Role of BimC in Actin-based Motility of *Burkholderia pseudomallei*

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Background: *Burkholderia pseudomallei*, a facultative intracellular bacterium, is the causative agent of melioidosis. The disease is recognized as a major public health problem in the northeastern part of Thailand. *B. pseudomallei* can subvert cellular actin dynamics to promote its movement within and between infected host cells, a process termed actin-based motility (ABM). The bacterial factor required for ABM of *B. pseudomallei* is BimA (Burkholderia intracellular motility A). A *bimA* knockout mutant was attenuated in a murine model suggesting the importance of ABM in *B. pseudomallei* virulence. Analysis of the *B. pseudomallei* genome indicated that the *bimA* gene was located downstream of a gene encoding a putative glycosyltransferase (*bimC, bpss1491*) and may be co-transcribed in the same operon. We hypothesize that BimC protein is required for BimA to induce ABM by *B. pseudomallei*. To investigate this hypothesis, a *B. pseudomallei* *bimC* deletion mutant was constructed and characterized for the ability to induce ABM compared with the wild-type strain.

Methods: A *B. pseudomallei* *bimC* deletion mutant (Δ*bimC*) was constructed using the pDM4 suicide replicon and *sacB*-mediated double recombination. Complementation was performed by cloning the *bimC* gene into plasmid pME6032 then inducing *bimC* expression in the mutant strain via IPTG induction. ABM of *B. pseudomallei* in infected J774A.1 murine macrophage-like cells was detected by immunostaining and confocal microscopy.

Results: A *B. pseudomallei* Δ*bimC* mutant was successfully constructed and verified by PCR and Southern blot analysis. The *bimC* deletion mutant and the wild-type strains were used to infect J774A.1 murine macrophages. At 8 hours post-infection, ABM was detected in *B. pseudomallei* wild-type strain. In contrast, *bimC* mutant failed to form actin tails, even though intracellular bacteria with polar expression of the BimA protein were detected. Trans-complementation of *B. pseudomallei* *bimC* mutant with *bimC* gene restored actin tail formation by the Δ*bimC* mutant.

Conclusion: *B. pseudomallei* BimC is required for the ABM of *B. pseudomallei*. Further studies to identify the importance of BimC on *B. pseudomallei* intracellular survival and multinucleated giant cell formation are under investigation. Moreover, we seek to determine if BimC modifies BimA by glycosylation, to identify the putative glycan moiety and to map the catalytic residues required for its activity.

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