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# **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  $\beta$ -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  $\alpha/\beta$  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  $37^{\circ}$ C.

# **Construction of expression vector**

A primer pair Beta (F): 5'-GCCCATATGTCATCCCTGCCTGTGATTG-3' (Ndel) and Beta (R): 5'-ATTAAGCTTTCATGCCGCGCTCCCGGTTCC-3' (HindIII) was designed for directional subcloning into expression vector  $pMS470\Delta8$ .

# Homology Modelling

The threading programs FUGUE and GenTHREADER were used for the identification of structural homologues and structure predictions. Alignments outputs from FUGUE and Gen-THREADER were subsequently used for model building using the external program MOD-ELLER. 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.

Lip1	1	MGEDSLFRLASVSKPI 16
Lip8	1	MSSFEREPGCGLAENVDQVIEAAFADQRLVGAVVLVAHRGQWLYRRAAGLADREAGRIMGEDSLFRLASVSKPI 74
LipZM4	1	MAVDSVFRLSSVTKPL 16
EstBp	1	MVLSSWSALGERVDAAIDAALAQRRLVGAVVLVARRGELAYRRAAGLADREAGVPMREDALFRFASVSKPI 71
EstPs	1	MRRIDGVWRRFVDQGRIVGGVLLLAQHGQLRYASARGWADREQQMPVSRDTRFRLASLTKLL 62
EstB	1	MTAASLDPTAFSLDAASLAARLDAVFDQALRERRLVGAVAIVARHGEILYRRAQGLADREAGRPMREDTLFRLASVTKPI 80
EstBL	1	-MSSLPVIARDSEPDVALHARLDDALERALADERIVGAVVMVARGGVLRYTRAAGLADREARTPMREDTLFRLASVTKPI 79
		A-Block
		90 100 110 120 130 140 150 <u>16</u> 0
Lip1	17	VSVAALSLVDEGRLALDEPIADWLPAFRPRLADGRE-RGITPROLLSHSAGLGYRFLEADADGPYARAGISDGMDLP-GF 94
- Lip8	75	VSVAALSLVDEGRLALDEPIADWLPAFRPRLADGRE-ARITPROLLSHSAGLGYRFLEADADGPYARAGISDGMDLP-GF 152
- LipZM4	17	VTAAALRLVEEGRISLDTPVTRWLPYFKPQFADAPETPVITLHHLLTHQSGLNYGFSLP-EDSIYHHLGISDGLDFCPAL 95
- EstBp	72	VSAAAMRAVAAGKLDLDASIARWLPAFTPALAGGRP-ARITAROLLSHTAGLGYRFLETHAHGPYARAGVSDGMDRA-GI 149
EstPs	63	TSVSVLRLCEVGVLNLNAAVTDWLPAFRPRLAGGRE-PLITLOOLLSHTAGLSYG-FERMPDNAYERGGVSDGLDCV-AF 139
EstB	81	VALAVLRLVARGELALDAPVTRWLPEFRPRLADGSE-PLVTIHHLLTHTSGLGYWLLEG-AGSVYDRLGISDGIDLR-DF 157
EstBL	80	VTAAAMRLVAAGRIALDEPVARWLPAFRPTLRDGTP-ARITLRHLLSHTAGLGYRFLEPDDDGPYARAGVSDGMDRT-PV 157
		B-Block C-Block
		170 180 190 200 210 220 230 240
Lip1	95	DLAENLRRLASVPLLYEPGRAWGYSLATDVLGALVERVDGRPLAEVLRORVGIPAGMRDSGFLCADACRLAAVYVSD 171
- Lip8	153	DLAENLRRLASVPLLYEPGRAWGYSLATDVLGALVERVDGRPLAEALRORVGIPAGMRDSGFLCADACRLAAVYVSD 229
- LipZM4	96	TLEENMRRLAKAPLFYYPTEGWAYSLGMDVVGAMLEKLCORPLPEIIADYVTHPLGLHSCRFWA-EADDLVVPYYNT 171
EstBp	150	SLAENLRRIASVPLLYEPGTSWAYSLATDVLGALIEAVCDKPLEDAVAEFVTTPLGMVDTRFYAHDAARLAAAYVDASDA 229
EstPs	140	GLOENLRRLAGLPLLFEPGSAWGYSLATDVLGAVIEOATGLALSEAIARMVTGPLRMSATSFRPMOGLPLASAYKDT 216
EstB	158	DLDENLRRLASAPLSFAPGSGWOYSLALDVLGAVVERATGOPLAAAVDALVAOPLGMRDCGFVSAEPERFAVPYHDG 234
EstBL	158	SLAENVRRIASVPLOFAPGTSGGYSLAIDVVGALIEAVDGRPLADAVAALVTTPLGMTDTAFVAPDATRFATPYVST 234
		D-Block E-Block
		<u> </u>
Lip1	171	RPRPRRMAG-RETVAPFEDSVGIRFEPSRAFEPSAYASGGAGMIGSAGDVLRLLEILRQGGAPLLTPGLVEEMGRDQV 248
Lip8	229	RPRPRRMAG-RETVAPFEDCVGIRFEPSRAFEPSAYASGGAGMIGSAGDVLRLLEILRQGGAPLLTPGLVEEMGRDQV 306
LipZM4	171	KSEPQRMGD-KEIYGGIIFSPRRATEKQAYPSGGAGMVGNAKDLLHFFEILRTGKDKFLSQETIDRMFTNHI 242
EstBp	230	AAPGGPRRMAA-LEIASPFPDTAGIRFEPARALDAHAFASGGAGMVGTASDVLALIEALRTGGDGWLPAARIDEMARIQP 308
EstPs	216	DGSPERIGDHGVLMLDSGRARLSPARAYDASAYPSGGAGLLGTADDYLSLLECLRLGGAPVLSPASTRRLLSNAI 291
EstB	234	QPEPVRMRDGIEVPLPEGHGAAVRFAPSRVFEPGAYPSGGAGMYGSADDVLRALEAIRAN-PGFLPETLADAARRDQA 311
EstBL	234	PGAPRRMAD-VDLVPVFDGTIGIRFEPARAFDVDAWPSGGAGMVGTARDCVTLLDTLRTGRDGWLGRAWTDEMARAOP 311
		F-Block
		330 340 350 360 370 380 390 400
Lip1	249	PGLELPA-NPGFGFGLGFSVLRDPAIAQSPEAPGTWRWGGAYGHSWFVDRARELSVVALTNTLFEGMSGRFVNDLRDAVY 327
- Lip8	307	PGLELPA-NPGFGFGLGFSVLRDPAIAQSPEAPGTWRWGGAYGHSWFVDRARELSVVALTNTLFEGMSGRFVNDLRDAVY 385

EstBp	309 GAEDLPT-APGYGFGLGFSVLRDPAAARSPESVGTWRWGGAYGHAWFVDRAAGLSVVALTNTAYEGMSGRFVADLRDAVY 387	
EstPs	292 GQTLVAARGPGWKFGLGPMILTDPSAAGQRQGAGSWSWCGLYGNHYWVDPVSAISLVAMTNTATTGAWGEFAKSMVDAIY 371	
EstB	312 GVGAETR-GPGWGFGYLSAVLDDPAAAGTPQHAGTLQWGGVYGHSWFVDRALGLSVLLLTNTAYEGMSGPLTIALRDAVY 390	
EstBL	312 GAHDLRD-APGFGFGLGFSVLRDPVAAQSPESVGTWRGGGAYGHTWFVDRAAGLTVVALSNTLYEGLNGRIVTCVRDAVY 390	
	G-Block H-Block	
	410	
Lip1	328 RSAELR 333	
Lip8	386 RSADVR 391	
LipZM4	322 ENIASRVRVRS 332	
EstBp	388 GAGAAAQERAA 398	
EstPs	372 IRSGRTACGNCSG 384	
EstB	391 AR 392	
EstBL	391 GVGTGSAA 398	

LipZM4 243 DPKISTD-KAGFGFGYGGSLVVDPDKVGSGQSEGTMQWGGVYGHRWFIDRKRAITVVSLTNTSFEGMNGAFTEDFTKAIY 321

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<sub>149</sub>-D-G and  $S_{74}$ -V-T-K. The estBL 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 pnitrophenyl and β-naphthyl esters of shorter chain length (C2-C4) while no activity against standard  $\beta$ -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  $\alpha/\beta$  fold topolology (Figure 4).



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



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

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

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