An Evolutionarily Divergent B3 Metallo-β-lactamase: A case study of Hypothetical Protein Bleg1_2437 from *Bacillus lehensis* G1 Alkaliphile

Yahaya M. Normi,[a] *Soo Huei Tan,*[a] *Adam Thean Chor Leow,*[a] *Abu Bakar Salleh,*[a] *Abdul Munir Abdul Murad,*[b] *Nor Muhammad Mahadi,*[c] *& Mohd Basyaruddin Abdul Rahman*[c,d]

[a] Enzyme and Microbial Biotechnology (EMTECH), Universiti Putra Malaysia, Serdang, Selangor, Malaysia; E-mail address: normi_yahaya@upm.edu.my

[b] School of Biosciences and Biotechnology, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia

[c] Malaysia Genome Institute, Ministry of Science, Technology and Innovation, Kajang, Selangor, Malaysia.

[d] Department of Chemistry, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

β-lactam antibiotics which are used as chemotherapeutic agents to treat bacterial infections are gradually becoming ineffective due to antibiotic resistance mechanism among pathogenic bacteria via the production metallo-β-lactamase (MBL). In the present study, a hypothetical protein (HP) termed Bleg1_2437 was discovered from the genome of alkaliphilic *Bacillus lehensis* G1 which exhibited properties similar to B3 subclass of MBLs, but evolutionary diverged from them. Domain and sequence analysis of Bleg1_2437 revealed that it contains highly conserved Zn^{2+}-binding residues namely H54, H56, D58, H59, H131 and H191 which are involved in the coordination of two Zn^{2+} ions important for catalysis, similar with subclass B3 MBLs. Built 3-D Bleg1_2437 structure exhibited an αββα sandwich layer similar to the well conserved global topology of MBL superfamily. Other notable features include a ceiling and floor in the model which are important for accommodation and orientation of β-lactam antibiotics. Docking of several β-lactam antibiotics to the protein model showed interactions with residues in the binding pocket of the protein. The ability of Bleg1_2437 in degrading β-lactam antibiotics was proven via enzymatic assay whereby its activity increased significantly in the presence of Zn^{2+}. Although Bleg1_2437 HP shared similar sequence features and enzymatic characteristics with B3 MBLs, it shares a closer evolutionary link to glyoxalase II with significant sequence conservation – thus diverging it from members of other circulating B3 MBLs. These findings point to a possible looming threat of such silent and evolutionary-divergent MBLs circulating in the environment within the pools of uncharacterized HPs.