Autotransporters and their role in the virulence of *Burkholderia pseudomallei* and *Burkholderia mallei*

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**Burkholderia pseudomallei** and *Burkholderia mallei* are closely related Gram-negative bacteria responsible for the infectious diseases melioidosis and glanders, respectively. Autotransporters (ATs) comprise a large and diverse family of secreted and outer membrane proteins that includes virulence-associated invasins, adhesins, proteases, and actin-nucleating factors. The *B. pseudomallei* K96243 genome contains 11 predicted ATs, eight of which share homologs in the *B. mallei* ATCC 23344 genome. This review distils key findings from *in silico*, *in vitro*, and *in vivo* studies on the ATs of *B. pseudomallei* and *B. mallei*. To date, the best characterized of the predicted ATs of *B. pseudomallei* and *B. mallei* is BirmA, a predicted trimeric AT mediating actin-based motility which varies in sequence and mode of action between *Burkholderia* species. Of the remaining eight predicted *B. pseudomallei* trimeric autotransporters, five of which are also present in *B. mallei*, two (BoaA and BoaB), have been implicated in bacterial adhesion to epithelial cells. Several predicted *Burkholderia* ATs are recognized by human humoral and cell-mediated immunity, indicating that they are expressed during infection and may be useful for diagnosis and vaccine-mediated protection. Further studies on the mode of secretion and functions of *Burkholderia* ATs will facilitate the rational design of control strategies.

**Keywords:** *Burkholderia pseudomallei*, *Burkholderia mallei*, autotransporter, secreted, protein

**BURKHOLDERIA PSEUDOMALLEI AND BURKHOLDERIA MALLEI**

Melioidosis, a febrile illness with disease states ranging from acute pneumonia or septicemia to chronic or localized abscess formation, was first documented by Whitmore and Krishnaswami (1912). The causative agent, *Burkholderia pseudomallei*, was subsequently identified as a motile, Gram-negative bacillus, which is principally a saprophyte found in soil and water in subtropical areas. The disease is acquired by inhalation, aspiration, subcutaneously, or occasionally via ingestion. Most cases occur in patients with immunosuppression, especially those with diabetes mellitus. Melioidosis has become an increasingly important disease in endemic areas such as South-East Asia and Northern Australia causing a significant number of deaths despite antibiotic treatment. Furthermore, *B. pseudomallei* is also recognized as a pathogen of numerous animal species (reviewed in Lazar Adler et al., 2009; Galyov et al., 2010).

*Burkholderia mallei* is closely related to *B. pseudomallei* and is the etiological agent of Glanders, an infectious equine disease that can be transmitted to humans. The disease may be acute or chronic, with the chronic form usually occurring in horses and the acute form occurring in mules and donkeys. Clinical signs include swollen lymphatics, a chronic nasal discharge, and ulcers. Contact with nasal discharge or infected ulcers is a major risk factor for infection. *B. mallei* is adapted to a narrower range of hosts than *B. pseudomallei* and is not considered able to persist for protracted periods in the environment. Horses are the primary carriers of disease and are largely responsible for transmission. Infections in humans frequently involve sepsis, pneumonia and abscess formation and have a high mortality rate. The incubation period ranges from days to weeks, and animals can die within a week of the onset of clinical symptoms (reviewed in Whitlock et al., 2007; Galyov et al., 2010).

**THE AUTOTRANSPORTER FAMILY**

Autotransporters (ATs) are a large and diverse family of bacterial secreted and outer membrane (OM) proteins that are translocated from the bacterial cytoplasm via the Type V pathway, one of seven recognized secretion pathways in Gram-negative bacteria. ATs play diverse roles, and many such proteins influence pathogenesis and immunity, for example by acting as invasins, adhesins, proteases, or actin-nucleating factors. ATs typically consist of a 20–400-kDa passenger domain that contains the effector functions and a 10–30-kDa β domain facilitating translocation across the OM (Dautin and Bernstein, 2007). ATs can be further divided into classical ATs and trimeric autotransported adhesins (TAAs), referred to as Type Va and Type Vc respectively. TAAs have a coiled-coil motif of unknown function, and are therefore referred to as oligomeric coiled-coil adhesins (Oca). Trimerization of TAAs is essential for their translocation and function as a single β barrel is formed from the three shorter 4-stranded β barrel domains which is superimposable with the classical ATs 12-stranded β barrel. While classical ATs have diverse effector functions, all characterized TAAs, with the notable exception of BirmA from *Burkholderia* spp., are adhesins.
A T s demonstrated an exception to the solenoid structure, with As implied in their name, A T s were originally believed to mediate the driving force for translocation of A T (Van Den Berg, 2010), while TAAs have a secretions (Cotter et al., 2005). Classical A T s form a right-handed helix which may contribute to protein folding after transport and/or allow effector domains to traverse LPS and capsule layers. This conserved shape creates a scaffold for various loops and domains without disturbing structural integrity and allows multiple interfaces to mediate interactions (Wells et al., 2007; Nishimura et al., 2010). Classical ATs form a right-handed β helical structure while TAAs have a β roll (two β strands contributing to a single superhelical turn); these structural differences affect curvature and binding specificity (Dautin and Bernstein, 2007). However, the recent full structure for the Pseudomonas aeruginosa classical AT EstA demonstrated an exception to the solenoid structure, with a α-β-α globular fold (Van Den Berg, 2010), which may relate to its function as a lipase rather than an adhesin.

ATs must cross both plasma membranes of Gram-negative bacteria. Classical ATs are then predominantly cleaved for secretion into the extracellular milieu whereas TAAs remain integrated in the bacterial OM via their β barrel domain. Firstly, ATs are targeted to the IM by their N-terminal signal sequence, where translocation occurs via the general secretory (Sec) pathway. The N-terminal signal sequence ranges from 20 to 60 aa, with the most elongated signal peptides being associated with larger (>1000 aa) passenger domains in TAAs (Dautin and Bernstein, 2007). Such extended signal peptides exhibit reduced interaction with the Sec complex promoting delayed post-translational translocation across the inner membrane which is proposed to allow partial folding for subsequent OM secretion (Peterson et al., 2006; Desvaux et al., 2007). Once in the periplasm, ATs can undergo modifications including glycosylation and lipidation and may also form disulfide bonds. As implied in their name, ATs were originally believed to mediate their own transport across the OM. However, recent research suggests that they are chaperoned by periplasmic proteins, such as SurA and DegP to the Bam (Omp85) complex located within the OM which may assist in translocation (Oomen et al., 2004; Dautin and Bernstein, 2007). Several ATs have been shown to interact directly with the Bam complex (Ieva and Bernstein, 2009; Sauri et al., 2009; Lehr et al., 2010), as well as periplasmic chaperones including SurA and DegP (Ieva and Bernstein, 2009; Ruiz-Perez et al., 2009). Furthermore, depletion of the essential BamA protein significantly affects AT secretion (Jain and Goldberg, 2007). BamA forms a 16-strand β barrel with a pore size of ~2.5 nm which is significantly larger than the AT β barrel (~1.2 nm) and would allow for translocation of partially folded ATs. Additionally, BamA oligomerizes in vitro and could possibly form oligomer pores capable of translocating folded ATs. These observations have led to two hypotheses: translocation of the unfolded passenger domain through the Bam-stabilized AT β barrel via a hairpin curvature or translocation of the entire AT, possibly partially folded, via the Bam complex (Sauri et al., 2009). Structural analysis indicates that sequential folding of the passenger domain may act as the driving force for translocation of AT (Van Den Berg, 2010), although evidence suggests that partially folded or misfolded ATs are still capable of translocation (Dautin and Bernstein, 2007). Therefore, the translocation of ATs is dependent on periplasmic chaperones and the Bam complex via a mechanism which remains to be elucidated but which appears to be mediated by interaction with the β domain of the ATs. This would account for the observation that the C-terminal domains of TAAs from diverse bacterial species are functionally interchangeable (Ackermann et al., 2008).

**AUTOTRANSPORTERS OF B. PSEUDOMALLEI AND B. MALLEI**

The B. pseudomallei K96243 genome contains 11 predicted ATs, annotated as such based on sequence similarity to known ATs (Table 1). Additionally, 10 of these ATs are conserved across the other three completed B. pseudomallei genomes, while BPSL1705 is missing from both the 1106a and 688 strains. The B. mallei ATCC 23344 genome has eight homologs of the B. pseudomallei ATs (Table 1). Six of these are conserved across the three additional B. mallei completed genomes, with BMA1027 being present only in B. mallei ATCC 23344, while the avirulent B. mallei SAVPI strain also lacks BMAA0649. Homologs for seven of the B. pseudomallei ATs are also found in avirulent B. thailandensis (Table 1). However, we caution that conservation of loci in pathogenic *Burkholderia* and *B. thailandensis* does not necessarily imply that the genes play no role in virulence or host cell-interactions, as demonstrated by our analysis of the *bsa* Type III secretion locus shared by such strains (Stevens et al., 2002, 2004). Only two ATs are predicted to be classical ATs: BPS0906/BMA1263 (a putative serine protease) and BPSL2237/BMA1647 (a putative lipase/esterase). The remainder are predicted TAAs (Tiyawisutsri et al., 2007). An in silico subtractive hybridization of pathogenic versus non-pathogenic *Burkholderia* species to generate a 650 gene virulome, identified six ATs (BPS09062, BPSL2237, BPSL1631, BPSL2063, BPS796, BPS0908; Schell et al., 2008). Furthermore, a “gain of function” genomic library screen identified five ATs as having a possible role in invasion and survival within macrophages (BPSL1705, BPSL2237, BPS0908, BPS0796, BPS1492; Dowling et al., 2010). Neither approach identified a common set of dominant ATs, which may indicate that the ATs have a cumulative importance. However, direct experimental evidence for a role of ATs in virulence or intramacrophage survival, as proposed by Schell et al. (2008) and Dowling et al. (2010), is lacking at the time of writing. Several predicted ATs are recognized by human convalescent sera, in some cases with a specificity adequate for serodiagnosis, and such data indicate that the proteins are expressed in vivo (Tiyawisutsri et al., 2007; Felgner et al., 2009; Suwannasaen et al., 2011). To date, the best characterized of the predicted ATs of *B. pseudomallei* and *B. mallei* is a factor influencing intracellular motility, BimA.

**BURKHOLDERIA INTRACELLULAR MOTILITY AND THE BIMA PROTEIN**

In common with selected species of *Listeria*, *Shigella*, *Rickettsia*, and *Mycobacterium marinum*, *B. pseudomallei* shares the ability to induce polymerization of actin at one pole of the bacterial cell to promote its movement within and between host cells (Figure 1). The formation of actin-rich bacteria-containing

**Table 1**

<table>
<thead>
<tr>
<th>AT</th>
<th>B. mallei</th>
<th>B. pseudomallei</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>AT2</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>AT3</td>
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<td>AT4</td>
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<td>AT5</td>
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<td>AT6</td>
<td>Present</td>
<td>Present</td>
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<td>AT7</td>
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<td>Present</td>
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<td>AT8</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>AT9</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>AT10</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>

*Note: ATs marked with a present are conserved across at least two of the three genomes.*
Table 1 | Autotransporters of \textit{B. pseudomallei} K96243 and \textit{B. mallei} ATCC 23344. The 11 \textit{B. pseudomallei} ATs and their eight \textit{B. mallei} homologs are listed below alongside relevant functional information.

<table>
<thead>
<tr>
<th>Bp K96243</th>
<th>Bm ATCC 23344</th>
<th>Size (aa)</th>
<th>Annotation</th>
<th>Biological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Classical autotransporters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPSL2237*</td>
<td>BMA1647</td>
<td>610, 610</td>
<td></td>
<td>Immunogenic (Bp; Tiyawisutsri et al., 2007)</td>
</tr>
<tr>
<td>BPSS0962*</td>
<td>BMAA1263</td>
<td>1131, 1129</td>
<td></td>
<td>Adhesive (Balder et al., 2010)</td>
</tr>
<tr>
<td><strong>TrimERIC autotransporter adhesins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPSL1631</td>
<td>BMA1027</td>
<td>1124, 1012</td>
<td></td>
<td>Adhesive (Balder et al., 2010)</td>
</tr>
<tr>
<td>BPSL1705</td>
<td>–</td>
<td>1606</td>
<td>BoaB</td>
<td>Immunogenic (Bp, Bm; Tiyawisutsri et al., 2007)</td>
</tr>
<tr>
<td>BPSL2063</td>
<td>BMA0840</td>
<td>1090, 1090</td>
<td></td>
<td>Adhesive (Balder et al., 2010), immunogenic (Bp, Bm; Tiyawisutsri et al., 2007)</td>
</tr>
<tr>
<td>BPSS0088*</td>
<td>–</td>
<td>1645</td>
<td></td>
<td>Serodiagnostic (Felgner et al., 2009)</td>
</tr>
<tr>
<td>BPSS0796</td>
<td>BMAA0649</td>
<td>1653, 1535</td>
<td>BoaB</td>
<td>Serodiagnostic (Felgner et al., 2009), immunogenic (Bp, Bm; Tiyawisutsri et al., 2007)</td>
</tr>
<tr>
<td>BPSS0908*</td>
<td>BMAA1324*</td>
<td>1324, 831</td>
<td></td>
<td>Characteristic TAA head crystal structure (Edwards et al., 2010), serodiagnostic (Felgner et al., 2009)</td>
</tr>
<tr>
<td>BPSS1434*</td>
<td>–</td>
<td>2634</td>
<td>BpaA</td>
<td></td>
</tr>
<tr>
<td>BPSS1439*</td>
<td>BMAA0810*</td>
<td>1530, 459*</td>
<td></td>
<td>Immunogenic (Bp; Tiyawisutsri et al., 2007)</td>
</tr>
<tr>
<td>BPSS1492*</td>
<td>BMAA0749*</td>
<td>516, 373</td>
<td>BimA</td>
<td>Actin-based motility (Stevens et al., 2005b), serodiagnostic (Felgner et al., 2009), immunogenic (Bp; Tiyawisutsri et al., 2007)</td>
</tr>
</tbody>
</table>

\*B. thailandensis homolog identified in E264 genome.
\*Putative pseudogene.

that suggested it may be involved in actin assembly (Stevens et al., 2005b). Actin-based motility of \textit{B. pseudomallei} in \textit{J744.2} cells was abolished by inactivation of \textit{bimA} and could be restored by transient expression of the gene \textit{in trans} (Stevens et al., 2005b). BimA is located at the pole of the bacterial cell at which actin polymerization occurs and polar localization is frequently seen in only one daughter cell at division (Figure 1), implying that polar targeting of BimA may rely on distinguishing the new and old poles of the cell (Stevens et al., 2005b).

\textit{Burkholderia pseudomallei} BimA possesses an unusually long signal sequence typical of autotransporters and a passenger domain containing several putative functional domains. Within this effector domain, the two WH2 motifs have each been found to influence actin binding and polymerization \textit{in vitro} (Sitthidet et al., 2011). In addition, \textit{B. pseudomallei} BimA contains between 2 and 7 predicted target sites for host cell casein kinase II (PDAX), with variation across sequenced strains (Sitthidet et al., 2008). The PDAX repeats have since been established to act in an additive manner to enhance actin polymerization \textit{in vitro} (Sitthidet et al., 2011). It remains unclear if such repeats are phosphorylated \textit{in vivo}, or what the functional consequences of post-translational modification of BimA may be. Interestingly a 13 aa repeat region of BimA was found to be required for intercellular spread of \textit{B. pseudomallei} but was dispensable for actin binding and polymerization (Sitthidet et al., 2011). Other bacterial factors involved in actin-based motility have been shown to promote intracellular survival through evasion of cytosolic killing by autophagy and further studies with defined mutants are required to determine if BimA or its domains confer such activity.

BimA orthologs exist in \textit{B. mallei} ATCC23344 (BMAA0749) and \textit{B. thailandensis} E264 (BTH_II0875) and can restore actin-based motility of a \textit{B. pseudomallei bimA} mutant (Stevens et al., 2005a). The BimA passenger domain of the three species differ markedly in primary sequence resulting in differences in the

FIGURE 1 | Actin-based motility of \textit{B. pseudomallei} in \textit{a J774.2} murine macrophage-like cell \textit{8 h} post-infection. \textit{B. pseudomallei} was detected with antibodies to its lipopolysaccharide (green) and BimA was detected with a panel of specific monoclonal antibodies (blue). Polymerized (F-) actin was stained red with fluorophore-labeled phalloidin.
number of WH2 domains, sequence of proline-rich motifs and the presence of a central and acidic (CA) domain, indicating that they may initiate actin assembly by distinct mechanisms (Stevens et al., 2005a). Many mechanisms of bacterial actin-based motility converge on activation of the cellular Arp (actin-related protein) 2/3 complex. Activation of the Arp2/3 complex requires cellular nucleation-promoting factors (NPFs) such as WASP-family members, and pathogenesis capable of actin-based motility often mimic the activity of NPFs or recruit and activate them at the bacterial pole (reviewed in Stevens et al., 2006). In recent years however, a number of virulence-associated factors have been identified in bacteria that initiate actin assembly in an Arp2/3-independent manner. For example, the AT Sca2 facilitates actin-based motility of certain Rickettsia species in an Arp2/3-independent manner akin to cellular formin proteins (Haglund et al., 2010; Kleba et al., 2010). It is notable that truncated recombinant B. pseudomallei BimA purified from E. coli polymerizes actin in vitro in the absence of any other cellular and bacterial co-factor in an Arp2/3-independent manner (Stevens et al., 2005b; Sitthidet et al., 2010); however, it remains unclear if such intrinsic activity would be adequate to propel the bacteria inside cells. In contrast, the ability of the B. thailandensis BimA ortholog to assemble actin in vitro is Arp2/3-dependent and requires the Arp2/3-recruiting CA domain (Sitthidet et al., 2010). Little is presently known about the mechanism of action of the B. mallei BimA ortholog; however, it is considered unlikely to be Arp2/3-dependent as it lacks an invariant tryptophan residue within an acidic stretch of residues required for Arp2/3 binding and activation (Stevens et al., 2005b). Intra-species conservation of BimA in natural populations of B. pseudomallei and B. thailandensis is high, with the exception of a geographically restricted subset of B. pseudomallei isolates harboring a B. mallei-like BimA variant (Sitthidet et al., 2008, 2010). The impact of sequence variation within BimA on pathogenesis and recognition by host immunity remains to be elucidated.

BimA has attracted much interest as a diagnostic tool. Recently, researchers have developed PCR-based methods to discriminate between Burkholderia species in a clinical setting (Ulrich et al., 2006a,b; Sitthidet et al., 2011), and protein microarray studies led to inclusion of BimA in a 10-protein multiplex classifier to improve diagnosis of human B. pseudomallei infection (Felgner et al., 2009; Suwannasaen et al., 2011). Curiously the B. mallei BimA protein was non-immunogenic in a test with serum from B. mallei-infected horses (Tiyawisutsri et al., 2007), yet B. pseudomallei BimA is one of the most immunogenic proteins recognized by sera from convalescent melioidosis patients (Felgner et al., 2009; Suwannasaen et al., 2011) and also serves as a T cell antigen (Felgner et al., 2009; Suwannasaen et al., 2011). The role of BimA in the pathogenesis and virulence of B. mallei and B. pseudomallei also appears to differ, with B. mallei BimA being dispensable for virulence in a Syrian hamster model of glanders (Schell et al., 2007) whilst B. pseudomallei BimA contributes to pathogenesis in murine models of melioidosis and confers protection against homologous challenge (Galyov et al., unpublished data). This may reflect differences in the animal species, dose and route of inoculation. BimA in B. mallei is regulated by the VirAG two-component system that is encoded nearby and which also controls expression of a virulence-associated Type VI secretion system (Schell et al., 2007). BimA appears to be poorly expressed in vitro and the stimuli for its expression are ill-defined.

CHARACTERIZATION OF OTHER CANDIDATE B. PSEUDOMALLEI AND B. MALLEI AUTOTRANSPORTERS

In addition to BimA, two predicted TAAs have been the subject of further characterization: BPSS0796/BMAA0649 and BPSL1705, re-annotated as BoaA and BoaB respectively, were expressed in a heterologous E. coli host where they were displayed on the bacterial surface and promoted attachment of the recombinant E. coli strains to epithelial cell lines (Balder et al., 2010). In common with the role of other TAA family members in adherence, B. pseudomallei and/or B. mallei mutants with disruptions in boaA or boaB exhibited reduced adherence to epithelial cells and a double mutant, but neither single mutant, displayed a growth defect in phagocytic cell lines (Balder et al., 2010). Though suggestive of a role in virulence, studies with defined mutant and repaired or complemented strains in animals are required to establish the role of BoaA and BoaB in pathogenesis.

Evidence of expression of selected TAAs has been obtained by screening of expression libraries with convalescent sera. Serum from B. mallei-infected horses identified multiple independent clones for four TAAs (BMA0840, BMA1027, BMA0649, and BMAA1324) suggestive of a strong antibody response (Tiyawisutsri et al., 2007). A similar screen with convalescent serum from human melioidosis patients identified five TAAs (BPSL2063, BPS0908, BPSS0796, BPS1439, and BPS1492); however, these were found at a low frequency indicative of a relatively poor immune response (Tiyawisutsri et al., 2007). Further studies are required to determine if this reflects genuine differences in immunogenicity of the predicted proteins, or the distinct hosts, nature of exposure and/or sampling times. Additionally, a protein microarray probed with pooled melioidosis patients sera identified 4 ATs (BPSS0088, BPSS0796, BPSS1434/BoaA, BPSS1492/BimA) as having serodiagnostic potential due to their ability to interact with melioidosis-specific antibodies (Felgner et al., 2009). The ATs identified by these two studies demonstrate minimal overlap although this may reflect the variation in in vitro bacterial gene expression or the host immune response. Microarray studies under 82 in vitro different conditions demonstrated that the 11 ATs of B. pseudomallei are constitutively expressed (Tan, personal commun.), while qRT-PCR analysis of the transcription of two AT genes (boaA, boaB) demonstrated low expression (Balder et al., 2010).

Alignment of the C-terminal β barrel domain regions of the putative B. pseudomallei and B. mallei TAAs demonstrates considerable conservation indicative of amplification of these adhesins through gene duplication events (Figure 2A). This is potentially significant given the importance of C-terminal regions of TAAs in translocation and trimer stability, as evidenced by the ability of C-terminal domains of YadA family members from diverse bacteria to functionally replace the cognate domain in Yersinia (Ackermann et al., 2008). A phylogenetic tree of the β barrel domains is supportive of this idea as the TAAs form two clusters suggesting that these genes may have been amplified from two original ancestral
genes (Figure 2B). Both clusters appear to be derived from a common ancestor to the prototypic Yersinia YadA TAA. Additionally, the two outliers in the alignment, BPSS0908/BMAA1324 and BPSS1434, display early separation in the phylogenetic tree. Interestingly, of the three unique B. pseudomallei TAAs, two (BPSS1434 and BPSS0088) are early branches in the phylogenetic tree suggesting that their absence from B. mallei is due to gene loss as later branching genes are present. Meanwhile BPSS1705, which is located within a genomic island (GI8), is very recently branched from BPSS0796/BMAA0649 and has been proposed to have originated as a duplication of BPSS0796/BMAA0649 (Tiyawisutsri et al., 2007). This duplication event may explain why BPSS1705 is the only AT not conserved across the four completed B. pseudomallei genomes. Furthermore, the β barrel domains of the B. pseudomallei and B. mallei TAA homologs show 100% identity although little similarity is seen between the passenger domains. However, the effector domains of TAAs are generally not conserved at a sequence level and the crystal structure analysis of a section of the passenger domain of BPSS1434 (re-annotated as BpaA, B. pseudomallei adhesion A) demonstrated quaternary structure similar to that of other TAAs (Edwards et al., 2010). This new annotation gives BPSS1434 the same name as the unrelated Type V two-partner secreted BpaA (Brown et al., 2004) for which no homolog exists in either B. pseudomallei K96243 or B. mallei ATCC 23344. The B. pseudomallei and B. mallei genomes contain homologs of BamA, BamB, and SurA which are proposed to assist in translocation of ATs across the OM (Ieva and Bernstein, 2009; Ruiz-Perez et al., 2009; Sauri et al., 2009; Lehr et al., 2010), and further studies are needed to evaluate the role of such factors in AT secretion and pathogenesis.

CONCLUDING REMARKS

On the basis of homology and the precedent of phenotypes for AT mutants in other bacteria, it is reasonable to infer that ATs predicted to be encoded in the genomes of pathogenic Burkholderia species may play a role in pathogenesis and immunity. In the short time since annotation of the genomes of B. pseudomallei and B. mallei, it has been established that several putative ATs mediate bacterial interactions with host cells. For example, BimA mediates intracellular actin-based motility, which in the case of B. pseudomallei is necessary for intercellular spread and full virulence, whereas BoaA and BoaB appear to function as adhesins. One should note that significant sequence divergence in effector domains can occur even among closely related species, and within species, as we have noted for BimA (Sitthidet et al., 2008). Such variation in BimA can markedly alter the mode of action (Sitthidet et al., 2011) and future studies will need to consider the extent to which the repertoire and sequence of ATs in prototype strains is typical of wider populations. Very few published studies have probed the role of ATs in pathogenesis or immunity during B. pseudomallei and B. mallei infection in model hosts, and further studies with defined mutants and complemented strains are needed. Attenuated mutants may be suitable as live-attenuated vaccines, and based on the ability of some ATs to act as B- and T-cell antigens (Tiyawisutsri et al., 2007), purified ATs may have merit as
subunit vaccines or as diagnostic tools. Importantly, the mode of translocation of Rickettsia ATs is inferred only from homolo-
gous systems, and studies are needed to address outstanding ques-
tions regarding their biological role. Future experiments should
evaluate the role of AT domains and specific residues in protein
folding, oligomerization, interaction and translocation across the
OM, with particular reference to the requirement for periplasmic
chaperones and the Bam complex. Analysis of the structure of
Rickettsia ATs may also provide clues to the mechanistic basis
of their secretion and function.

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