Biochemical and Biophysical Research Communications 437 (2013) 342–348

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Functional characterisation of a metagenome derived family VIII esterase with a deacetylation activity on β-lactam antibiotics

Nobalanda Mokoena [a], Kgama Mathiba [a], Tsepo Tsekoa [a], Paul Steenkamp [a,b], Konanani Rashamuse [a,*]

[a] CSIR Biosciences, Scientia Campus, P.O. Box 395, Pretoria 0184, South Africa
[b] Biochemistry Department, University of Johannesburg, Auckland Park 2006, South Africa

ARTICLE INFO

Article history:
Received 14 June 2013
Available online 1 July 2013

Keywords:
Functional metagenomics
Family VIII carboxylesterase
β-Lactam substrate

ABSTRACT

Family VIII esterases represent a poorly characterised esterase family, with high sequence identity to class C β-lactamases, peptidases and penicillin binding proteins. This study reports on the metagenomic library screening and biochemical characterisation of a novel esterase (Est22) derived from an acidic Leachate environment. The enzyme is 423 amino acids in length and contained 22 aa signal peptide. The Est22 primary structure revealed the presence of N-terminus S-x-x-K sequence, which is also highly conserved in class C β-lactamases, peptidases as well as carboxylesterases belonging to family VIII. Phylogenetic analysis using the representative sequences from class C β-lactamases and family VIII esterases indicated that Est22 is a member of family VIII esterases. Substrate specificity profiling using p-nitrophenyl esters (C2–C16) indicated that Est22 preferred shorter chain p-nitrophenyl esters (C2–C5), a characteristic that is typical for true carboxylesterases. In addition of hydrolysing Nitrocefin, Est22 also hydrolysed first generation cephalosporin based derivatives. Detailed selectivity study using different cephalosporin based substrates indicated that Est22 selectively hydrolyse the ester bond of a cephalosporin derivatives leaving the amide bond of the β-lactam ring intact. The selective nature of Est22 makes this enzyme a potential candidate for the use in the synthesis and modification cephalosporin based molecules.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Microbial lipolytic enzymes comprise of two enzyme groups, namely lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) and carboxylesterases (carboxylester hydrolases, EC 3.1.1.1) [1]. Lipases catalyse the hydrolysis of triacylglycerols with subsequent release of diacylglycerols, monoacylglycerols, free fatty acids and glycerol [2]; while carboxylesterases catalyse the hydrolysis of ester bonds of carboxyl ester molecules to form an alcohol and carboxylic acid [3]. Currently, microbial lipolytic enzymes (including both esterases and lipases) are classified into eight families (Family I–VIII) [1]. This classification scheme is based on a comparison of amino acid sequences and some fundamental biological properties of lipolytic enzymes of different origin.

Family VIII esterases (F-VIII-Ests) represent an ill-defined family with high sequence identity to class C β-lactamases, peptidases, and penicillin binding protein [1]. Like all other reported carboxylesterases, all reported members belonging to F-VIII-Ests are serine hydrolases [2]. The primary structures of these enzymes contain a highly conserved S-x-x-K motifs (where x-denotes any amino acid) located at the N-terminus part of the primary structure. This S-x-x-K sequence is also highly conserved in a number of peptidases [4] and β-lactamases [5] and the serine residue within this sequence has been shown to be catalytic nucleophile residue. In F-VIII-Ests, a number of site directed mutagenesis studies have shown that the serine residue located within the S-x-x-K motif is also responsible for catalysis [6,7].

Despite high sequence identity to class C β-lactamases, most members of family VIII esterases reported to date lack activity against standard β-lactam substrates. The exception is with EstB [7] and EstU1 [8] which in addition of hydrolysing Nitrocefin (a chromogenic substrate used to determine β-lactamase activity) also exhibits β-lactamase activity towards cephalosporin based β-lactam substrates. In this study we report on the functional characterisation of an esterase (Est22) which selectively hydrolyses the ester bond and not the amide bond of the β-lactam substrates.

2. Materials and methods

2.1. Metagenomic library screening

A metagenomic library used for screening esterase activities was previously constructed from an aqueous acidic leachate (pH 4.2) collected from the Chloorkop landfill site East of Johannesburg South Africa (26°03′17.50″S) using the EpiFOS™ Fosmid Library

* Corresponding author.
E-mail address: KRashamuse@csir.co.za (K. Rashamuse).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.06.076

Production Kit (Epicentre Biotechnologies, USA) [9]. Functional screening of the recombinant esterase positive clones in *Escherichia coli* EPI100-T1^{R} was performed on LB agar plates supplemented with isopropyl-β-D-thiogalactopyranoside (IPTG, 0.1 mM), chloramphenicol (12.5 μg ml^{-1}), tributyrin 1% (v/v) and Gum Arabic 0.1% (w/v), followed by incubation at 37 °C. Esterase positive clones were identified by the presence of zone of clearance around the colony margins.

2.2. DNA manipulation and sequencing

Recombinant DNA techniques were carried out as described by Sambrook and Russell [10]. DNA sequencing using 454-pyrosequncing technology and oligonucleotide synthesis services were provided by Inqaba Biotech (South Africa). Sequence analysis and manipulation were performed using CLC Combine Workbench software (CLCBIO, Denmark) and Bioedit [11] with the aid of BLASTP search [12]. The signal peptide predictions were conducted using SignalP 3.0 server located at http://www.cbs.dtu.dk/services/SignalP/ [13].

2.3. Phylogenetic analysis

The evolutionary relationship between Est22, family VIII esterases and class C β-lactamases was inferred using the Neighbor-Joining method conducted with MEGA5 [14]. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method [15], and are in the units of the number of amino acid substitutions per site.

2.4. Recombinant protein expression and purification

The est22 gene was amplified with and without signal peptide using F8F/F8R and F8FF/F8R primer pairs, targeting the full length and truncated (without leader peptide) version of the gene respectively (Supplementary Data: Table S1). Both primer pairs introduced the *Nde*I and *Xho*I sites at the 5′- and -3′ end of the gene respectively. Amplified PCR products were digested with *Nde*I/*Xho*I, followed by ligation into pET28a linearised with the same enzymes. The recombinant *est*22 gene (with and without leader peptide) was placed in-frame with the 6x-His tag sequence at the 3′-end of the gene. Expression vectors were propagated in *E. coli* BL21 (DE3) cells and the recombinant clones were selected on Kanamycin (50 μg/ml). Protein Expression studies were performed using the EnBase technology (BioSilta, Finland). The soluble esterase (Est22) protein fraction was loaded onto the immobilised metal affinity column (IMAC) packed with Protino Ni-TED resin (Macherey-Nagel, Germany). The bound Est22 was eluted with 250 mM imidazole. Eluted fractions were passed through VIVASPIN 10 kDa cut-off spin columns (Vivascience, U.K.) for imidazole removal and protein concentration. Protein concentration of the purified samples was determined by the Bradford [16] method, using bovine serum albumin (BSA) as a standard, while the purity of the samples was analysed on denaturing sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) [17]. Molecular weight of Est22 was determined with Superdex™ 200 10/30 GL using 30% Acetonitrile with 0.1% TFA buffer. The gel filtration standard (Bio–Rad, USA) contained the mixture of thyroglobulin, γ-globulin, ovalbumin, myoglobin, and vitamin B12.

2.5. Functional assays

2.5.1. Esterase Assays

Unless otherwise stated all enzyme assays were performed in triplicate. Esterase activity assays were performed by a standard colorimetric method measuring the release of p-nitrophenol from p-nitrophenyl esters at 410 nm [9], using a Beckman DU850 UV/visible spectrophotometer with a temperature controller. Described enzyme activity was measured at 30 °C in 20 mM Tris–HCl, pH 7.5 with 1 mM p-nitrophenyl butyrate (dissolved in isopropanol) as the substrate. The extinction coefficient of p-nitrophenol under these conditions was 13,800 M^{-1} cm^{-1}.

2.5.2. β-Lactamase assay

The β-lactam hydrolytic activity of Est22 was determined spectrophotometrically using 0.1 mM Nitrocefin [3-(2, 4 dinitrostyrl)-(6R, 7R-7-(2-thienylacetamido)-ceph-3-em-4-carboxylic acid, E-isomer)] as a substrate (Oxoid kit manual, 6th edition 1990, Unipath Ltd., Basingstoke, UK). The activity of Est22 against non-chromogenic β-lactam included the following substrates: 7-Aminocephalosporanic acid, Cephalosporin C and Cephalotin. The purified Est22 and a positive control β-lactamase from *Bacillus cereus* (Sigma) were incubated with antibiotic substrates at 1 mM in 50 mM Tris–HCl (pH 8.0) for 1 h at 30 °C. The reaction mixtures were analysed by HPLC, using a Hewlett Packard 1100 HPLC (Agilent Technologies Incorporated, Lovedale, CO, USA) equipped with a binary pump autosampler, column oven, UV diode array detector and ChemStation Chromatography Management software (For detailed HPLC conditions, refer to the Supplementary File).

2.7. Biochemical characterisation

To investigate substrate specificity of Est22, enzyme activity was determined using standard esterase assay in the presence of 1 mM of the specified p-nitrophenyl esters of various chain lengths: p-nitrophenyl acetate (C2), p-nitrophenyl butyrate (C4), p-nitrophenyl caprylate (C8) and p-nitrophenyl laurate (C12).

Optimum temperature of the Est22 was determined by measuring the rate of p-nitrophenyl butyrate hydrolysis over a temperature range (30–70 °C). Where necessary the pH adjustments at set temperatures were performed to take into account the effect that changing temperature has on pH.

A thermostability profile of Est22 was generated by incubating the enzyme at three temperature points 30-, 50- and 70 °C, followed by measuring residual activities after every 30 min using the standard esterase assay. The influence of pH on the p-nitrophenyl butyrate hydrolysis was tested from pH 4.0–9.5 at 30 °C.

2.8. Accession number

The esterase (est22) gene nucleotide coding sequence has been deposited in the GenBank under the Accession number: KF052088.

3. Results

3.1. Library screening and sequencing

A fosmid library from leachate environment (library size, average insert size) was previously constructed using a copy-controlled pEpiFOS-5 vector and subsequently screened for esterase positive colonies resulting in one fosmid (FOS8) with esterolytic activity (For detailed screening protocol adopted in this study refer to Supplementary File). In order to locate the gene(s) encoding an esterase phenotype within the pFOS8 fosmid, a random shotgun sequencing of a complete insert DNA was performed.

3.2. Primary structure analysis and phylogenetic classification

The est22 gene (ORF22 which is located at the -3 strand at position 31174-29906) is 1269 pb has a GC content of 52%.

Translational analysis of the ORF22 revealed a polypeptide of 423 amino acids encoding a putative protein of 47 kDa. The est22 also encoded a pre-protein containing a 22 amino acid signal peptide as predicted with SignalP 3.0 [13]. The maximum cleavage site was predicted to be 0.9 between Ala22 and Gln23, which could be cleaved to form a mature protein of 402 aa with predicted molecular weight and pI values of 44.7 kDa and 5.0, respectively.

Deduced Est22 protein sequence revealed the S-M-T-K sequence (amino acid positions 100–103), compatible with the conserved S-x-x-K motif in class C β-lactamases (Fig. 1A) [5], penicillin binding proteins (PBPs) [4]. The Est22 protein was identified as β-lactamase by BALST analysis with the top five highest identity score from *Methylobacterium* sp. (46%, 187/407; ACA19666.1), *Opitutus terrae* (46%, 187/409; ACB74974.1), *Methylobacterium nodulans* (46%, 191/404; ACL60913.1), *Acetobacteraceae bacterium* (45%, 184/409; EHM01916.1) and *Candidatus Koribacter* (43%, 180/423; ABF39642.1). However, Arpigny and Jaeger [1] esterase classification scheme indicated that ORF22 is related to family VIII esterases. Furthermore neighbour joining analysis of Est22 using MEGA indicated that this enzyme is a member of family VIII esterases, as it clustered with reported sequences belonging to this family (Fig. 1B).

3.3. Est22 expression and purification

Recombinant Est22 was produced in a biologically active form in the soluble cytoplasmic fraction of *E. coli* cells using EnBase technology with EnPresso™ tablet cultivation set based on the principle described by Krause et al. [19], which allows an enzymatic slow release of nutrients. The intracellular Est22 enzyme was purified in a single step IMAC procedure since the expression construct was designed to allow recombinant protein to be fused with the C- terminal 6× histidine tag (Fig. 2A). The molecular mass of the purified Est22 was estimated to be 45 kDa, consistent with the estimated molecular weight calculated from the translated nucleotide sequence of the mature protein. The monomeric size of Est22 (45 kDa) was within the 42–45 kDa range which has been reported for other family VIII esterases [6–9]. Est22 was purified

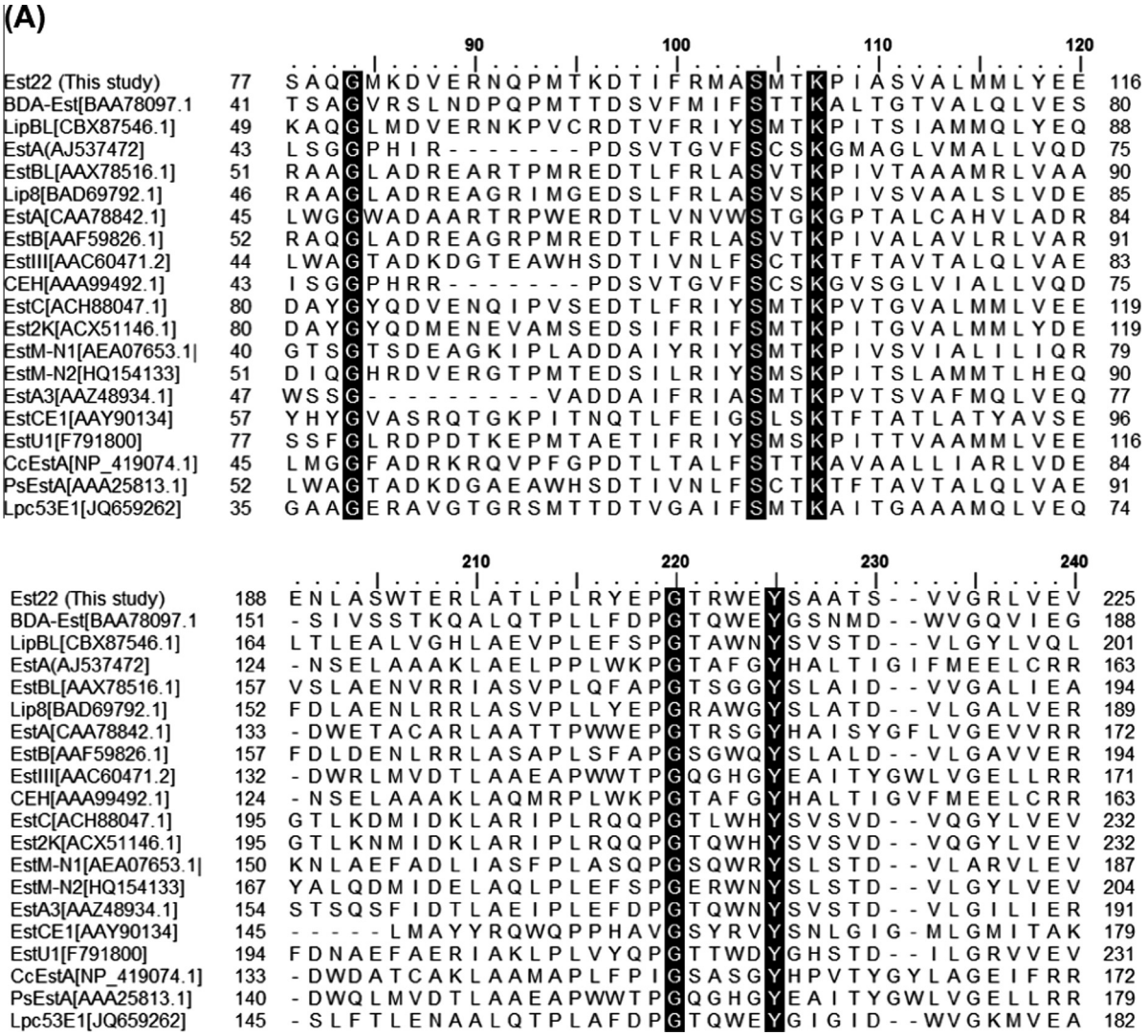

Fig. 1. (A) Multiple alignment of Est22 with related family VIII esterases and class β-lactamases showing the S-X-X-K motif and other conserved residues (conserved residues are shaded in black). Family VIII carboxylesterases are represented by BDA-Est, LipBL, EstA, EstBL, Lip8, EstB, EstIII, CEH, EstC, Est2K, EstM-N1 EstM-N2, EstA3, EstCE1, pLR1 and EstU1 (accession numbers; (BAA78097.1, CBX87546.1, AJ537472, AAX78516.1, BAD69792.1, AAF59826.1, AAC60471.2, AAA99492.1, ACH88047.1, AEA07653.1, HQ154133, AAZ48934.1, AAY90134, AEM45130.1 and F791800, respectively). Beta-lactamases are represented by CcEstA, PsEstA and Lpc53E1 (accession numbers; NP419074.1, AAA25813.1 and JQ659262, respectively). (B) The evolutionary history of Est22 in relation to family VIII esterases and class C β-lactamases inferred using the Neighbor-Joining method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances and the percentage of replicate trees in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances are in the units of the number of amino acid substitutions per site.

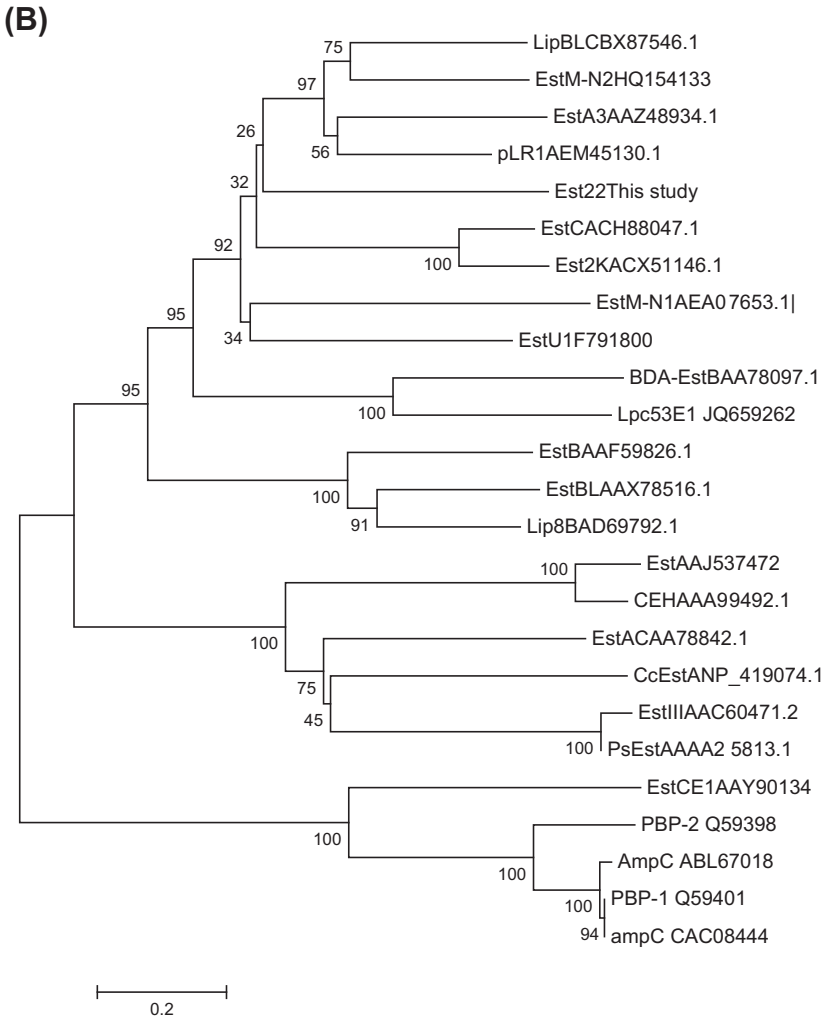

Fig. 1 (*continued*)

with 2-fold enrichment and a yield of 87% and specific activity of 60.04 U/mg. A zymogram assay involving the staining of PAGE gel α-naphthol acetate substrate solution in the presence of fast blue B dye exhibited single dark-red protein band (Fig. 2B), indicating the biological activity of the purified Est22. However, the size of Est22 band on the native gel was migrating at higher molecular weight than the calculated subunit. This necessitated investigation of the globular structure of Est22 using size exclusion chromatography. Based on the analytical size exclusion molecular weight of Est22 was 153 kDa, suggesting a global trimetric structure of Est22 (Supplementary Data: Fig. S1).

3.4. Biochemical characterisation

Substrate specificity: When hydrolytic activity towards p-nitrophenyl esters was examined, Est22 enzyme showed the highest activity with p-nitrophenyl butyrate (Fig. 3A). Activity towards medium- and long-chain acyl substrates was poor substrates; Est22 showed no activity against p-nitrophenyl ester substrates with acyl chains C10 and longer (Fig. 3A).*pH, temperature and thermostability profile*: Kinetic parameters were determined with p-nitrophenyl acetate, and Est22 had the optimal temperature and pH at 30 °C (data not shown) and 8 (Fig. 3B), respectively. The enzyme was not active at higher temperatures. Thermostability studies revealed that the Est22 enzyme is thermo-labile with an estimated half-life of 30 min at temperatures 50 °C and higher (Supplementary data: Fig. S2).

β-Lactamase activity: Deduced Est22 protein sequence revealed the S-x-x-K motif which is conserved in class C β-lactamases. Therefore Est22 β-lactam hydrolytic activity was preliminarily determined using Nitrocefin as the substrate. Est22 showed low β-lactam activity against Nitrocefin as evidenced by slow colour change from yellow to red (data not shown). Hydrolytic activity of Est22 against nitrocefin promoted further investigation against non-chromogenic β-lactam cephalosporin derivatives. Est22 showed activity towards these three cephalosporin derivatives. While the HPLC profile of the β-lactamase positive control indicated hydrolysis of the amide bond of the β-lactam ring, the Est22 profile indicated that the enzyme is hydrolysing a different bond of the three β-lactam cephalosporin derivatives. HPLC spectra showed that retention times obtained with the β-lactamase positive control and the Est22 reactions were different (Fig. 4A–C). These results suggested that Est22 is hydrolysing only the ester bond on the substrates and not the amide bond.

To validate the results acetic acid was measured. Results of the acetic acid production were inconclusive and this necessitated the use of more reliable and conclusive technique. The reaction mechanisms of Est22 against the cephalosporin derivatives were analysed by UPLC-TOF MS analysis. Cephalosporin alcohols were identified from all the reactions indicating that Est22 selectively hydrolyse the ester bond and not the amide bond (Supplementary

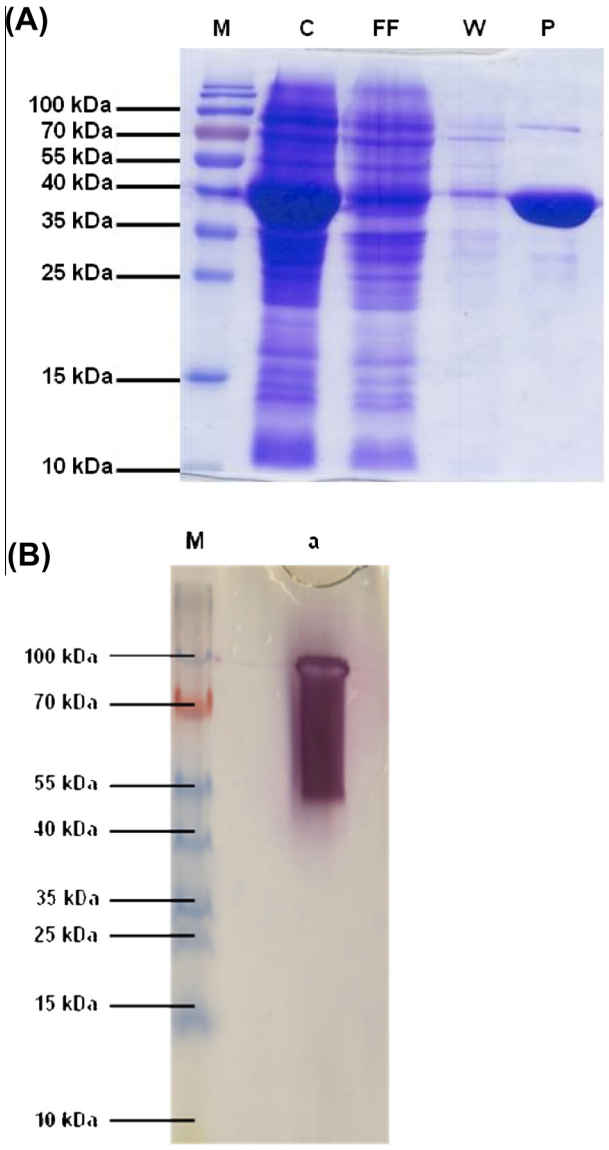

Fig. 2. Denaturing SDS–PAGE and native PAGE for activity analysis, (A) IMAC purification of Est22, crude sample is represented on lane 1, the flow through and the wash fractions on lanes 2 and 3, respectively and the purified Est22 is on lane 4. (B) Purified Est22 zymogram, lane (a) indicate Est22 stained with α-naphthol acetate/fast blue B-stained and lanes M on both gels represents protein ladders. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

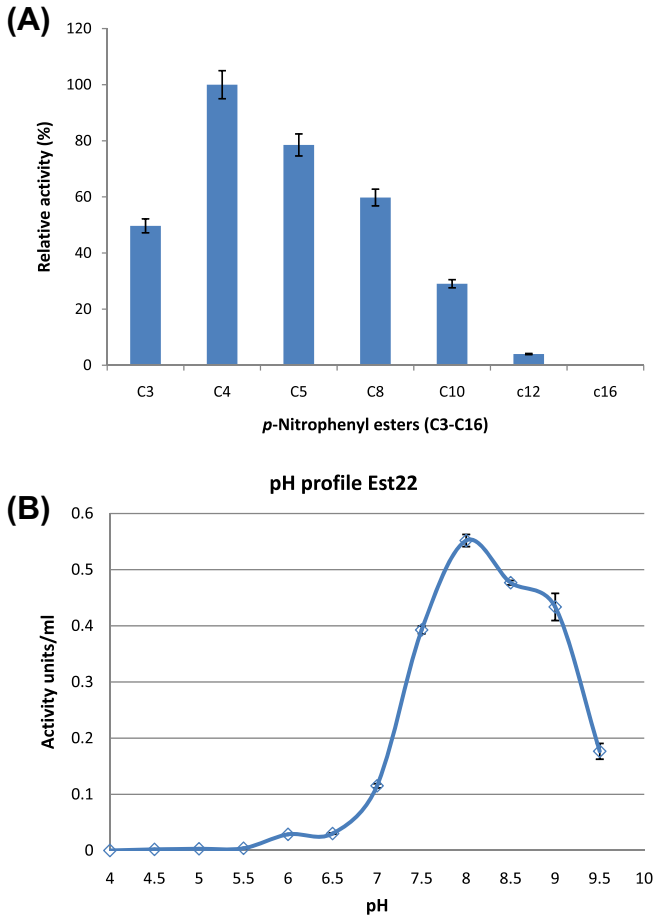

Fig. 3. Biochemical characterisation of recombinant Est22: (A) The Est22 substrate specificity (B) thermostability profiling using p-nitrophenyl butyrate as a substrate. (C) Effect of pH on Est22 activity using p-nitrophenyl butyrate as a substrate.

data: Fig. S3). The MS data clearly revealed that Est22 was attached the ester bond of cephalosporin derivatives leaving the amide bond.

4. Discussions

Traditionally esterase gene candidates from the environment have been discovered using culture enrichment approaches [20]. The classical culture enrichment approaches, generally involve the cultivation of microorganisms and the subsequent screening of the pure strains for the desired catalytic activity [21]. This is typically achieved by simply including the substrate of interest in the agar plate, usually tributyrin (1% v/v) for screening of esterase activities and olive oil and rhodamine B for lipase activities [22].

In recent years, a number of culture-independent approaches have been developed to access the diversity of novel esterase gene sequences that fit a particular industrial performance profile [21]. These include strategies that involve a combination of functional and sequence-based screening approaches designed to rapidly clone and express genes from metagenome libraries [9] and the PCR-based sequence-independent techniques that prospect for novel enzyme genes directly from community DNA [20,21]. In this study we demonstrated the application of functional metagenomic approach in the isolating a novel esterase (Est22) with a primary structure related to C β-lactamase [1], transpeptidases [4] and penicillin binding protein PBPs [5].

The Est22 enzyme was identified as a class C β-lactamase through BLAST analysis. However, a phylogenetic analysis employing amino acid sequences representing class C β-lactamases and family VIII esterases evidently showed that Est22 primary structure clustered together with family VIII esterases representative sequences and not with class C lactamases (Fig. 1B). All previously reported carboxylesterases belonging to family VIII are serine hydrolases, with the catalytic serine residue located within the conserved S-x-x-K motif [3, 7, 8, and 9]. The S-x-x-K motif is also highly conserved in class C β-lactamases [5] and some carboxylpeptidases [4]. In the Est22 primary structure, the corresponding the S-x-x-K motif was represented by the following amino acid residues (S^{100}-M^{101}-T^{102}-K^{103}) (Fig. 1A).

The mechanism of ester hydrolysis involving Ser residue (within the S-x-x-K sequence as a catalytic nucleophile) by F-VIII-Ests was proposed from the crystal structure of EstB from *Burkolderia gladioli* [23]. The EstB crystal structure revealed that during the ester hydrolysis of substrate molecule at least three residues are involving in hydrogen bonding network, namely Ser and Lys residues (which are part of the S-x-x-K motif) and Tyr residue which is highly conserved and located in the middle (position

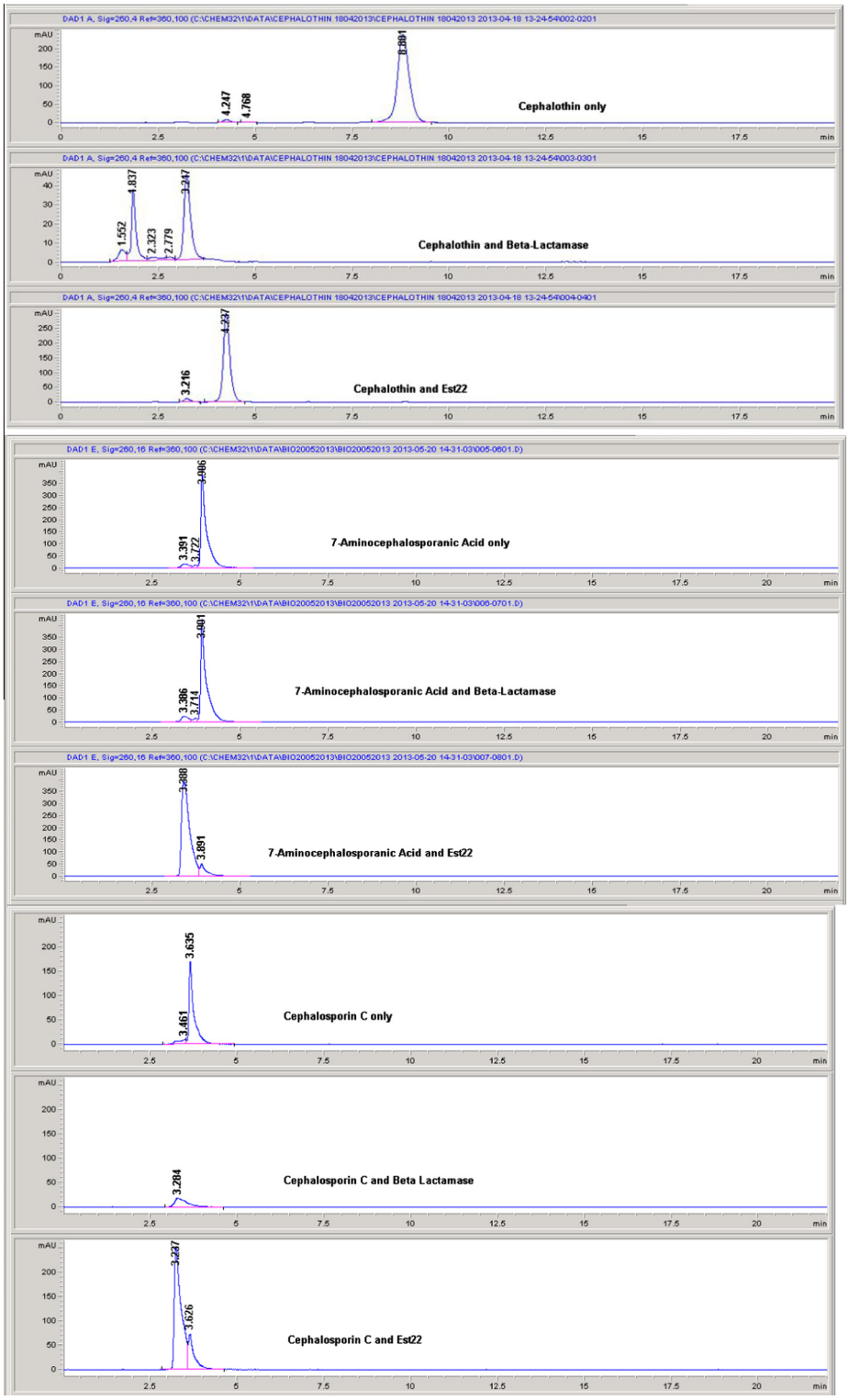

Fig. 4. HPLC analysis of the cephalosporin derivatives reactions performed with β-lactamase and Est22 separately, (A) Cephalothin substrate (B) 7-amino-cephalosporanic acid and (C) Cephalosporin C. Substrates only were also analyzed with the reaction products to compare retention times. Retention times for Cephalothin, 7-amino-cephalosporanic acid and Cepaholosporin were 8.801, 3.906 and 3.635, respectively.

180–200), of the primary structure. Although the roles of serine and Lys residues in the consensus S-x-x-K motif as catalytic nucleophile and base respectively were clearly elucidated in the EstB structure [23], it is the involvement of the Tyr residue which is not yet clear. Multiple sequence analysis based on the published primary structures belong to Family VII revealed that Tyr residue (Tyr 225) is highly conserved in all the primary structures (Fig. 1A). The conserved residue corresponds to Tyr212 in Est22 primary structure. The site directed mutagenesis studies coupled with homology modelling [9,18] proposed that the role of the Tyr residue to be analogous to that proposed for Tyr150 from the X-ray crystal structure of the C. freundii class C β-lactamase [24]. It has been suggested that the Tyr212 residue (in Est22 primary structure) plays a role in the catalytic mechanism of Est22 as a

result of its optimally positioning to act as a proton acceptor for the hydrogen atom of catalytic Ser100, thereby activating Ser100 for nucleophilic attack of the ester carbonyl of the substrate molecule [9]. At the same time, such reaction consequently position Tyr212 favourably to act as a proton donor to the main-chain NH groups of residues Ser100 during the release of the tetrahedral intermediate. Furthermore, the primary structures of some of F-VIII-Est members such as that of EstU1 [8] and EstC [9] also encode 25–29 aa N-terminal leader (secretion signal) peptide. Analysis of Est22 primary structure also revealed the presence of a 22 aa N-terminal leader peptide consistent with the primary structure of EstC, Est2k and EstU1. This observation suggests that Est22 could be an extracellular enzyme that is secreted through the Xep-dependent pathway mechanism mediated by the N-terminal leader peptide [13].

Esterases are generally differentiated from lipase counterparts on the basis of substrate preferences, and they only hydrolyse water-soluble short acyl chain esters of less than 10 carbon atoms [3]. Substrate specificity profiling with p-nitrophenyl esters (pNP-esters) of different carbon chain lengths (C2–C16) revealed that Est22 is a "true" esterases with a strong preference for short acyl chains esters, C4. Due to high sequence identity of Est22 to class C β-lactamases; the enzyme was further investigated for its ability to hydrolyse β-lactam substrates. Some members of family VIII esterases have been reported to exhibit low activity towards nitrocefin (a chromogenic substrate used to determine β-lactamase activity) namely; EstC [9], EstB [7] and EstU1 [8] EstM-N1 and EstM-N2 [25]. However, very few few esterases have been reported to date that catalyse the hydrolysis of β-lactam substrates. The exceptions include the EstB [7] and EstU1 [8] which exhibit hydrolysing activity towards β-lactam substrates. With the EstB enzyme it has been demonstrated clearly that the enzyme selectively deacetylates cephalosporin base substrates leaving the amide bond of the β-lactam ring intact [7]. However with EstU1 it was not clear from the HPLC data if the observed pattern against cephalosporin substrates was due to deacetylation or amide bond hydrolysis of the β-lactam ring. The Est22 enzyme reported in this study was only specific for the ester bond of the β-lactam substrates (7-Aminocephalosporanic acid, Cephalosporin C and Cephalothin).This could be verified by the HPLC spectrum that was clearly different from that of β-lactamase positive control (Fig. 4A–C). Furthermore, the findings were further confirmed by the presence of cephalosporin corresponding alcohols based on the UPLC-TOF MS analysis (Supplementary data: Fig. S3) and none of the products corresponding to the hydrolysis of β-lactam amide were identified.

In this study we have shown conclusively using UPLC-TOF MS analysis that Est22 selectively deacetylate the first generation β-lactam antibiotics by hydrolysing the ester bond and not the amide bond in these β-lactam substrates. Our data is in agreement with that reported by Petersen et al. [7], whereby EstB was also showed to display the same substrate profile as Est22 with respect to the first generation β-lactam antibiotics. However, recently report EstU1 [8] was inconclusively reported to also hydrolyse the amide bond of cephalosporin based derivative substrates in addition of the ester bond. The study by Jeon and co-workers [8] therefore represents the first family VIII esterase to hydrolyse both the amide and ester bonds of the cephalosporin based molecules. In concluding, the selectivity of the Est22 enzyme with a β-lactam fold provides a potential application of this enzyme in the modification of cephalosporin based molecules.

Acknowledgments

The authors would like to thank CSIR and CSIR-Bioscience BIDC members who were actively involved in the stimulating discussions towards conceptualizing this project. We also would like to thank the NRF-NEP as well as the University of Johannesburg who co-funded the purchase of the Waters UPLC-QTOF MS instrumentation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.06.076.

References

[1] K.L. Arpigny, K.E. Jaeger, Bacterial lipolytic enzymes: classification and properties, J. Biochem. 343 (1999) 177–183.
[2] K.E. Jaeger, B.W. Dijkstra, M.T. Reetz, Bacterial biocatalysis: molecular biology, three-dimensional structures, and biotechnological applications of lipases, Annu. Rev. Microbiol. 53 (1999) 315–351.
[3] U.T. Bornscheuer, Microbial carboxylesterases: classification, properties and application in biocatalysis, FEMS Microbiol. Rev. 26 (2002) 73–81.
[4] R.F. Pratt, Substrate specificity of bacterial DD-peptidases (penicillin-binding proteins), Cell. Mol. Life Sci. 65 (2008) 2138–2155.
[5] F.K. Majiduddin, I.C. Materon, T.G. Palzkill, Molecular analysis of beta-lactamase structure and function, Int. J. Med. Microbiol. 292 (2002) 127–137.
[6] D. Pérez, F. Kovacic, S. Wilhelm, et al., Identification of amino acids involved in hydrolytic activity of lipase LipBL from *Marinobacter lipolyticus*, Microbiology 158 (2012) 2192–2203.
[7] E.I. Petersen, G. Valinger, B. Solkner, et al., A novel esterase from *Burkholderia gladioli* shows high deacetylation activity on cephalosporins is related to β-lactamases and DD-peptidases, J. Biotechnol. 89 (2001) 11–25.
[8] H.J. Jeon, S.J. Kim, H.S. Lee, et al., Novel metagenome-derived carboxylesterase that hydrolyzes β-lactam antibiotics, Appl. Environ. Microbiol. 77 (2011) 7830–7836.
[9] K. Rashamuse, V. Magomani, T. Ronneburg, et al., A novel family VIII carboxylesterase derived from a leachate metagenome library exhibits promiscuous beta-lactamase activity on nitrocefin, Appl. Microbiol. Biotechnol. 83 (2009) 491–500.
[10] J. Sambrook, D.W. Russell, Molecular Cloning: A Laboratory Manual, third ed., Cold Spring Harbor, Cold Spring Harbor Laboratory, 2001.
[11] T.A. Hall, BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, Nucl. Acids Symp. Ser. 41 (1990) 95–98.
[12] S.F. Altschul, T.S. Madden, A.A. Schäffer, et al., Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucl. Acids Res. 25 (1997) 3389–3402.
[13] J.D. Bendten, H. Nielsen, G. von Heijnie, et al., Improved prediction of signal peptide: SignalP 3.0, J. Mol. Biol. 340 (2004) 783–795.
[14] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4 (1987) 406–425.
[15] K. Tamura, D. Peterson, N. Peterson, et al., MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, Mol. Biol. Evol. 28 (2011) 2731–2739.
[16] M.M. Bradford, A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding, J. Anal. Biochem. 72 (1976) 248–254.
[17] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[18] K. Rashamuse, S. Burton, W. Stafford, et al., Molecular characterization of a novel family VIII esterase from *Burkholderia multivorans* UWC10, J. Mol. Microbiol. Biotechnol. 13 (2007) 181–188.
[19] M. Krause, K. Ukkonen, T. Haataja, et al., A novel fed-batch based cultivation method provides high cell-density and improves yield of soluble recombinant proteins in shaken cultures, Microb. Cell Fact. 9 (2010), http://dx.doi.org/10.1186/1475-2859-9-11.
[20] D. Wahler, J.L. Reymond, Novel methods for biocatalyst screening, Curr. Opin. Chem. Biol. 5 (2001) 152–158.
[21] P. Lorenz, K. Liebeton, F. Niehaus, et al., Screening novel enzymes for biocatalytic processes: accessing the metagenome as a resource of novel functional sequences space, Curr. Opin. Biotechnol. 13 (2002) 572–577.
[22] G. Kouker, K.E. Jaeger, Specific and sensitive plate assay for bacterial lipases, Appl. Environ. Microbiol. 53 (1987) 211–213.
[23] U.G. Wagner, E.I. Petersen, H. Schwab, C. Kratky, EstB from *Burkholderia gladioli:* a novel esterase with a β-lactamase fold reveals steric factors to discriminate between esterolytic and β-lactam cleaving activity, Prot. Sci. 11 (2002) 467–478.
[24] C. Oefner, A. D'Arcy, J.J. Daly, K. Gubernator, et al., Refined crystal structure of beta-lactamase from *Citrobacter freundii* indicates a mechanism for beta-lactam hydrolysis, Nature 343 (1990) 284–288.
[25] E.Y. Yu, M.A. Kwon, M. Lee, et al., Isolation and characterization of cold-active family VIII esterases from an arctic soil metagenome, Appl. Microbiol. Biotechnol. 90 (2011) 573–581.