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X-Ray Crystallographic Studies on BPSL2418 from Burkholderia pseudomallei

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Abstract : Melioidosis has emerged as a lethal disease. Unfortunately, the molecular mechanisms of virulence and pathogenicity of Burkholderia pseudomallei remain unknown. However, proteomics research has selected putative targets in B. pseudomallei that might play roles in the B. pseudomallei virulence. BPSL 2418 putative protein has been predicted as a free methionine sulfoxide reductase and interestingly there is a link between the level of the methionine sulfoxide in pathogen tissues and its virulence. Therefore in this work, we describe the cloning expression, purification, and crystallization of BPSL 2418 and the solution of its 3D structure using X-ray crystallography. Also, we aimed to identify the substrate binding and reduced forms of the enzyme to understand the role of BPSL 2418. The gene encoding BPSL2418 from B. pseudomallei was amplified by PCR and reclone in pETBlue-1 vector and transformed into E. coli Tuner DE3 pLacI. BPSL2418 was overexpressed using E. coli Tuner DE3 pLacI and induced by 300µM IPTG for 4h at 37°C. Then BPS2418 purified to better than 95% purity. The pure BPSL2418 was crystallized with PEG 4000 and PEG 6000 as precipitants in several conditions. Diffraction data were collected to 1.2Å resolution. The crystals belonged to space group P2 21 21 with unit-cell parameters a = 42.24Å, b = 53.48Å, c = 60.54Å, α=γ=β= 90Å. The BPSL2418 binding MES was solved by molecular replacement with the known structure 3ksf using PHASER program. The structure is composed of six antiparallel β-strands and four α-helices and two loops. BPSL2418 shows high homology with the GAF domain fRMsrs enzymes which suggest that BPSL2418 might act as methionine sulfoxide reductase. The amino acids alignment between the fRmsrs including BPSL 2418 shows that the three cysteines that thought to catalyze the reduction are fully conserved. BPSL 2418 contains the three conserved cysteines (Cys^{75}, Cys^{85} and Cys^{109}). The active site contains the six antiparallel β-strands and two loops where the disulfide bond formed between Cys^{75} and Cys^{109}. X-ray structure of free methionine sulfoxide binding and native forms of BPSL2418 were solved to increase the understanding of the BPSL2418 catalytic mechanism.

Keywords : X-Ray Crystallography, BPSL2418, Burkholderia pseudomallei, Melioidosis
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