



## Partial characterization of bacteriocin-like compound (BLIS) produced by *Burkholderia stagnalis* strain K23/3 against *Burkholderia pseudomallei*

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### ABSTRACT

**Aims:** *Burkholderia pseudomallei*, the human pathogen that causes melioidosis, is intrinsically resistant towards a wide range of antibiotics and there have been reports of acquired resistance towards antibiotics used for melioidosis treatments. Antimicrobial peptides (AMP) such as bacteriocins are gaining the interests of researchers as alternative for treating infections caused by multidrug resistant bacteria. In this study, we aimed to identify *Burkholderia* spp. isolated from soil in Sarawak that possess the potential in inhibiting the growth of *B. pseudomallei* and to further characterize the antagonistic compound produced.

**Methodology and results:** A total of 50 *Burkholderia* spp. isolates of environmental origin and two isolates of *Ralstonia solanacearum* were screened against five clinical isolates of *B. pseudomallei* using spot-on-lawn assay and flip streak method. *Burkholderia stagnalis* isolate K23/3 showed clear zones of inhibition (ZOI) in both preliminary tests. Cell-free supernatant (CFS) was obtained from *B. stagnalis* K23/3 broth culture and was tested via agar well diffusion assay (AWDA). The antagonistic compound secreted at the early log phase of the bacterial growth was shown to be stable in a wide range of temperatures and pH. Treatment with different enzymes revealed that it was sensitive towards proteinase K, suggesting that it is proteinaceous. The bacteriocin-like-substance (BLIS) was subjected to ammonium sulfate precipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE gel was overlaid with indicator *B. pseudomallei* isolates where the active protein was shown to be less than 7.1 kDa.

**Conclusion, significance and impact of study:** *Burkholderia stagnalis* isolate K23/3 was able to secrete bacteriocin-like-substance (BLIS) that has the potential in biocontrol of *B. pseudomallei* in the environment or as potential treatment for melioidosis.

**Keywords:** Antimicrobial peptides, bacteriocin, *Burkholderia pseudomallei*, melioidosis, SDS-PAGE

### INTRODUCTION

Melioidosis is a potentially fatal infectious disease that is caused by the bacterium *Burkholderia pseudomallei*. With ineffective antimicrobial treatment, the fatality rate of melioidosis is expected to exceed 70% (Limmathurotsakul *et al.*, 2016), making it a life-threatening infectious disease. Melioidosis has been recognized as one of the important human infections in Malaysia (Nathan *et al.*, 2018), Singapore and across the north of Australia (White, 2003) and Southeast Asia (Limmathurotsakul *et al.*, 2016). Over the past years, this disease has been emerging in tropical developing countries that were not previously reported such as Africa (Birnie *et al.*, 2015), Puerto Rico (Doker *et al.*, 2015) and Brazil (Rolim *et al.*, 2018). A person could be infected by *B. pseudomallei* through inhalation, open wound inoculation or ingestion of contaminated environmental sources (Barnes and Ketheesan, 2005). According to Currie *et al.* (2000), the

incubation period for melioidosis is estimated to be 1–21 days and may extend to months and even years. It is influenced by the amount of inoculum, route of infection and underlying host risk factors (Ngauy *et al.*, 2005). However, *B. pseudomallei* has the tendency of staying dormant in humans for a prolonged period (Wiersinga *et al.*, 2012).

*Burkholderia pseudomallei* is intrinsically resistant to many classes of commonly used antibiotics such as  $\beta$ -lactams, aminoglycosides, macrolides and fluoroquinolones (Lipsitz *et al.*, 2012). Currently, meropenem, trimethoprim/sulfamethoxazole (TMP-SMX) and doxycycline are used for melioidosis treatment (Dance, 2014). However, there were reported possible resistance towards TMP-SMX (Wuthiekanun *et al.*, 2005; Yong *et al.*, 2016) and meropenem (Yong *et al.*, 2016). Although yet to be verified through comprehensive in-depth studies, concerns of possible resistance towards antibiotics is a major concern to the public health. Thus,

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