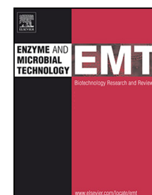
Enzyme and Microbial Technology 85 (2016) 1–11

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

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



Characterization of a novel cold active and salt tolerant esterase from *Zunongwangia profunda*

Mohammad Asadur Rahman [a], Umma Culsum [a], Wenhao Tang [b], Shao Wei Zhang [a], Gaobing Wu [c], Ziduo Liu [a,*]

[a] *State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, People's Republic of China*
[b] *Hubei Vocational College of Bio-technology, Wuhan 430070, People's Republic of China*
[c] *College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, People's Republic of China*

ARTICLE INFO

Article history:
Received 16 August 2015
Received in revised form
18 December 2015
Accepted 29 December 2015
Available online 4 January 2016

Keywords:
Novel esterase
Cold-active
Salt tolerant
Organic solvent tolerant

ABSTRACT

A novel cold active esterase, EstLiu was cloned from the marine bacterium *Zunongwangia profunda*, over-expressed in *E. coli* BL21 (DE3) and purified by glutathione-S transferase (GST) affinity chromatography. The mature esterase EstLiu sequence encodes a protein of 273 amino acids residues, with a predicted molecular weight of 30 KDa and containing the classical pentapeptidase motif from position 156 to 160 with the catalytic triad Ser158-Asp211-His243. Although, EstLiu showed 64% similarity with the hypothetical esterase from *Chryseobacterium* sp. StRB126 (WP_045498424), phylogenetic analysis showed it had no similarity with any of the established family of lipases/esterases, suggesting that it could be considered as a new family. The purified enzyme showed broad substrate specificity with the highest hydrolytic activity against *p*-nitrophenyl butyrate (C4). EstLiu showed remarkable activity (75%) at 0 °Cand the optimal activity at pH 8.0 and 30 °C with good thermostability and quickened inactivation above 60 °C. EstLiu retained 81, 103, 67 and 78% of its original activity at 50% (v/v) in ethanol, isopropanol, DMSO and ethylene glycol, respectively. In the presence of Tween 20, Tween 80 and Triton X-100, EstLiu showed 88, 100 and 117% of relative activity. It is also co-factor independent. The high activity at low temperature and desirable stability in organic solvents and salts of this novel family esterase represents a good evidence of novel biocatalyst. Overall, this novel enzyme showed better activity than previously reported esterases in extreme reaction conditions and could promote the reaction in both aqueous and non-aqueous conditions, indicating its great potential for industrial applications.

© 2016 Published by Elsevier Inc.

1. Introduction

Microorganisms maintain the biogeochemical cycles of earth and environment and marine microorganisms contain phylogenetic diversity with distinctive genetic information because of extreme ecological conditions of the marine environment [1]. Additionally, marine microorganisms are also a great potential source of novel enzymes.

Esterases (EC 3.1.1.1) and lipases (EC 3.1.1.3) are lipolytic enzymes widespread in nature and very crucial in biotechnology. They both belong to the α/β hydrolase superfamily as well as serine hydrolase family. The catalytic triad is mostly arranged in the order of Ser-Asp-His and the serine residue has a consensus sequence of Gly-X-Ser-X-Gly, known as nucleophilic elbow [2–4]. Esterases could be differentiated from lipases by the preferences of substrates as esterases hydrolyze short chain fatty acids no more than 10 carbons and lipases prefer water insoluble long chain fatty acids with more than 10 carbons [5,6].

Enzymes with novel characteristics are in great demand these days owing to their numerous biotechnological applications. Industrially used lipolytic enzymes are mostly derived from bacteria and fungi and they account for a great percentage of industrial enzymes with a global market about billions of dollars a year [7]. Esterases have a broad spectrum of industrial uses including food and dairy products, detergents, pharmaceuticals, the synthesis of optically pure compounds, degradation of pollutants and production of perfumes [7,8].

* Corresponding author.
E-mail addresses: nayongeb@gmail.com (M.A. Rahman), eshageb@gmail.com (U. Culsum), 530278919@qq.com (W. Tang), zhangzhsw@163.com (S.W. Zhang), wgb@mail.hzau.edu.cn (G. Wu), Lzd308@yahoo.cn, lzd@mail.hzau.edu.cn (Z. Liu).

http://dx.doi.org/10.1016/j.enzmictec.2015.12.013
0141-0229/© 2016 Published by Elsevier Inc.

Esterases with novel properties are desirable in various aspects for industrial applications. Cold active esterases can not only increase the industrial perform efficiently at low temperature, but also save energy with minimum energy use [9–12]. Esterases active in organic solvents are desirable when substrates are insoluble to water and they are also necessary for biodiesel production through *trans* esterification reaction [13]. Salt tolerant esterase could catalyze the reactions and degrade organic matters under high salt concentrations [14–16]. Till now, very few reports are available on esterases with novel characteristics such as cold active, salt tolerant and organic solvent tolerant [16–25], because an esterase with a combination of many characteristics is desirable but rare.

In this study, we have reported the molecular cloning, purification and characterization of a novel family esterase from the marine bacterium *Zunongwangia profunda.* This enzyme shows not only high activity at low temperature but also tolerance to salt and organic solvents. It is also cofactor independent. This enzyme would enrich the results of lipolytic enzyme research and have potential biotechnological uses with its multiple extreme properties.

2. Material and methods

2.1. Materials

The *p*-nitrophenyl esters: *p*-nitrophenyl acetate (*p*NPA, C2), *p*-nitrophenyl butyrate (*p*NPB, C4), *p*-nitrophenyl hexanoate (*p*NPH, C6), *p*-nitrophenyl caprylate (*p*NPC, C8), *p*-nitrophenyl laurate (*p*NPL, C12), and *p*-nitrophenyl palmitate (*p*NPP, C16) were purchased from Sigma-Aldrich (St Louis, MO, USA). Taq DNA polymerases, T4 DNA ligases, restriction enzymes, and DNA markers were purchased from TaKaRa (Dalian, China). The primers and fragments were synthesized and sequenced, by TSINGKE Co. (Wuhan, China). Gel purification kit was purchased from AXYGEN (USA). All the other chemicals and buffers used were of high purity and analytical grade.

2.2. Strains, vectors and culture conditions

The marine bacterium *Z. profunda* is an aerobic, gram negative bacterium was isolated from the surface seawater in the coastal area of Fujian, China and was grown in high-salt Luria-Bertani medium (HLB) (peptone 1%, yeast extract 0.5%, NaCl 2%) at 26 °C. *E. coli* strains DH5α and BL21 (DE3) were used as hosts for gene cloning and protein expression, respectively. The plasmid pGEX-6P-1 (GE Healthcare, USA) was used as vector for gene cloning and protein expression.

2.3. Gene cloning

The putative esterase coding gene EstLiu encodes a protein (GenBank Accession No. ADF51938.1) with signal peptide. So the mature EstLiu coding gene was amplified using the genomic DNA of *Z. profunda* as template with the following primers, EstLiu F: 5′-CGCGGATCC CAAGATGAGGTGATCAAACTTTACGAC-3′ and EstLiu R: 5′-CCGCTCGAGTTAT TTATTGAGGTATTTATATTG-3′ with restriction enzyme sites of *Bam*HI and *Xho*I, (underlined) respectively. PCR was performed in a thermal cycler programmed with 95 °C for 30 s, 30 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 45s and a final elongation of 72 °C for 10 min. Then the PCR products were purified using 1% agarose gel using gel mini purification kit according to the manufacturer's instructions (AXYGEN, USA). Vector and purified PCR product was digested with *Bam*HI and *Xho*I and was purified using gel mini purification kit (AXYGEN, USA). The digested and purified gene was cloned into the digested and purified pGEX-6P-1 vector using T4 DNA ligase and transformed into the competent *E. coli* DH5α cells, which were grown in LB medium supplemented with 100 μg/ml of ampicillin at 37 °C overnight. After the recombinant plasmids were extracted, the correct insert of the plasmid was confirmed by sequencing, and the recombinant plasmid of pGEX-6P-1-EstLiu was used for further study.

2.4. Heterologous expression and purification

The recombinant plasmid pGEX-6p-1-EstLiu was transformed into *E. coli* BL21 (DE3) competent cells for expression. *E. coli* BL21 (DE3) cells carrying the recombinant plasmid pGEX-6P-1-EstLiu were grown in the liquid LB medium containing 100 μg/ml ampicillin at 37 °C overnight. Then the overnight grown cells were inoculated at 1% in fresh LB liquid medium containing 100 μg/ml ampicillin and then grown at 37 °C until the OD_{600} reached 0.6, followed by the addition of 1 mM (final concentration) IPTG was added and cultured in a shaking incubator for 15 h at 18 °C and 180 rpm. Next, the cells were collected and washed twice with PBS buffer (0.8% NaCl, 0.02% KCl, 0.142% Na_2HPO_4, 0.027% KH_2PO_4; pH 7.4) by centrifugation at 8000 rpm for 10 min and again resuspended in PBS (pH 7.4). After that, the cells were disrupted by a French press and the crude enzyme was obtained by centrifugation at 12000 rpm for 40 min at 4 °C. Finally, glutathione-S transferase (GST)-tagged esterase GST-EstLiu was purified according to manufacturer's instructions through affinity chromatography using Glutathione Sepharose 4B columns (GE Healthcare, USA). The GST tag was removed by digestion with a 3C protease solution (PreScission; Pharmacia) and the purified protein was eluted with 1 ml PBS (pH 7.4). The quantification of the protein was determined using the Bradford reagent (Sigma, USA) with bovine serum albumin (BSA) as a standard [26] and the proteins were analyzed by 12% SDS-PAGE which revealed the pure protein with a molecular weight of about 30 KDa.

2.5. Sequence analysis

To detect the presence and cleavage site of the signal peptide, SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/) was used. To study the localization of EstLiu, Cello, http://cello.life.nctu.edu.tw/, and Cell-PLoc (http://www.csbio.sjtu.edu.cn/bioinf/Gneg/), databases were used. The sequence similarity was examined by Basic Local Alignment Search Tool (BLAST) program from the server at the National Centre of Biotechnology, USA (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignment was performed using the Clustal W Method in the BioEdit Sequence Alignment Editor program. Phylogeny analysis was carried out using the sequences of previously determined families of esterases/lipases [5] with MEGA 6.0 program [27].

2.6. Enzyme activity assay

One unit of esterase activity was determined by the production of 1 μmol of *p*-nitrophenol from *p*-nitrophenyl butyrate in 1 min. The volume of each standard reaction mixture was 200 μl consisting of 4 μl of substrate (25 mM), 2 μl of appropriately diluted pure enzyme (20 μg/ml) and 194 μl Tris–HCl buffer (50 mM, pH 8.0), and reaction mixture without the addition of any enzyme was considered as control. The reaction continued for 5 min at 30 °C and the absorbance of the released *p*-nitrophenyl was measured at 405 nm in a 96-well plate using a Thermo Scientific Multiscan Spectrum Spectrophotometer [19,20].

2.7. Substrate specificity assay

The substrate specificity of EstLiu was studied with the *p*-NP esters with different acyl chain lengths from C2 to C16 using the standard method. The substrates used were *p*-nitrophenyl acetate

Download English Version:

https://daneshyari.com/en/article/16864

Download Persian Version:

https://daneshyari.com/article/16864

Daneshyari.com