Ecology and Evolution of Virulent *Burkholderia pseudomallei*

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Abstract

Burkholderia pseudomallei (*Bp*), the etiological agent of melioidosis, is a gram-negative soil-dwelling pathogen endemic to Thailand and other parts of southeast Asia. Despite the great threat it poses to endemic regions as a multidrug resistant pathogen, its presence and eco-environmental properties as an inhabitant of the soil rhizosphere are not well understood. We have adapted a rapid method for estimating *Bp* diversity which utilizes PCR and gel electrophoresis to distinguish among sequence types. Among 79 cultured environmental isolates originating in small fields in Thailand with ties to confirmed cases of melioidosis, we identified two major clades. These findings will allow us to explore the relationship between different strain types and virulence. However, not all bacteria in the environment are culturable. The environmental and clinical relevance of this viable but non-culturable (VBNC) state in *Bp* is not well understood. We used a combination of culture-based enumeration of cells and the variable binding of nucleic acid stains to live and dead cells to quantify the proportion of VBNC cells in cultures of the model system *B. thailandensis* after heat shock and cold treatment and found that both heat shock and cold treatment rapidly induced the VBNC state. Characterization of VBNC *Bp* may augment our understanding of the ecological drivers of *Bp* pathogenicity and the potential threat it poses to human populations.

Introduction

Burkholderia pseudomallei (*Bp*) is a soil-dwelling, Gram-negative bacterium which is the etiological agent of melioidosis.

Average mortality rate of melioidosis is 54% and relapse is common[1,2,3,4].

B. pseudomallei is a Tier 1 Select Agent with a history of development as a potential biological weapon[5].

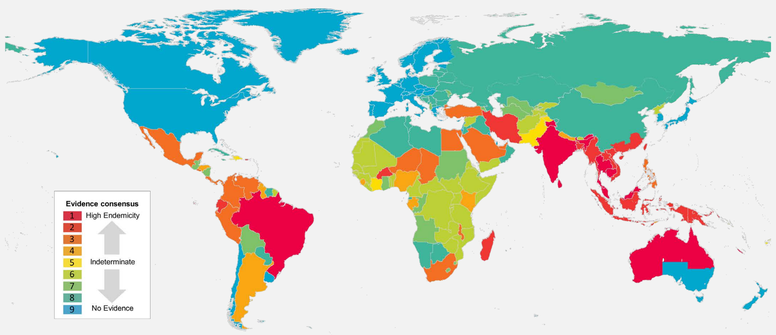


Figure 1. Global evidence consensus for *B. pseudomallei* and melioidosis endemicity[1]

Formation of Multi-Nuclear Giant Cells

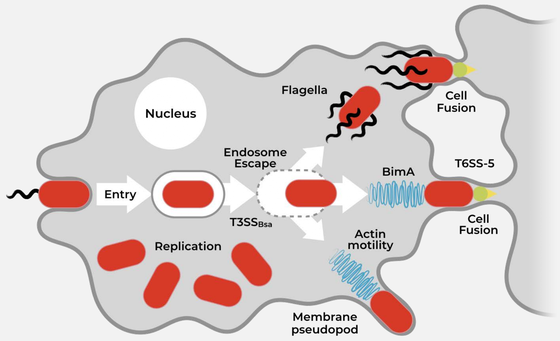


Figure 2. Intracellular lifecycle in mammalian cells

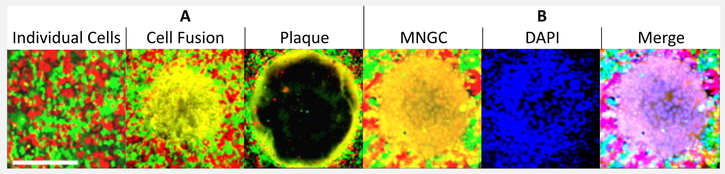
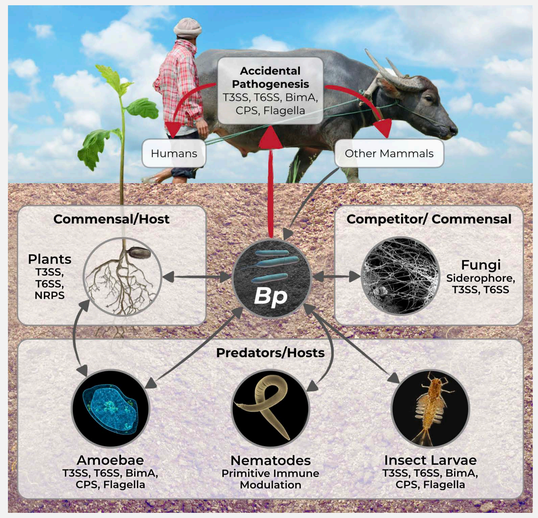


Figure 3. Intracellular spread and plaque formation occur through cell fusion[6]

Host, Predator, or Prey: What Promotes *B.pseudomallei* virulence?

Bp displays a wide host range, as infections in fungi[7], plants[8], insects[9], nematodes[10], and protozoa[11], have been reported, and these organisms are plentiful in the rhizosphere interactome.



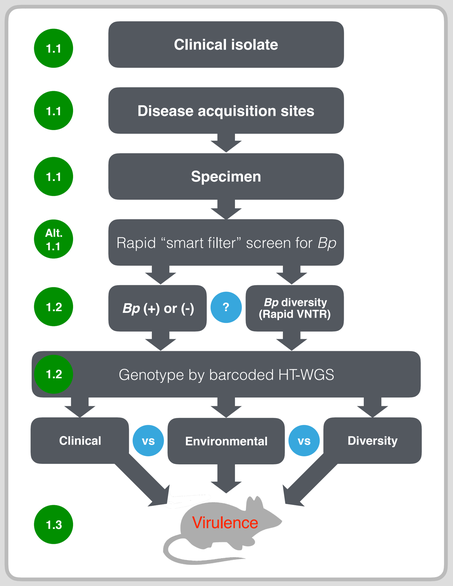
Hypothesis:

Virulence traits of *B. pseudomallei* are maintained through interactions with environmental predator, competitor, or host species.

Endemic regions can be categorized by environmental conditions and the presence of co-endemic fauna.

Figure 4. *B. pseudomallei* natural environment

Presence, Diversity, and Virulence



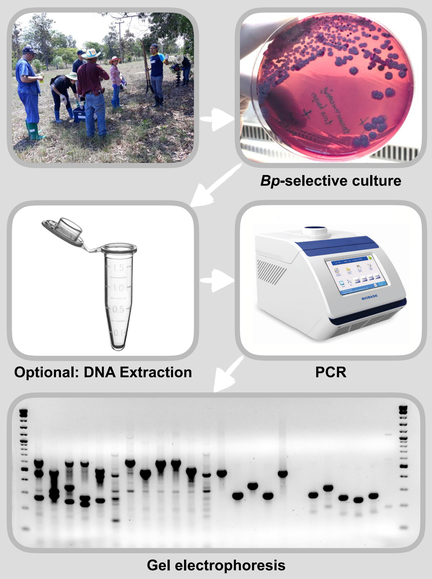
1.1 Identify sites linked to human infections and **confirm the presence of *B. pseudomallei* (*Bp*).**

1.2 Implement a rapid screening, identification and classification strategy to **Identify *Bp* strain diversity.**

1.3 Evaluate environmental and clinical strains for **differential virulence** in BALB/c mice.

Figure 5: Strategies to investigate relationships between environmental conditions, strain diversity, and virulence in mammals

Rapid Assessment of *Bp* Diversity in Environmental Samples

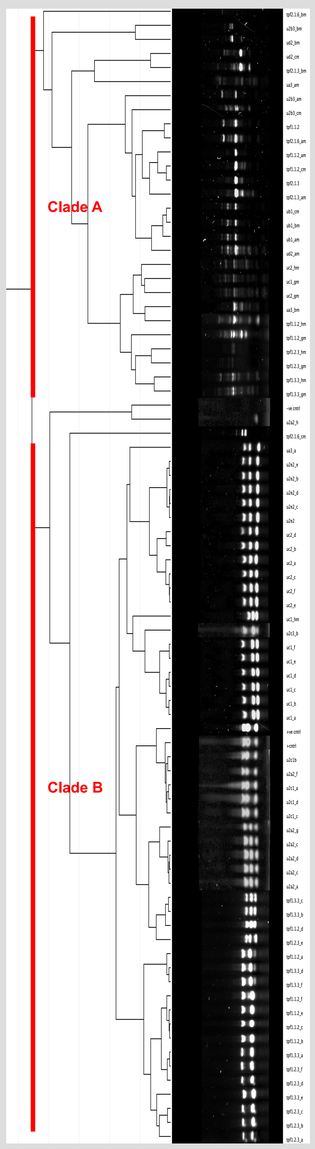


Rapid Multiplex Variable Number Tandem Repeat Assay

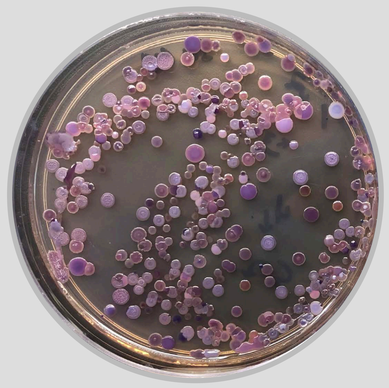
1. Sample sites in Thailand for *Bp*.
2. Isolate *Bp* using selective culture on Ashdown's medium and categorize soil isolates by colony morphology, color and growth rate.
3. Perform multiplex PCR amplifying loci 1367 k, 1934 k, and 2971 k[12] on extracted DNA or boiled colonies.
4. Gel electrophoresis reveals RAM-VNTR pattern

Figure 3. RAM-VNTR Workflow

VNTR Reveals Phylogenetic Relationships



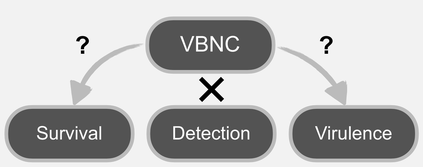
Predominant colony morphology is purple & mucoid. Likely *B. pseudomallei* near-neighbor species.



Predominant colony morphology is opaque & light blue, indicative of *B. pseudomallei*

Figure 7. RAM-VNTR performed on 79 bacterial isolates from Nakhon Si Thammarat and Ubon provinces in Thailand. Phylogenetic analysis conducted with GelJ[13].

Investigation of Viable but non-Culturable (VBNC) *Bp*



VBNC *Bp* escapes culture-based detection
VBNC role in *Bp* environmental survival and virulence is unknown
Cold and heat shock accelerate VBNC formation

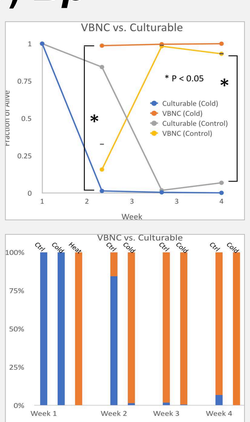


Figure 8. Top. Fraction of *B. thailandensis* cells induced to the VBNC state by cold (4°C) treatment for 21 days (>0.99). **Bottom.** Data as a stacked bar graph that also includes heat (70°C) treatment for 30 min, which appears to be as effective as cold treatment at inducing VBNC.

Acknowledgements

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