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Current Opinion in Microbiology

Intracellular replication of the well-armed pathogen *Burkholderia pseudomallei*

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The *Burkholderia* genus contains a group of soil-dwelling Gram-negative organisms that are prevalent in warm and humid climates. Two species in particular are able to cause disease in animals, *B. mallei* primarily infects *Equus* spp. and *B. pseudomallei* (BPS), that is able to cause potentially life-threatening disease in humans. BPS is naturally resistant to many antibiotics and there is no vaccine available. Although not a specialised human pathogen, BPS possesses a large genome and many virulence traits that allow it to adapt and survive very successfully in the human host. Key to this survival is the ability of BPS to replicate intracellularly. In this review we highlight recent advances in our understanding of the intracellular survival of BPS, including how it overcomes host immune defenses and other challenges to establish its niche and then spread the infection. Knowledge of these mechanisms increases our capacity for therapeutic interventions against a well-armed foe.

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Introduction

Burkholderia pseudomallei (BPS) is a motile, Gram-negative saprophyte that dwells in the soil and surface water of hot and humid countries, notably in South East Asia and Northern Australia. It is capable of infecting a wide variety of mammals and causes the emerging disease, melioidosis, in humans. Pathogenicity manifests as either a chronic pulmonary disease when particles of contaminated soil are inhaled, alternatively as life-threatening septicaemia if the bacteria are introduced into the blood stream through dermal puncture.

Humans are an accidental host and the lack of human-to-human transmission precludes host–pathogen evolution. Yet, BPS is very successful at survival *in vivo*, in part due to genomic islands acquired through horizontal gene transfer from other bacteria [1], and in part from adaptation to environmental stress factors that confer dual protection against host immune mechanisms. BPS possesses a large genome for a bacterium of around 7.25 million bases spread over two chromosomes. Accordingly, it possesses numerous virulence factors and demonstrates remarkable plasticity in adapting to microenvironmental conditions.

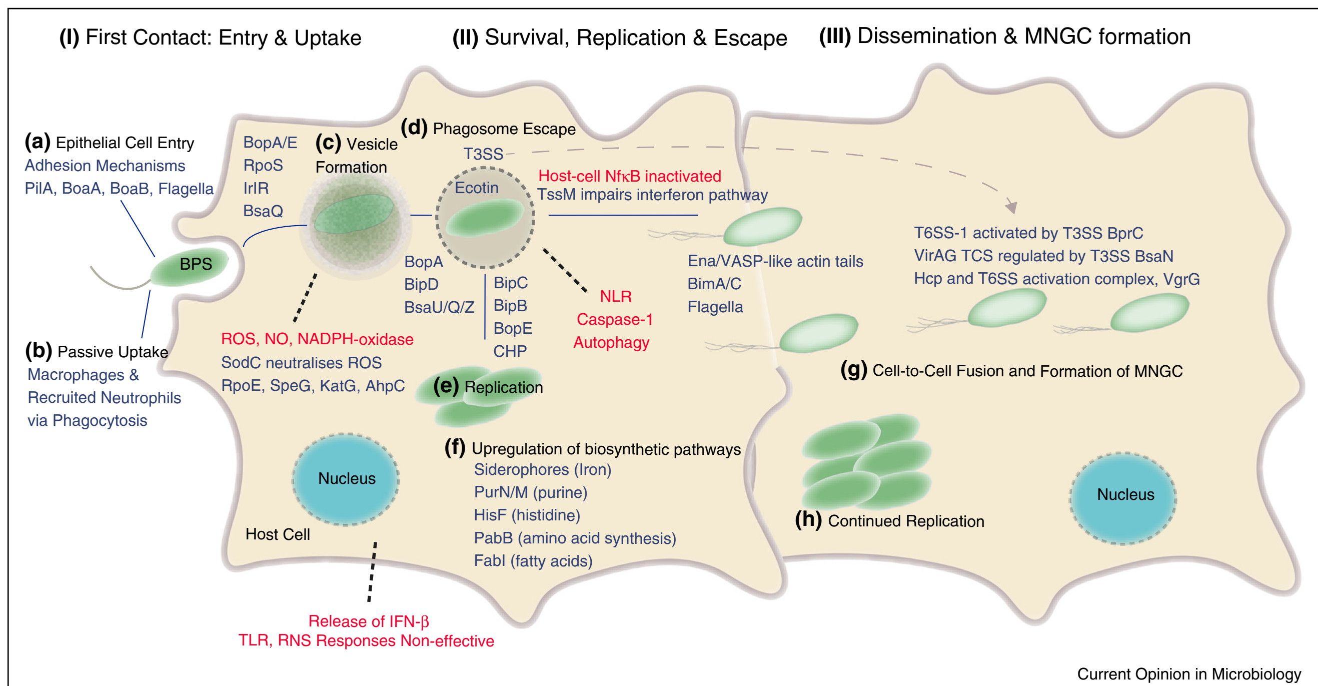
Several tools are available that help to identify virulence factors in the context of intracellular survival. A common approach is to compare the genome of BPS with a closely related avirulent species, *B. thailandensis*. A conceptually similar experiment is to compare virulent and attenuated clinical isolates derived from the same strain. Molecular based approaches such as PCR-based subtractive hybridisation have identified factors such as the membrane transporter, BPSL2033 that reduces the ability of BPS to replicate in macrophages [2]. More recently, the creation of initially small transposon-mediated signature-tagged mutant libraries in BPS [3] and now large scale libraries coupled with next generation sequencing [4] have opened new avenues to reveal the genes required by BPS to survive intracellularly.

In this review, we highlight recent reports relating to the mechanisms employed by BPS to survive and thrive inside host cells; summarised in Figure 1 and Table 1. Not only is this characteristic an essential requirement for pathogenicity, it also presents targets for the development of novel antimicrobial therapeutics against a hardy organism that naturally tolerates many existing antibiotics.

First contact with the host cell

BPS is not an obligate intracellular pathogen, but if cellular defense mechanisms are overcome it offers several advantages over an extracellular lifestyle, such as the evasion of humoral immunity, reduced exposure to antibiotics, and the potential establishment of latency. Intracellular living is also essential for localised spread from the site of infection, and eventual systemic dissemination.

Figure 1



Intracellular lifecycle summary of *Burkholderia pseudomalleii*. *B. pseudomallei* (BPS) gains access to epithelial or phagocytic cells via flagella and adhesion-related virulence factors. BPS then goes on to expresses an arsenal of virulence factors to combat the host response. (I) *Uptake and entry*: either via phagocytic cells or epithelial cells. When entering non-phagocytic cells such as epithelial cells, BPS can attach and enter host cells. Flagella, pilli, and other adhesins BoaA and BoaB are involved in this process. Passive uptake by tissue resident macrophages or neutrophils also occurs. (II) *Survival and escape*: BPS once taken up by the host cell is enveloped in an endocytic vesicle or phagasome. Upon internalisation, *B. pseudomallei* is held within a phagosome, where the T3SS is required for BPS escape. Escape occurs before maturation and killing. Resistance to oxidative stress response from the host, and production of the serine protease ecotin are also crucial steps for phagosome survival and subsequent escape. (III) BPS replication and dissemination to surrounding cells, and fusion of neighbouring cells into MNGC's. Once outside the phagosome, *B. pseudomallei* is able to replicate host actin is utilized for the polymerisation of elegantly structured Ena/VASP-like tails. These projections alongside the T6SS-1 result in successful dissemination to surrounding cells, resulting in the formation of multi-nucleated giant host cells (MNGC). Black text describes intracellular process of BPS, navy text lists genes important for intracellular survival are listed, references for which can be found in Table 1. Some of the host-combative responses are shown in red text, and the dashed-arrow indicates the connectivity between T3SS and T6SS. Actin tails are not structurally accurate.

The initial infection of a host cell depends upon invasion and adhesion factors that have been well reviewed [5]. In brief, BPS has the capability to invade cells either passively in the case of uptake by phagocytes such as resident tissue macrophages and recruited neutrophils; or actively in the case of epithelial cell infection. An advantageous trait of BPS in this regard are its flagellae, which in wet soil or aquatic environments propel the bacilli against a concentration gradient toward nutrients that may otherwise be scarce; and in the mammalian host, enable penetration of protective mucous linings and surfactant to reach epithelial cells. Other factors such as the expression of Type IV pilli are well-recognized adhesion factors, and BPS also possesses two further proteins, BoaA and BoaB that promote attachment to epithelial cells [6]. Host cell factors also play a role, such as protease-activated receptor-1, expressed by various cell types, that promotes cell ingress and is associated with BPS growth and dissemination [7].

The benefits of intracellular growth seem at odds with the characteristic of BPS to induce cell death of the infected cell. BPS is cytotoxic to dendritic cells (DC), monocytes and macrophages within hours of infection, with an absolute requirement for internalisation, caspase-1 activation, and the Type Three Secretion System (T3SS) in what has been described as a pyroptotic cell death mechanism [8]. The BPS flagellar hook-associated protein also induces cytotoxicity [9]. This rapid cell death is strongly linked to the multiplicity of infection and cell type. For example, epithelial cells seem somewhat resistant to BPS-mediated killing. Therefore, a low level infection that includes infection of epithelial cells may contribute to chronic persistence in the host, in contrast to highly inflammatory acute infection. Concurrently, BPS inhibits mechanisms of cell death that may control the infection though bacterial killing; infected neutrophils, for example, normally undergo apoptosis and are subsequently engulfed and degraded by macrophages, destroying their

Table 1

Selection of major *B. pseudomallei* intracellular virulence traits and advancements in the literature. BPS locus ID from Burkholderia.com (Winsor *et al*., 2008)

Genetic feature	General role	Reference(s)	BPS locus ID
Entry and uptake into host cells			
Boa *boaB*	Cell attachment; adhesin; autotransporter	Balder *et al*. [6] Lazar Adler *et al*. [74] Lazar Adler *et al*. [70]	BPSL1705
bpaC	Cell attachment; autotransporters; adhesin; Type V secretion system; protects from complement killing	Campos *et al*. [75] Lazar Adler *et al*. [70]	BPSL1631
Type IV pilli, pilA	Adhesion factor and intracellular mobility	Essex-Lopresti *et al*.; Boddey *et al*.	Fla1 and fla2 locus.
Metabolism and phagosome escape			
fabI -1 fabI-2	Two enoyl-ACP reductases, bpmFabI-1 and bpmFabI-2, found in BPS one on each chromosome, involved in antibacterial activity	Liu *et al*. [53] Cumming *et al*. [52]	BPSL2204 BPSS0721 (unclear if active)
sodC	Superoxide dismutase/oxidoreductase	Vanaporn *et al*. [21]	BPSL1001
ahpC	Alkyl hydroperoxide reductase; related to T-cell immunity	Loprasert *et al*. [76] Reynolds *et al*. [77]	BPSS0492
CHBP	Delays host cell maturation and apoptosis; ATP/GTP binding protein unique to BPS	Gourlay *et al*. [9]	BPSS1385
bopA *bopE*	Type three secretion system; involved in epithelial cell invasion; attenuated in murine model of infection	Stevens *et al*. [78] Pumirat *et al*. [79] Vander Broek *et al*. [80]	BPSS1525
bprD	Intracellular survival, type three secretion effector, virulence regulator	Vander Broek *et al*. [80] Chirakul *et al*. [81]	
spR	Regulators of T3SS and T6SS LexA repressor	Holden *et al*. [82] Chen *et al*. [63•] Chen *et al*. [83]	BPSL1840
eco	Serine protease activity releases BPS from vesicle, inhibits elastase	Ireland *et al*. [33]	BPSL1051
bsaN	Transcriptional regulator involved in modulation of T3SS and T6SS-1	Chen *et al*. [83] Sun *et al*. [50]	BPSS1546
BPSS1504	Affects Hcp1 secretion and integrity of the T6SS-1 apparatus	Hopf *et al*. [64]	BPSS1504
bsaS	T3SS-3 ATPase/ATP synthase SpaL	Gong *et al*. [84]	BPSS1541
vgrG-5	T6SS spike protein; membrane fusion; intracellular spread	Toesca *et al*. [66] Schwarz *et al*. [67•]	BP1026B_II1596
virAG	Two component system, T6SS regulators, sensor and histidine kinase.	Chen *et al*. (2013)	BURPS1710b_ A0530-A0532
bsaU	Involved in early onset activation of caspase-1 pathway in macrophages; T3SS	Sun *et al*. [50] Bast *et al*. [40]	BPSS1539
purM *purN*	Phosphoribosylglycinamide formyltransferase; phosphoribosylaminoimidazole synthetase purine biosynthetic pathway	Breitbach *et al*. [54]	BPSL0908 BPSL2818
fabI	Enoyl-ACP reductase; type II fatty acid biosynthesis pathway	Liu *et al*. [53]	BPSL2204
pabB/lipB	Lipoate-protein ligase B; essential for intracellular life cycle and virulence in murine model	Breitbach *et al*. [54]	BPSL0413
bipD, C, B	T3SS translocon proteins; phagolysosome survival; cell invasion protein	Gong *et al*. [36•] Vander Broek *et al*. [80]	BPSS1529 BPSS1531 BPSS1532
bpscN (formerly sctC)	TTSS1 ATPase; intracellular survival; murine pathogenesis	Warawa and Woods [85] D'Cruze *et al*. [86]	BPSS1390
sctN	Type III secretion system ATPase	Warawa and Woods [85]	BPSS1394
bsaQ	Type III secretion system; cell cycle inhibiting; invasion and escape from endocytic vesicles	Muangsombut *et al*. [35] Pumirat *et al*. [10]	BPSS1543
Spread and MNGC formation			
hcp1	Tail-spike T6ss1, MNGC cell formation, hemolysin-coregulated protein substrate and or a secretion tube (also part of T6SS)	Burtnick *et al*. [87] Lim *et al*. [62]	BPSS1498

Table 1 (*Continued*)

Genetic feature	General role	Reference(s)	BPS locus ID
bimA	Escape from the phagosome; autotransporter, actin tail formation	Stevens *et al*. [88] Sitthidet *et al*. [89] Lazar Adler *et al*. [74] Sarovich *et al*. [90] Lazar Adler *et al*. [70] Benanti *et al*. [60*]	BPSS1942
BPSL1528	Hypothetical protein with role in actin tail formation, reduced intracellular survival, and high-grade attenuation of virulence	Pilatz *et al*. [47]	BPSL1528

contents within a few hours, but this is delayed by BPS infection [10,11].

The host cell response to BPS infection

The engagement of host pattern recognition receptors during BPS infection have been well characterized, and it has been found that the toll-like receptor repertoire offer little protective benefit or worse, contribute to disease severity and mortality [12–17]. This may in part be due to the ability of BPS to resist various TLR-dependent innate immune killing mechanisms. One such mechanism, particularly in the murine model of infection, is the generation of reactive nitrogen species (RNS). In contrast to other Gram-negative bacteria, the LPS in the cell wall of BPS is a weak inducer of the enzyme, iNOS, partly due to its inability to stimulate IFN-β [18]. Accordingly, impairment of iNOS activity in a murine model of infection does not significantly alter killing capability [19]. A related mechanism is the respiratory burst, triggered by the engagement of pathogen associated molecular patterns (PAMP) by TLR. Reactive oxygen species (ROS) are able to denature enzymes as well as damage fatty acids and DNA, and direct exposure is lethal for BPS. While the effect of ROS-mediated killing of BPS in *ex vivo* isolated macrophages or macrophage-like cell lines is modest, *in vivo* studies have shown a significant role of ROS in host survival [19].

Unlike RNS, BPS is an inducer of ROS, perhaps unavoidably so. A key protein in BPS invasion of host cells is BopE, which acts as a guanine nucleotide exchange factor for RhoGTPases, promoting actin cytoskeleton rearrangement (reviewed by Allwood *et al.* [5]). However, RhoGTPases also stimulate NAPH oxidase [20], promoting the respiratory burst. Therefore, the ability of BPS to neutralise ROS using proteins such as superoxide dismutase is a key aspect of their virulence *in vivo* [21]. Whole genome analysis has further revealed that during oxidative stress, BPS upregulates genes involved in cell wall repair and biosynthesis, as well as the peroxide scavenging enzymes, KatG and AhpC. Also discovered was an oxidative stress regulon under the control of the sigma factor, RpoE. RpoE is conserved among Gram-negative bacteria, and deletion in BPS resulted in reduced intracellular survival in macrophages and virulence in the murine model. RpoE seems to be important for maintaining expression of spermidine acetyltransferase, *speG*, under oxidative stress. Spermidine is useful for the bacteria as it acts as a free radical scavenger but excessive levels are toxic, and so SpeG is required to prevent its accumulation to damaging levels [22,23].

Restricting the exposure of BPS to toxic levels of ROS in monocytes and macrophages is potentially realised through an additional mechanism. During the initial hours post-BPS infection, there is a reduction in CFU followed by growth recovery [24–26]; this temporally correlates with activation of NADPH-oxidase that causes rapid, but only transient ROS production. Although direct modulation of NADPH-oxidase by BPS has not been experimentally demonstrated, its activity, as well as a large component of TLR-mediated signalling, depends on the transcription factor, NfκB, which BPS has been shown to actively inhibit. By undermining the transcriptional response to TLR-activation, BPS is able to disarm a key aspect of host immunity. The BPS virulence factor, TssM, inactivates NfκB through deubiquitinisation of its inhibitor, IκB, thus also impairing downstream interferon pathway activation [27]. TssM is located upstream of the T3SS, and downstream of the T6SS gene clusters. Deletion of the T3SS *bsa* cluster reduces its secretion, as does removal of the Type-Two Secretion-associated outer membrane channel, GspD [27,28].

It has been observed over many years that there is a clinical correlation between non-obese diabetes, predominantly Type 1 diabetes mellitus, and BPS infection; it is a recognized risk factor in endemic regions. Diabeties mellitus is an auto-immune disease with pleiotropic effects that generally impair the ability of the diabetic host to control bacterial infection. For example, a decreased capacity of macrophages to phagocytose and kill [29], LPS-induced generation of CD4 T-regulatory cells and impairment of TLR-mediated MyD88 inflammatory signalling [30].

Research into the molecular and immunological mechanisms that link diabetes with BPS in particular is beginning to reveal new insights into clinical observations. For example, a further mechanism by which BPS moderates

NfκB activity is by phosphorylation, and subsequent inactivation of, glycogen synthase kinase 3β (GSK3β), which usually supports NfκB activity. This is dysregulated in a rat model of diabetes, resulting in excessive TNF-α and IL12 cytokine production by peripheral blood mononuclear cells that may contribute to the higher risk of septic shock in diabetic patients with melioidosis [31]. Remarkably, BPS can alter the immune function of not only the infected host cell, but also uninfected bystander cells. BPS-infected neutrophils are able to locally inhibit $CD4^{+}$ T-cell proliferation and IFN-γ production via increased surface expression of programmed death ligand-1 (PDL1) [32•]. This trait is exacerbated diabetes mellitus patients, and the resulting loss of effective $CD4^{+}$ T-cell function may contribute to disease severity and the ability to prevent disease progression.

Phagosome escape and the type three secretion system

Following uptake by a professional phagocyte, BPS is contained in a membrane-bound vesicle that matures through endosomal contact to eventual fusion with the lysosome, an organelle that by virtue of acidic pH and degradative enzymes destroys the contents of the phagosome, allowing denatured proteins to be loaded onto MHCII molecules for surface expression and engagement of CD4 T-cell immunity.

As the phagosome matures, BPS encounters host proteases that are able to kill the compartmentalised invader. To counter this, BPS expresses a serine protease inhibitor called ecotin that enables growth in macrophages and contributes to virulence in the murine model [33]. Ultimately, however, BPS is able to escape the phagosome before phagolysosomal fusion occurs, and for this, the T3SS is essential.

The role of the T3SS in BPS intracellular fitness, and therefore virulence cannot be overstated. To provide some context, the capsule of BPS is a well-studied virulence factor that confers protection against antibiotics and host immune effectors such as complement and antibody deposition. In a murine model of acute respiratory BPS infection, capsule mutants were shown to be attenuated 6.8-fold, while T3SS mutants were attenuated 290-fold [34]. Not only did T3SS mutants show reduced survival, but were also poorly able to disseminate beyond the site of infection. Mutation of genes encoding T3SS structural components, such as *bsaQ*, impair BPS invasion of macrophages and multi- nucleated giant cell (MNGC) formation due to their inability to evade lysosomal degradation [35], as well as reducing BPS-induced cytotoxicity [8]. For this reason, T3SS-related proteins have become attractive therapeutic targets [36•].

The secreted protein effectors that are exported via the T3SS have diverse effects. For example, a cell-cycle inhibitor, CHBP (BPSS1385), is able to arrest host cell division, delay apoptosis and modify the cytoskeleton [10]. BipC, located within the *bsa* locus, contributes to host cell adhesion and invasion, intracellular replication, cytotoxicity, phagosomal escape [37]. Adjacent to *bipC* on the chromosome is *bipB*, which has similarly been reported for its role in intracellular BPS infection, including induction of MNGC and cell-to-cell spread [38•].

The T3SS-dependent liberation of BPS from the phagosome into the cytoplasm exposes the bacilli to intracellular, TLR-independent, pattern recognition receptors, namely the NOD-like receptors (NLR) and by extension to the machinery of the inflammasome [39]. Sensing of BPS PAMP, such as flagellin or the T3SS-associated proteins, BopE and BsaU by NLR4 leads to activation of caspase-1 in early infection [40], subsequently inducing rapid pyroptosis of infected cells, thus partly explaining the mechanism of BPS-induced cytotoxicity and the pre-requirement of T3SS-dependent phagosomal escape. While BopE is a secreted effector of the T3SS, BsaU is a component of the secretion apparatus itself, and its disruption compromises the secretion of other effectors, reducing NfκB-mediated inflammation in the host cell [41].

BPS infection additionally upregulates NOD2 expression [42], engagement of which leads to NfκB activation. While NOD2 is important for limiting early bacterial replication and partially contains BPS infection, it seems not to ultimately affect the outcome of survival *in vivo* [17]. BPS-mediated dysregulation of NfκB may play a role, as described above, as well an apparent NOD-2-dependent expression of suppressor of cytokine signalling 3 [42].

NOD2 activation has been reported to promote autophagy, a homeostatic process by which cellular contents are degraded and recycled. An autophagosome is formed around cytoplasmic material, meaning bacteria that have escaped the initial phagosome may once again become membrane entrapped. Autophagy has been shown to be induced by BPS infection in a T3SS-dependent manner, and is able to kill the bacteria [43,44]. However, the effectiveness of autophagy is undermined by expression of the T3SS effector protein, BopA. Although its mechanism of action remains to be fully elucidated, BopA is required for evasion of autophagy, and contributes to intracellular virulence [36•,44].

Metabolic challenges in the intracellular environment

Any intracellular organism finds itself in a conflict for resources with the host to maintain its metabolic activity. This is especially true for essential micronutrients such as iron, which is incorporated into molecules involved in multiple biological processes. Pathogens such as *Mycobacterium tuberculosis* achieve the sequestration of iron through the expression of siderophores that have a higher

affinity for the molecule than equivalent host molecules such as transferrin and lactoferrin. Ferric siderophores are then recognized by specific receptors on the bacterial surface. The BPS malleobactin siderophore family is crucial for its virulence and intracellular survival. They appear to be remarkably diverse in their peptide structure, suggesting characteristic adaptability of BPS to different microenvironmental conditions [45]. Genes that encode malleobactins as well as other BPS proteins relating to iron binding and uptake have been shown to be upregulated in experimentally induced models of iron deprivation, including oxidative stress [23,46,47]. Besides the dedicated iron-binding molecules, BPS expresses proteins with dual function such as BPSL2825 (PabB), a *para* aminobenzoate synthetase component important for metabolism and growth *in vitro* that takes on a critical role utilizing hemin as an iron source *in vivo* [47].

Metabolic flexibility is key to the ability of BPS to adapt as it moves from *in terra* to *in vivo* and it remains one of its defining features. While several *Burkholderia* spp. are similarly well equipped to thrive on multiple carbon sources, it has been postulated based on genomic interrogation that virulent species may regulate these pathways differently from avirulent species, with consequential effects on the expression of virulence factors [48]. An important requirement of pathogens, whether they are obligate or facultatively intracellular is their ability to metabolise fatty acids [49–51]. In bacteria, fatty acids are required for the formation of cell wall phospholipids and may be acquired either from existing host-derived fatty acids, through *de novo* synthesis or a combination of both. Since there is no evidence that BPS is able to acquire host-derived fatty acids, *de novo* fatty acid synthesis an attractive target for chemotherapeutic intervention. FabI is an enoyl-acyl carrier protein reductase, a key enzyme of the type II fatty acid synthesis system, and has been shown to be required for BPS virulence in the murine model of infection. It has since been successfully targeted by specific chemical inhibitors [52,53], representing a promising approach to drug design.

In addition to these processes, anabolic pathways, while perhaps not traditionally considered virulence factors are nonetheless essential for intracellular fitness. Anabolism requires ATP energy, for which the synthesis of purine, encoded by the *pur* gene cluster is required, purine also being a vital component of nucleic acids [54]. BPS differs from its host in that it is able to synthesise several amino acids and vitamins that humans cannot and instead acquire from dietary intake. This also makes them an attractive target for antimicrobial therapy. For example, histidine synthesis, encoded by the *his* gene cluster, is required for intracellular growth of BPS, and this area is also being explored for potential future vaccine design [54].

Cell to cell spread and the Type Six Secretion System

BPS is able to spread beyond the site of initial infection, disseminating through the host and potentially infecting any organ or tissue. One strategy is to use the infected host cell itself as a vehicle. BPS has been shown to remain viable within, and induce maturation and migration of, immature DCs *in vitro*, a phenomenon seen in other infections as a route for transport to secondary lymphoid organs *in vivo*; although seemingly only a minority of BPS-infected DCs translocate to the lymph *in vivo* [55].

More localised spread occurs via a distinctive feature of BPS infection, namely its ability to create MNGC. Arising from the fusion of cell membranes between an infected cell and a neighbouring, uninfected cell, it is a phenomenon that enables continued intracellular replication, and cell to cell spread without the requirement of an extracellular step and the consequences that entails regarding host immunity. BPS is able to form MNGC in both phagocytic and non-phagocytic cells within hours of infection [56], and the eventual death of the MNGC results in plaque formation [57•] that is damaging to the host, and may serve as a niche for further BPS replication.

It is well established that cell–cell spread and induction of MNGC requires bacterial motility. In addition to the two flagellin encoding genes, *fla2* on chromosome 2 and *fla1* on chromosome 1, BPS achieves intracellular motility through the action of the autotransporter, BimA. Localising to one pole of the bacillus, it binds host actin, polymerisation of which pushes the bacteria through the cytoplasm, leaving in its wake characteristic 'comet tails'. BimA localisation is not itself directed to the poles, rather it is transported equally across the bacterial inner membrance [58]. BimA localisation depends on the polarity of its partner protein, BimC, which functions through an iron-dependent mechanism that remains to be fully described.

Interestingly, BimA is also expressed by *B. thailandensis* which is avirulent in humans. Recent published work has revealed the finer distinction responsible for virulence: whereas *B. thailandensis* BimA mimics the host Arp3/3 actin polymerase complex, BimA from BPS and *B. mallei*, another pathogenic member of the genus that causes the disease, glanders, in horses, instead mimic the far more efficient host Ena/VASP complex to initiate and elongate actin filaments [59,60•]. The result is that while *B. thailandensis* BimA is able to generate branched actin networks, BPS BimA generates multiple bundles of filaments; moreover, BPS BimA is able to generate more actin tails and larger MNGC compared with *B. mallei* BimA, highlighting the key importance of actin-based motility for cell–cell spread in the host.

In addition to bacterial motility, a key requirement for cell fusion and MNGC formation is the Type Six

Secretion System (T6SS), disruption of which attenuates virulence *in vivo*. BPS possesses a predicted six T6SS gene clusters, which are thought to have evolved as a weapon for inter-bacterial competition [61] within the rhizosphere, the native microbiome of BPS.

While the T3SS is not directly required for MNGC formation, it is a prerequisite that the bacteria are able to escape the phagocytic vacuole [39,57•]. Furthermore, there is cross-talk between the T6SS and T3SS; for example, T6SS1 expression is regulated by BprC, which is located in the T3SS gene cluster. The T6SS is upregulated by several orders of magnitude upon infection of host cells, and this is largely regulated by the two-component histidine kinase sensor, VirAG [62], which is itself regulated by a T3SS regulator, BsaN [63•]. Mutagenesis studies have revealed that abrogation of both *virAG* and *bprC* function attenuates virulence in the murine model of BPS infection [63•].

VirAG activates the promoter of a key T6SS effector, hemolysin-coregulated protein (Hcp), located in gene cluster five out of the six BPS T6SS gene clusters [63•]. Solving the crystal structure of BPS Hcp has revealed unique variations in its tertiary structure that are not present in orthologues from avirulent species, hinting at its key role in virulence.

Hcp polymerases the hexameric ring structures that comprise the T6SS, forming an overall structure that is akin to the bacteriophage tailspike. Hcp is also secreted by the T6SS, dependant on the protein, BPSS1504 [64], and functions as a chaperone for other secreted effectors, localising to the infected cell plasma membrane. Exogenous recombinant Hcp also localises to the host cell surface, preferentially targeting antigen presenting cells. Accordingly, it has been found to be highly immunogenic and reacts strongly with sera from melioidosis patients [65]. The consequence of this is not clear, but it has been reported that binding of Hcp induces the anti-inflammatory cytokines, IL-10 and TGF-β, potentially interfering with the mounting of a robust immune response to other epitopes besides Hcp.

Activation of T6SS is a dynamic, multi-step process that begins with assembly of the inner tube, comprised of Hcp, its outer sheath and baseplate at the bacterial cell wall-host cell membrane interface. The tip of the T6SS spike is comprised of a trimer of the protein, Valine-glycine repeat protein G (VgrG) [66]. VgrG tranverses the inner membrane, periplasmic space and peptidoglycan layer of the bacterial cell wall, while the elongation of the tail spike extends into the bacterial cytoplasm.

Upon contact with the host cell membrane, the T6SS outer sheath contracts and provides the kinetic energy for the inner tube to propel the VgrG tip, penetrating the host cell and potentially into the neighbouring cell membrane [67•]. At this stage, T6SS effector proteins are also delivered. The recruitment of ATPase then disassembles the outer sheath proteins, which are recycled [68]. The contraction of the T6SS may serve to pull the two membranes close enough whereupon, according to the hemifusion paradigm, membrane fusion occurs when proximity is $<$1 nM [69], resulting in a fused, multinucleated cell. Although VgrG has been shown to be functionally conserved across *Burkholderia* spp., some species such as *B. oklahomensis* lack the ability of cell–cell spread, suggesting other requisites than VgrG alone [66].

Conclusions and future perspectives

Much has been learned in recent years about how BPS is able to infect human cells, evade immune killing mechanisms and replicate intracellularly. This review is not an exhaustive list of BPS virulence factors. Several are the subject of ongoing research to fully explain their function, such as *boaB*, *bpaC* and *bpaE*, which all contribute to virulence in the murine infection model [70]. Still more targets remain to be identified and understood, and newly emerging tools will help to expedite these efforts. For example, transposon-directed insertion-site sequencing (TraDIS) also known as TnSeq, has facilitated the discovery of the essential gene set of BPS [4] and *B. thailandensis* [71] and has very recently been applied to elucidate virulence genes in the acute model of *in vivo* infection [72]. A particular remaining challenge is to understand the establishment of chronic infection with BPS, which may demand novel treatment therapies in the clinical setting [73]. We have gained important insights into the pathogenicity of BPS, for example the significance of the T3SS and T6SS, and some of the basic cell biology of infection. Further work is needed to understand the subtleties of host-pathogen interactions that result in, or fail to result in, protective host immunity. Together these efforts will contribute to our ability to counteract the emerging threat of this well-armed pathogen.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest

1. Tumapa S *et al.*: ***Burkholderia pseudomallei* genome plasticity associated with genomic island variation**. *BMC Genom* 2008, **9**:190.

2. Puah SM *et al.*: **Molecular characterization of putative virulence determinants in *Burkholderia pseudomallei***. *Scientific World J* 2014, **2014**:590803.

3. Cuccui J *et al.*: **Development of signature-tagged mutagenesis in *Burkholderia pseudomallei* to identify genes important in survival and pathogenesis**. *Infect Immun* 2007, **75**:1186-1195.

4. Moule MG *et al.*: **Genome-wide saturation mutagenesis of *Burkholderia pseudomallei* K96243 predicts essential genes and novel targets for antimicrobial development**. *MBio* 2014, **5**:e00926-13.

5. Allwood EM *et al.*: **Strategies for intracellular survival of *Burkholderia pseudomallei***. *Front Microbiol* 2011, **2**:170.

6. Balder R *et al.*: **Identification of *Burkholderia mallei* and *Burkholderia pseudomallei* adhesins for human respiratory epithelial cells**. *BMC Microbiol* 2010, **10**:250.

7. Kager LM *et al.*: **Deficiency of protease-activated receptor-1 limits bacterial dissemination during severe Gram-negative sepsis (melioidosis)**. *Microbes Infect* 2014, **16**:171-174.

8. Sun GW *et al.*: **Caspase-1 dependent macrophage death induced by *Burkholderia pseudomallei***. *Cell Microbiol* 2005, **7**:1447-1458.

9. Gourlay LJ *et al.*: **From crystal structure to in silico epitope discovery in the *Burkholderia pseudomallei* flagellar hook-associated protein FlgK**. *FEBS J* 2015, **282**:1319-1333.

10. Pumirat P *et al.*: **Analysis of the prevalence, secretion and function of a cell cycle-inhibiting factor in the melioidosis pathogen *Burkholderia pseudomallei***. *PLoS One* 2014, **9**:e96298.

11. Chanchamroen S *et al.*: **Human polymorphonuclear neutrophil responses to *Burkholderia pseudomallei* in healthy and diabetic subjects**. *Infect Immun* 2009, **77**:456-463.

12. West TE *et al.*: **Activation of Toll-like receptors by *Burkholderia pseudomallei***. *BMC Immunol* 2008, **9**:46.

13. West TE *et al.*: **Toll-like receptor 4 region genetic variants are associated with susceptibility to melioidosis**. *Genes Immun* 2012, **13**:38-46.

14. Wiersinga WJ *et al.*: **Toll-like receptor 2 impairs host defense in gram-negative sepsis caused by *Burkholderia pseudomallei* (Melioidosis)**. *PLoS Med* 2007, **4**:e248.

15. West TE *et al.*: **Impaired TLR5 functionality is associated with survival in melioidosis**. *J Immunol* 2013, **190**:3373-3379.

16. West TE, Hawn TR, Skerrett SJ: **Toll-like receptor signaling in airborne *Burkholderia thailandensis* infection**. *Infect Immun* 2009, **77**:5612-5622.

17. Myers ND *et al.*: **The role of NOD2 in murine and human melioidosis**. *J Immunol* 2014, **192**:300-307.

18. Utaisincharoen P *et al.*: **Involvement of beta interferon in enhancing inducible nitric oxide synthase production and antimicrobial activity of *Burkholderia pseudomallei*-infected macrophages**. *Infect Immun* 2003, **71**:3053-3057.

19. Breitbach K *et al.*: **Role of inducible nitric oxide synthase and NADPH oxidase in early control of *Burkholderia pseudomallei* infection in mice**. *Infect Immun* 2006, **74**:6300-6309.

20. Bokoch GM, Diebold BA: **Current molecular models for NADPH oxidase regulation by Rac GTPase**. *Blood* 2002, **100**:2692-2696.

21. Vanaporn M *et al.*: **Superoxide dismutase C is required for intracellular survival and virulence of *Burkholderia pseudomallei***. *Microbiology* 2011, **157(Pt 8)**:2392-2400.

22. Limsuwun K, Jones PG: **Spermidine acetyltransferase is required to prevent spermidine toxicity at low temperatures in *Escherichia coli***. *J Bacteriol* 2000, **182**:5373-5380.

23. Jitprasutwit S *et al.*: **Transcriptional profiles of *Burkholderia pseudomallei* reveal the direct and indirect roles of Sigma E under oxidative stress conditions**. *BMC Genom* 2014, **15**:787.

24. Wikraiphat C *et al.*: **Activation of NADPH oxidase is essential, but not sufficient, in controlling intracellular multiplication of *Burkholderia pseudomallei* in primary human monocytes**. *Pathog Dis* 2014, **71**:69-72.

25. Arjcharoen S *et al.*: **Fate of a *Burkholderia pseudomallei* lipopolysaccharide mutant in the mouse macrophage cell line RAW 264.7: possible role for the O-antigenic polysaccharide moiety of lipopolysaccharide in internalization and intracellular survival**. *Infect Immun* 2007, **75**:4298-4304.

26. Charoensap J *et al.*: **Differential intracellular fate of *Burkholderia pseudomallei* 844 and *Burkholderia thailandensis* UE5 in human monocyte-derived dendritic cells and macrophages**. *BMC Immunol* 2009, **10**:20.

27. Tan KS *et al.*: **Suppression of host innate immune response by *Burkholderia pseudomallei* through the virulence factor TssM**. *J Immunol* 2010, **184**:5160-5171.

28. Burtnick MN, Brett PJ, DeShazer D: **Proteomic analysis of the *Burkholderia pseudomallei* type II secretome reveals hydrolytic enzymes, novel proteins, and the deubiquitinase TssM**. *Infect Immun* 2014, **82**:3214-3226.

29. Rich J, Lee JC: **The pathogenesis of *Staphylococcus aureus* infection in the diabetic NOD mouse**. *Diabetes* 2005, **54**:2904-2910.

30. Wang J *et al.*: **Multiple mechanisms involved in diabetes protection by lipopolysaccharide in non-obese diabetic mice**. *Toxicol Appl Pharmacol* 2015, **285**:149-158.

31. Maniam P *et al.*: **Regulatory role of GSK3beta in the activation of NF-kappaB and modulation of cytokine levels in *Burkholderia pseudomallei*-infected PBMC isolated from streptozotocin-induced diabetic animals**. *Trop Biomed* 2015, **32**:36-48.

32. • Buddhisa S *et al.*: **Programmed death ligand 1 on *Burkholderia pseudomallei*-infected human polymorphonuclear neutrophils impairs T cell functions**. *J Immunol* 2015, **194**:4413-4421.
This article is notable for describing a mechanism by which BPS may suppress host signalling to hamper adaptive immunity, particularly in type 2 diabetes, a major risk factor for melioidosis.

33. Ireland PM *et al.*: **The serine protease inhibitor Ecotin is required for full virulence of *Burkholderia pseudomallei***. *Microb Pathog* 2014, **67–68**:55-58.

34. Gutierrez MG, Pfeffer TL, Warawa JM: **Type 3 secretion system cluster 3 is a critical virulence determinant for lung-specific melioidosis**. *PLoS Negl Trop Dis* 2015, **9**:e3441.

35. Muangsombut V *et al.*: **Inactivation of *Burkholderia pseudomallei* bsaQ results in decreased invasion efficiency and delayed escape of bacteria from endocytic vesicles**. *Arch Microbiol* 2008, **190**:623-631.

36. • Gong L *et al.*: **The *Burkholderia pseudomallei* type III secretion system and BopA are required for evasion of LC3-associated phagocytosis**. *PLoS One* 2011, **6**:17852.
This article highlights the importance of the type three secretion systems in the evasion of host innate immunity through the transport of effector proteins such as BopA and BipD.

37. Kang WT *et al.*: **Functional characterizations of effector protein BipC, a type III secretion system protein, in *Burkholderia pseudomallei* pathogenesis**. *J Infect Dis* 2015, **211**:827-834.

38. • Suparak S *et al.*: **Multinucleated giant cell formation and apoptosis in infected host cells is mediated by *Burkholderia pseudomallei* type III secretion protein BipB**. *J Bacteriol* 2005, **187**:6556-6560.
This article describes a notable feature of BPS infection, the formation of multinucleated giant cells, and the role of the protein, BipB.

39. Teh BE *et al.*: **Type three secretion system-mediated escape of *Burkholderia pseudomallei* into the host cytosol is critical for the activation of NFkappaB**. *BMC Microbiol* 2014, **14**:115.

40. Bast A *et al.*: **Caspase-1-dependent and -independent cell death pathways in *Burkholderia pseudomallei* infection of macrophages**. *PLoS Pathog* 2014, **10**:e1003986.

41. Hii CS *et al.*: **Interleukin-8 induction by *Burkholderia pseudomallei* can occur without Toll-like receptor signaling but requires a functional type III secretion system**. *J Infect Dis* 2008, **197**:1537-1547.

42. Pudla M *et al.*: **Nucleotide-binding oligomerization domain-containing protein 2 regulates suppressor of cytokine signaling 3 expression in *Burkholderia pseudomallei*-infected mouse macrophage cell line RAW 264.7**. *Innate Immun* 2011, **17**:532-540.

43. Rinchai D *et al.*: **Macroautophagy is essential for killing of intracellular *Burkholderia pseudomallei* in human neutrophils**. *Autophagy* 2015, **11**:748-755.

44. Cullinane M *et al.*: **Stimulation of autophagy suppresses the intracellular survival of *Burkholderia pseudomallei* in mammalian cell lines**. *Autophagy* 2008, **4**:744-753.

45. Franke J, Ishida K, Hertweck C: **Plasticity of the malleobactin pathway and its impact on siderophore action in human pathogenic bacteria**. *Chemistry* 2015, **21**:8010-8014.

46. Alice AF *et al.*: **Genetic and transcriptional analysis of the siderophore malleobactin biosynthesis and transport genes in the human pathogen *Burkholderia pseudomallei* K96243**. *J Bacteriol* 2006, **188**:1551-1566.

47. Pilatz S *et al.*: **Identification of *Burkholderia pseudomallei* genes required for the intracellular life cycle and in vivo virulence**. *Infect Immun* 2006, **74**:3576-3586.

48. Challacombe JF *et al.*: **Interrogation of the *Burkholderia pseudomallei* genome to address differential virulence among isolates**. *PLoS One* 2014, **9**:e115951.

49. Lee W *et al.*: **Intracellular *Mycobacterium tuberculosis* exploits host-derived fatty acids to limit metabolic stress**. *J Biol Chem* 2013, **288**:6788-6800.

50. Sun Y, O'Riordan MX: **Branched-chain fatty acids promote *Listeria monocytogenes* intracellular infection and virulence**. *Infect Immun* 2010, **78**:4667-4673.

51. Vaughan AM *et al.*: **Type II fatty acid synthesis is essential only for malaria parasite late liver stage development**. *Cell Microbiol* 2009, **11**:506-520.

52. Cummings JE *et al.*: **The *Burkholderia pseudomallei* enoyl-acyl carrier protein reductase FabI1 is essential for in vivo growth and is the target of a novel chemotherapeutic with efficacy**. *Antimicrob Agents Chemother* 2014, **58**:931-935.

53. Liu N *et al.*: **Mechanism and inhibition of the FabI enoyl-ACP reductase from *Burkholderia pseudomallei***. *J Antimicrob Chemother* 2011, **66**:564-573.

54. Breitbach K, Kohler J, Steinmetz I: **Induction of protective immunity against *Burkholderia pseudomallei* using attenuated mutants with defects in the intracellular life cycle**. *Trans R Soc Trop Med Hyg* 2008, **102(Suppl. 1)**:S89-S94.

55. Williams NL *et al.*: **Migration of dendritic cells facilitates systemic dissemination of *Burkholderia pseudomallei***. *Infect Immun* 2014, **82**:4233-4240.

56. Pegoraro G *et al.*: **A high-content imaging assay for the quantification of the *Burkholderia pseudomallei* induced multinucleated giant cell (MNGC) phenotype in murine macrophages**. *BMC Microbiol* 2014, **14**:98.

57. • French CT *et al.*: **Dissection of the Burkholderia intracellular life cycle using a photothermal nanoblade**. *Proc Natl Acad Sci U S A* 2011, **108**:12095-12100.
This research uses a novel technology to bypass host cell surface receptors to analyse the intracellular host-pathogen interactions. Key differences between Bth and BPS are elucidated and contribute to our knowledge of MNGC formation.

58. Lu Q *et al.*: **A polar-localized iron-binding protein determines the polar targeting of Burkholderia BimA autotransporter and actin tail formation**. *Cell Microbiol* 2015, **17**:408-424.

59. Gouin E, Torres JJ, Cossart P: **Intracellular bacteria find the right motion**. *Cell* 2015, **161**:199-200.

60. • Benanti EL, Nguyen CM, Welch MD: **Virulent *Burkholderia* species mimic host actin polymerases to drive actin-based motility**. *Cell* 2015, **161**:348-360.
The authors describe key differences between Bm, Bth, and Bps in regards to actin based motility and the action of BimA. This sheds light on the evolution of a key virulence feature.

61. Ooi WF *et al.*: **The condition-dependent transcriptional landscape of *Burkholderia pseudomallei***. *PLoS Genet* 2013, **9**:e1003795.

62. Lim YT *et al.*: **Extended loop region of Hcp1 is critical for the assembly and function of type VI secretion system in *Burkholderia pseudomallei***. *Sci Rep* 2015, **5**:8235.

63. • Chen Y *et al.*: **Regulation of type VI secretion system during *Burkholderia pseudomallei* infection**. *Infect Immun* 2011, **79**:3064-3073.
Is a conclusive structure study of Bps Hcp1 protein and correlates Hcp1 binding of antigen presenting cells and intracellular infection following by MNGC formation with a key structural loop that, when abolished, diminishes or oblates these traits.

64. Hopf V *et al.*: **BPSS1504, a cluster 1 type VI secretion gene, is involved in intracellular survival and virulence of *Burkholderia pseudomallei***. *Infect Immun* 2014, **82**:2006-2015.

65. Chieng S, Mohamed R, Nathan S: **Transcriptome analysis of *Burkholderia pseudomallei* T6SS identifies Hcp1 as a potential serodiagnostic marker**. *Microb Pathog* 2015, **79**:47-56.

66. Toesca IJ, French CT, Miller JF: **The Type VI secretion system spike protein VgrG5 mediates membrane fusion during intercellular spread by pseudomallei group *Burkholderia* species**. *Infect Immun* 2014, **82**:1436-1444.

67. • Schwarz S *et al.*: **VgrG-5 is a Burkholderia type VI secretion system-exported protein required for multinucleated giant cell formation and virulence**. *Infect Immun* 2014, **82**:1445-1452.
Presents support for the different Bthai T6SS playing different roles for Bthai indicating T6SS-5 during eukaryotic cell infection or T6SS-1 for battling other bacteria in an environmental or bacterial community life-style.

68. Zoued A *et al.*: **Architecture and assembly of the Type VI secretion system**. *Biochim Biophys Acta* 2014, **1843**:1664-1673.

69. Martens S, McMahon HT: **Mechanisms of membrane fusion: disparate players and common principles**. *Nat Rev Mol Cell Biol* 2008, **9**:543-556.

70. Lazar Adler NR *et al.*: **Systematic mutagenesis of genes encoding predicted autotransported proteins of *Burkholderia pseudomallei* identifies factors mediating virulence in mice, net intracellular replication and a novel protein conferring serum resistance**. *PLoS One* 2015, **10**:e0121271.

71. Gallagher LA *et al.*: **Sequence-defined transposon mutant library of *Burkholderia thailandensis***. *MBio* 2013, **4**:e00604-13.

72. Gutierrez MG, Yoder-Himes DR, Warawa JM: **Comprehensive identification of virulence factors required for respiratory melioidosis using Tn-seq mutagenesis**. *Front Cell Infect Microbiol* 2015, **5**:78.

73. Nandi T, Tan P: **Less is more: *Burkholderia pseudomallei* and chronic melioidosis**. *MBio* 2013, **4**:e00709-13.

74. Lazar Adler NR *et al.*: **Autotransporters and their role in the virulence of *Burkholderia pseudomallei* and *Burkholderia mallei***. *Front Microbiol* 2011, **2**:151.

75. Campos CG, Byrd MS, Cotter PA: **Functional characterization of *Burkholderia pseudomallei* trimeric autotransporters**. *Infect Immun* 2013, **81**:2788-2799.

76. Loprasert S *et al.*: **Compensatory increase in ahpC gene expression and its role in protecting *Burkholderia pseudomallei* against reactive nitrogen intermediates**. *Arch Microbiol* 2003, **180**:498-502.

77. Reynolds C *et al.*: ***T* cell immunity to the alkyl hydroperoxide reductase of *Burkholderia pseudomallei*: a correlate of disease outcome in acute melioidosis**. *J Immunol* 2015, **194**:4814-4824.

78. Stevens MP *et al.*: **A *Burkholderia pseudomallei* type III secreted protein, BopE, facilitates bacterial invasion of epithelial cells and exhibits guanine nucleotide exchange factor activity**. *J Bacteriol* 2003, **185**:4992-4996.

79. Pumirat P *et al.*: **Global transcriptional profiling of *Burkholderia pseudomallei* under salt stress reveals differential effects on the Bsa type III secretion system**. *BMC Microbiol* 2010, **10**:171.

80. Vander Broek CW *et al.*: **Quantitative proteomic analysis of *Burkholderia pseudomallei* Bsa type III secretion system effectors using hypersecreting mutants**. *Mol Cell Proteomics* 2015, **14**:905-916.

81. Chirakul S *et al.*: **Characterization of BPSS1521 (bprD), a regulator of *Burkholderia pseudomallei* virulence gene expression in the mouse model**. *PLoS One* 2014, **9**:e104313.

82. Holden MT *et al.*: **Genomic plasticity of the causative agent of melioidosis, *Burkholderia pseudomallei***. *Proc Natl Acad Sci U S A* 2004, **101**:14240-14245.

83. Chen Y *et al.*: **Characterization and analysis of the *Burkholderia pseudomallei* BsaN virulence regulon**. *BMC Microbiol* 2014, **14**:206.

84. Gong L *et al.*: ***Burkholderia pseudomallei* type III secretion system cluster 3 ATPase BsaS, a chemotherapeutic target for small-molecule ATPase inhibitors**. *Infect Immun* 2015, **83**:1276-1285.

85. Warawa J, Woods DE: **Type III secretion system cluster 3 is required for maximal virulence of *Burkholderia pseudomallei* in a hamster infection model**. *FEMS Microbiol Lett* 2005, **242**:101-108.

86. D'Cruze T *et al.*: **Role for the *Burkholderia pseudomallei* type three secretion system cluster 1 bpscN gene in virulence**. *Infect Immun* 2011, **79**:3659-3664.

87. Burtnick MN *et al.*: **The cluster 1 type VI secretion system is a major virulence determinant in *Burkholderia pseudomallei***. *Infect Immun* 2011, **79**:1512-1525.

88. Stevens MP *et al.*: **Identification of a bacterial factor required for actin-based motility of *Burkholderia pseudomallei***. *Mol Microbiol* 2005, **56**:40-53.

89. Sitthidet C *et al.*: **Actin-based motility of *Burkholderia thailandensis* requires a central acidic domain of BimA that recruits and activates the cellular Arp2/3 complex**. *J Bacteriol* 2010, **192**:5249-5252.

90. Sarovich DS *et al.*: **Variable virulence factors in *Burkholderia pseudomallei* (melioidosis) associated with human disease**. *PLoS One* 2014, **9**:e91682.