Molecular Characterization of a Novel Family VIII Esterase from *Burkholderia multivorans* UWC10

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INTRODUCTION

Family VIII esterases represent an ill-defined family with high homology to class C β-lactamases and penicillin binding protein (Arpigny and Jaeger, 1999). The primary structures of these enzymes contain both G-x-S-x-G and S-x-x-K motifs, which normally harbor the catalytic serine in other esterase families and β-lactamases respectively. Site direct mutagenesis studies have shown that the serine residue of the S-x-x-K motif is essential for catalysis (Sakai *et al*., 1999, Petersen *et al*., 2001). However, members of family VIII esterases studied to date show no activity against standard β-lactamases substrates (Sakai *et al*., 1999, Petersen *et al*., 2001). Esterase (EstB) from *Burkholderia gladioli* represents the only 3D structure solved from this family to date (Wagner *et al*., 2002). Like other serine hydrolyses, EstB revealed a classical α/β fold topolology (Ollis *et al*., 1992). Here we report the cloning, purification, and 3D model of a novel family VIII esterase from *Burkholderia multivorans* UWC10. To our knowledge no report of esterolytic activity from *B. multivorans* is currently available.

METHODOLOGY

Library construction and screening

B. multivorans UWC10 gene library was constructed using shotgun cloning technique. Esterase positive clones were screened on 1% (v/v) tributyrin and agar plates at 37°C.

Construction of expression vector

A primer pair Beta (F): 5′-GCCCATATGTCATCCCTGCCTGTGATTG-3′ (*Nde*I) and Beta (R): 5′-ATTAAGCTTTCATGCCGCGCTCCCGGTTCC-3′ (*Hind*III) was designed for directional subcloning into expression vector pMS470Δ8.

Homology Modelling

The threading programs FUGUE and GenTHREADER were used for the identification of structural homologues and structure predictions. Alignments outputs from FUGUE and GenTHREADER were subsequently used for model building using the external program MODELLER. Structural modelling of *B. multivorans* EstBL was based on the structure of *Burkholderia gladioli* esterase PDB code 1CI8 (Wagner *et al*., 2002), which showed 52% amino acid sequence identity.

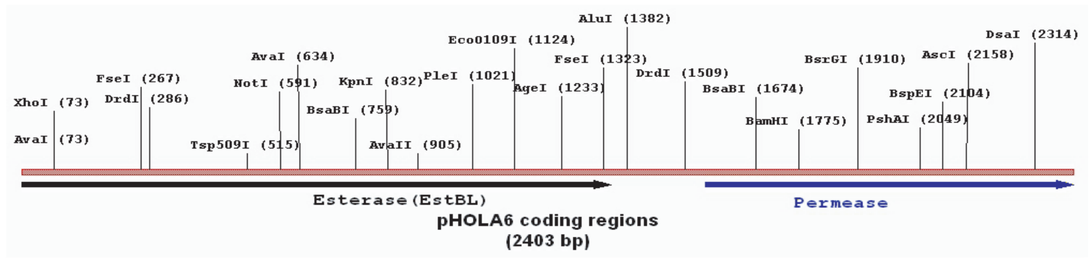

Figure 1: Physical map and genetic organization of the pHOLA6B insert DNA. Arrows indicate the proposed direction and extension of the putative

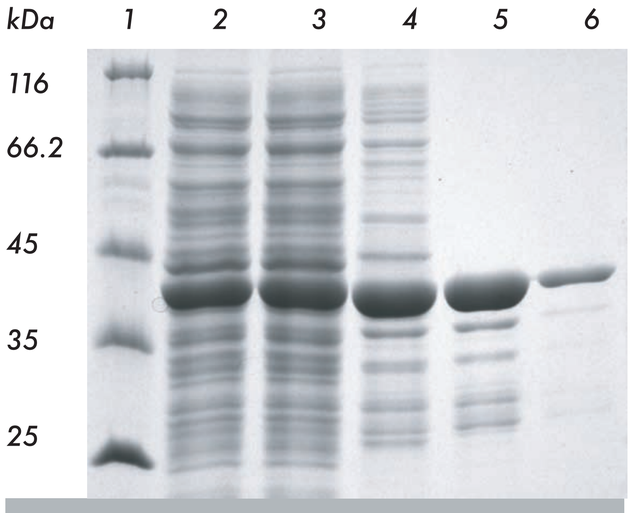

Fig. 3: SDS-PAGE electrophoregram of EstBL from different purification steps: Lane 1: Molecular weight markers, Lane 2: Crude cell free fraction, Lane 3: Ammonium sulphate fraction, Lane 4: Phenyl-Sepharose hydrophobic interaction fraction, Lane 5: Q-Sepharose ion exchange fraction, Lane 6: Superdex 75 size exclusion fraction

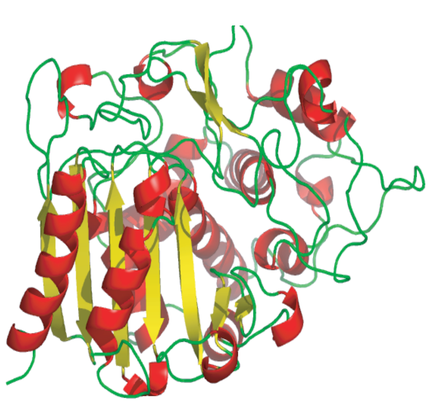

Fig 4: Cartoon representation of the *B. multivorans* esterase (EstBL) structural model

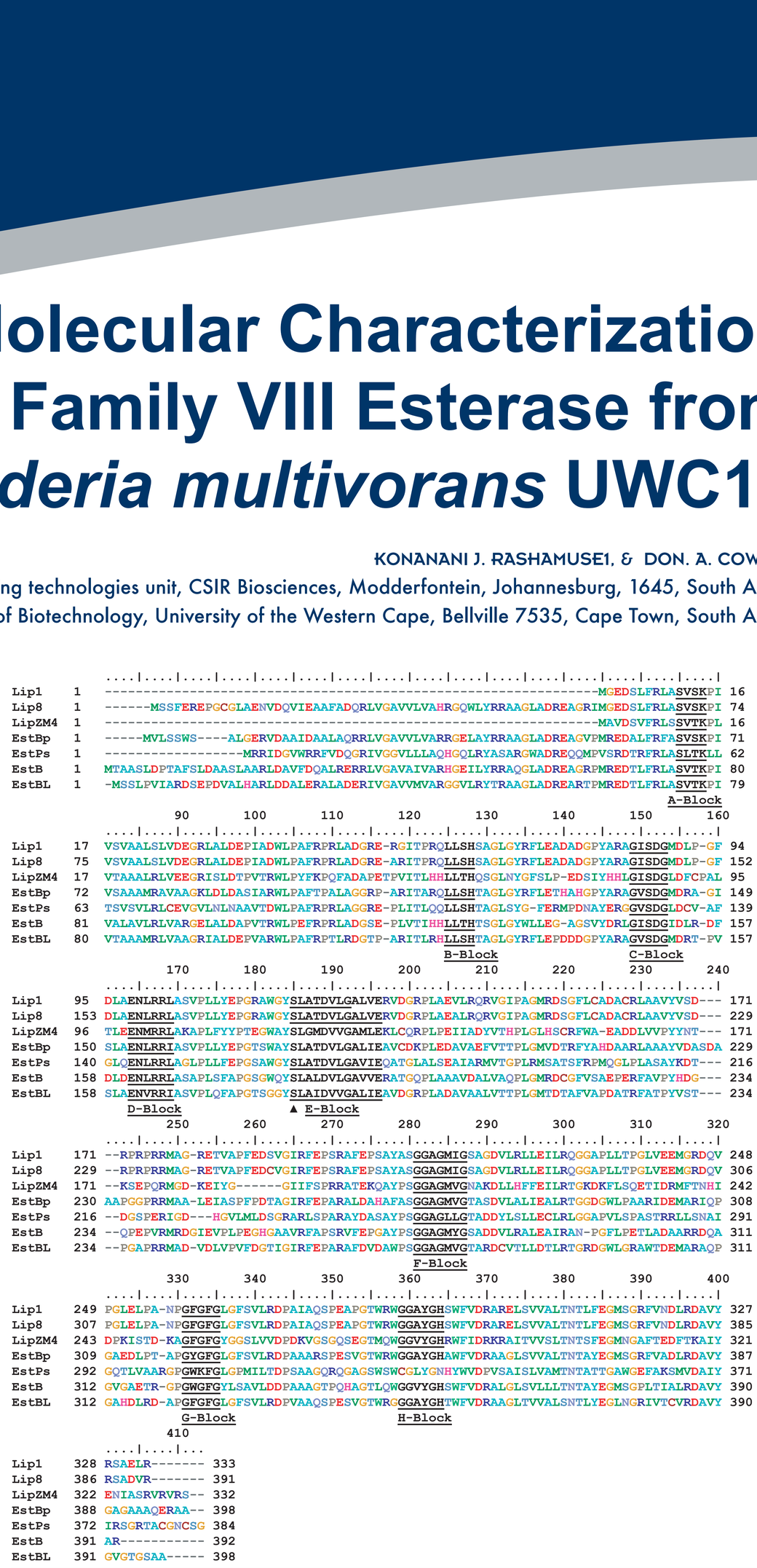

Fig. 2: Multiple sequence alignment of *B. multivorans* esterase EstBL (AAV97951) and other related proteins: Lip1 from *Pseudomonas* sp. nov. 109 (CAA43847), Lip8 from *Ps. aeruginosa* LST-03 (BAD69792), LipZM4 from *Zygomonas mobilis* ZM4 (AAG42401), EstBp from *B. mallie* (AAU49131), EstPs from *Ps. syringae* (AA056448) and EstB from *B. gladioli* (AAF59826)). Motifs are underlined

RESULTS

An esterase-producing *Burkholderia multivorans* UWC10 strain was isolated by culture enrichment strategies. A shotgun library of *B. multivorans* UWC10 genomic DNA (prepared in *E. coli*/pUC18) was screened for esterase activity on tributyrin agar plates. A recombinant clone Hola6, conferring an esterolytic phenotype, was identified. Full-length sequencing of the DNA insert was performed using sub-cloning and prime walk methods. Nucleotide sequence analysis revealed that pHola6 plasmid DNA consisted of two open reading frames (ORF1 and ORF2), encoding putative proteins of 398 and 353 amino acids respectively (Figure 1). Database searches and multiple sequence alignments revealed that the putative protein (termed EstBL) encoded by ORF1 had a high homology to family VIII esterases (Figure 2). The EstBL primary structure included two serine motif sequences G-V-S_{149}-D-G and S_{74}-V-T-K. The *est*BL gene was successfully over-expressed in E. coli and purified by a combination of ammonium sulphate fractionation, hydrophobic interaction, ion exchange, and size exclusion chromatography(Figure 3). EstBL activity showed a preference for ρ-nitrophenyl and β-naphthyl esters of shorter chain length (C2-C4) while no activity against standard β-lactam substrates was detectable (data not shown). A 3D model based on the primary structure and comparative modelling revealed that EstBL tertiary structure adopts the α/β fold topolology (Figure 4).

ACKNOWLEDGEMENTS

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REFERENCES

1. Arpigny and Jaeger, (1999) J. Biochem. 343: 177-183
2. Ollis et al., (1992) Protein Eng. 5: 197-211
3. Petersen et al., (2001) J. Biotech. 89: 11-25
4. Sakai et al., (1999)Biosci. Biotechnol.Biochem. 63: 688-267
5. Sali, 1995). Curr. Opin. Biotechnol. 6: 437-451
6. Wagner et al., (2002) Protein Sci.11: 467-478