated otherwise.

2.2. DNA extraction from environmental samples

Turpan region sits in a desert basin at the southern foot of the Tian Mountains and has a continental desert climate, as a result, the main characteristics of the Turpan Basin soil are desertification and salinization. For the construction of metagenomic library, a soil sample from the Turpan Basin (42°56′N, 89°11′E) was collected and stored at −80 °C until the DNA extraction was performed. The total DNA was extracted using the direct method developed by Zhou et al. [18] with slight modification.

2.3. Metagenomic library construction and functional screening for lipolytic gene

The purified DNA was partially digested with *Bam*H I, DNA fragments of approximately 2.5–10 kb were pooled and ligated into pUC118, which had been previously digested with *Bam*H I and dephosphorylated. The ligated product was purified and transformed into *E. coli* DH5α via electroporation. The transformants were plated onto Luria–Bertani (LB) agar plates containing 100 μg mL^{-1} ampicillin, 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG), 8 μg mL^{-1} rhodamine B, and 1% emulsified tributyrin [19]. After cultivation at 37°C for 2–4 days, clones showing lipolytic activity were identified by the formation of clear zones (halos) around the colonies. Then these clones were selected and further tested for the ability to hydrolyze ρ-NP acetate. 19 transformants with lipolytic activity were obtained and reconfirmed.

2.4. Lipolytic gene analysis and phylogenetic tree construction

The recombinant plasmids derived from all 19 positive clones were sequenced by Shanghai Invitrogen Biotechnology Co., Ltd. ORFs in each sequence were identified using ORF FINDER program (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). A database homology search was performed with BLAST program (http://blast.ncbi.nlm.nih.gov) provided by National Center for Biotechnology Information (NCBI). Multiple sequence alignment was performed using CLUSTAL W (http://www.ebi.ac.uk/clustalw/) [20], in combination with GENEDOC (http://www.psc.edu/biomed/genedoc). Phylogenetic tree was generated using neighbor joining method with MEGA 5.0 software [21]. Bootstrapping based on 1000 replicates was used to evaluate the reliability of the tree [22].

2.5. Expression and preparation of the recombinant esterase/lipase

The lipolytic gene *estWSD* from positive clone was amplified via PCR with the primer of estWSD-f (5′-CTGATATC-GGATCCATGGGCTTCACCGCGCGCGTGGC, in which the *Bam*H I site is underlined) and estWSD-r (5′-CCGTCGACAAGCTTTCACC-GCTCGAGAAACGCCGCCAGC, in which the *Hin*d III site is underlined). The PCR fragments were digested with *Bam*H I–*Hin*d III and ligated with *Bam*H I–*Hin*d III treated expression vector pET32a(+). The ligation product was transformed into *E. coli* BL21(DE3) and the transformants were plated onto LB agar plates supplemented with 100 μg mL^{-1} ampicillin. After confirming by sequencing, the correct recombinant clone was inoculated to 100 mL of LB medium supplemented ampicillin and grown to an OD_{600} of 0.8 at 37 °C with agitation, then the culture was added with 0.5 mM IPTG to induce the expression of the target gene. After cultivation continued at 30 °C for 6 h with agitation, the cells were harvested at 12,000 × *g* for 2 min and resuspended in ice-cold lysis buffer (100 mM phosphate buffer, pH 7.0). The suspension was subjected to ultrasonic treatment and the extract was centrifuged at 12,000 × *g* for 5 min at 4 °C, and the supernatant was purified using His Bind Purification Kit (Novagen, Madison, WI) by following the instructions of the manufacturer. The purified enzyme was stored in phosphate buffer (100 mM, pH 7.0) at 4 °C and remained fully active throughout. Protein concentrations were determined according to the method of Bradford [23] using bovine serum albumin as standard. The purified enzyme solutions were employed for the lipolytic activity assay.

2.6. Molecular mass determination

The subuint molecular mass of EstWSD was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions, using the protein molecular weight marker (Takara, Dalian, China) as reference proteins. All protein bands were stained with Coomassie brilliant blue (CBB) G-250 for visualization.

2.7. Analysis of substrate specificity

To analyze the lipolytic activity of recombinant EstWSD, ρ-NP esters of fatty acids (C_2, ρ-NP acetate; C_4, ρ-NP butyrate; C_6, ρ-NP caproate; C_8, ρ-NP caprylate; C_{10}, ρ-NP caprate; C_{12}, ρ-NP laurate; C_{14}, ρ-NP myristate; C_{16}, ρ-NP palmitate; C_{18}, ρ-NP stearate) were used as substrates. The reaction mixture was composed of 10 μL of the enzyme solution, 10 μL of 50 mM ρ-NP esters dissolved in acetonitrile and 480 μL of phosphate buffer (100 mM, pH 7.0). After incubation at 37 °C for 5 min, absorbance of ρ-NP was measured at 405 nm using a spectrophotometer. One unit (U) of lipolytic activity was defined as the amount of enzyme needed to produce 1 μmol of ρ-NP min^{-1} under the assay condition.

2.8. Effect of pH and temperature on enzymatic activity and stability

For the determination of optimum pH of the enzyme, lipolytic activity was measured in a pH range of 5.0–10.0 using ρ-NP butyrate (C_4) as substrate. The following buffers were used: 100 mM sodium acetate (pH 5.0–6.0), 100 mM phosphate (pH 6.0–9.0), 100 mM glycine–sodium hydroxide (pH 9.0–10.0). Further study on the pH stability of the enzyme was carried out by incubating the enzyme solutions at 4 °C for 1–5 h in the buffer system mentioned above in the absence of substrate. The optimum temperature for the

Download English Version:

https://daneshyari.com/en/article/6531267

Download Persian Version:

https://daneshyari.com/article/6531267

Daneshyari.com