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The 4-hydroxyl-3-methyl-2-alkenylquinoline biosynthesis enzyme HmqD is involved in pathogenesis of Burkholderia pseudomallei

Sherry Mou, Jennifer Chua, Steven Tobery and David DeShazer

Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, USA

Introduction

Burkholderia pseudomallei is a pathogenic gram-negative bacterium that causes fatal melioidosis, a disease that is endemic in Southeast Asia and northern Australia. *B. pseudomallei* is an environmental saprophyte. Many mechanisms that enable *B. pseudomallei* to survive and adapt to the everchanging environmental factors translate to efficient virulence mechanisms when it enters a host cell, resulting in persistence and survival in the host cells.

There has been much attention placed on the HMAQs produced by *Burkholderia spp*. (1,2). The *Burkholderia hmqA-G* gene cluster encodes the enzymes responsible for generating HMAQs from anthranilic acid and β-keto fatty acid (3).

Recently, we reported that 4-hydroxy-3-methyl-2-alkenylquinolines(HMAQs) have antimicrobial activity and may provide a competitive advantage in the environment, but nothing is known about the role of HMAQs in the pathogenesis of *B. pseudomallei*. In this study, we generate a *hmqD* gene deleted mutant in *B. pseudomallei* JW270 strain, in vitro and in vivo assays were employed to explore the role of HMAQs in the pathogenesis of *B. pseudomallei*.

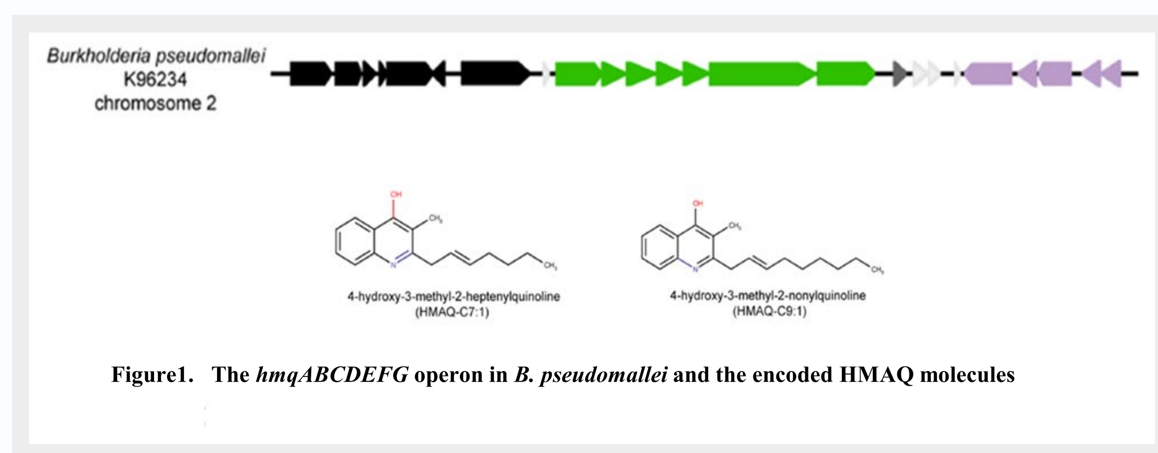

Figure1. The *hmqABCDEFG* operon in *B. pseudomallei* and the encoded HMAQ molecules

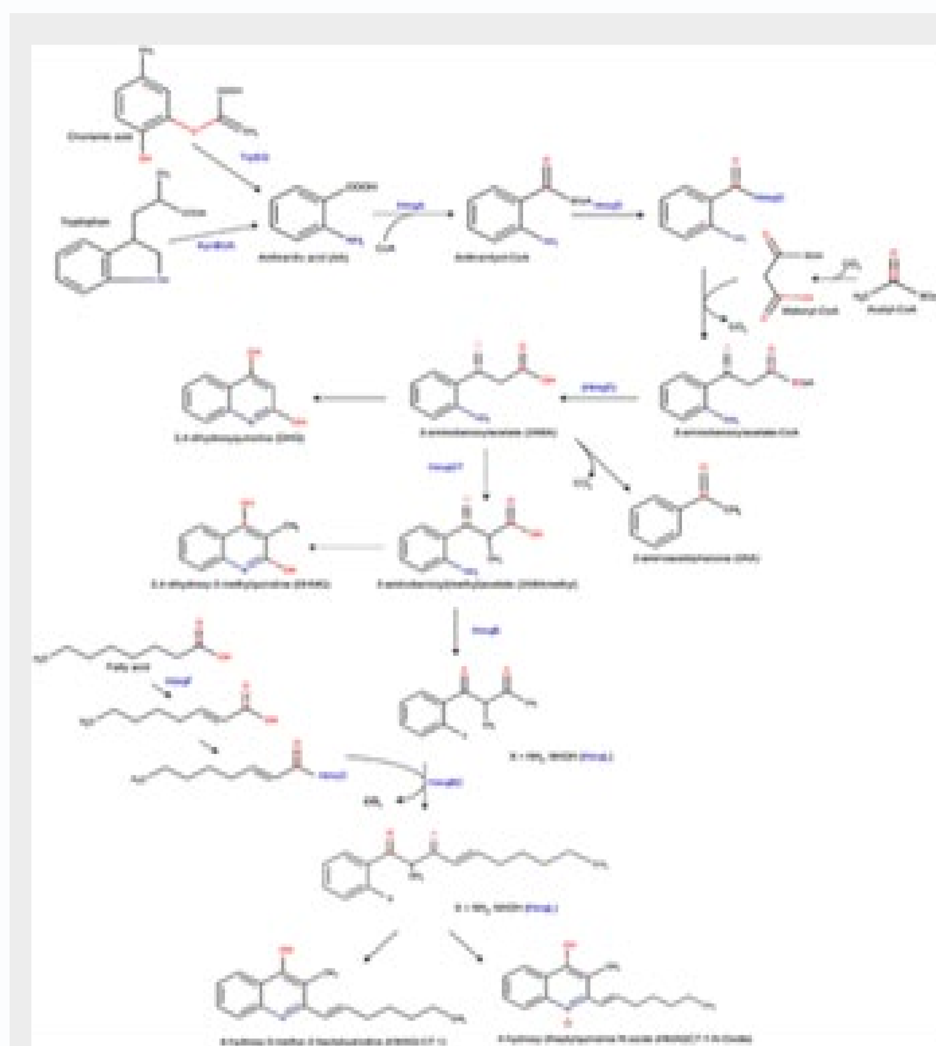

Figure2. Updated version of proposed Hmq biosynthesis pathway, based on HAQ biosynthesis in *P. aeruginosa*, produce HHQ and HMAQ both with a saturated or unsaturated acyl chain.

Methods

Bacterial strains and culture condition

The *B. pseudomallei* JW270 and mutant strain were grown in Difco Luria Broth (Lennox) at 37°C. When appropriate, antibiotics were added at the following concentration: 25μg of streptomycin (Sm) and 25μg of kanamycin (Km) per mL for *Escherichia coli* S17-1 and 25μg of polymyxin B (Pm) and 500μg of Km for *B. pseudomallei* JW270.

Mutagenesis and plasmid conjugations

Gene replacement experiments with *B. pseudomallei* JW270 were performed using *sacB*-based vector pMo130. The pMo130-*ΔhmqD* was conjugated to *B. pseudomallei* JW270 by using *E. coli* S17-1 as the donor strain. After 48 h of incubation at 37°C, individual colonies were incubated in LB broth and grown at 37°C for 16 to 18 h. Twenty-five microlite of saturated culture was spread onto LB agar lacking NaCl and containing 15% (wt/vol) sucrose and incubated at 25°C for 4 to 5 days to resolve the *sacB*-containing plasmid integrant. *B. pseudomallei* JW270 deletion mutants were identified by PCR.

Replication within macrophage-like cell line

For proliferation assays, the macrophage-like RAW 267.4 cells were infected at a MOI of 50:1 bacteria/cell. After 2 hours, cells were washed 1x with phosphate-buffered saline (PBS) (Thermo Fisher Scientific) and fresh medium containing 500 μg/ml kanamycin was added to kill the extracellular bacteria. Cells were lysed with 0.1% SDS at designated time point and the number of intracellular bacteria were enumerated by serial dilution and plating.

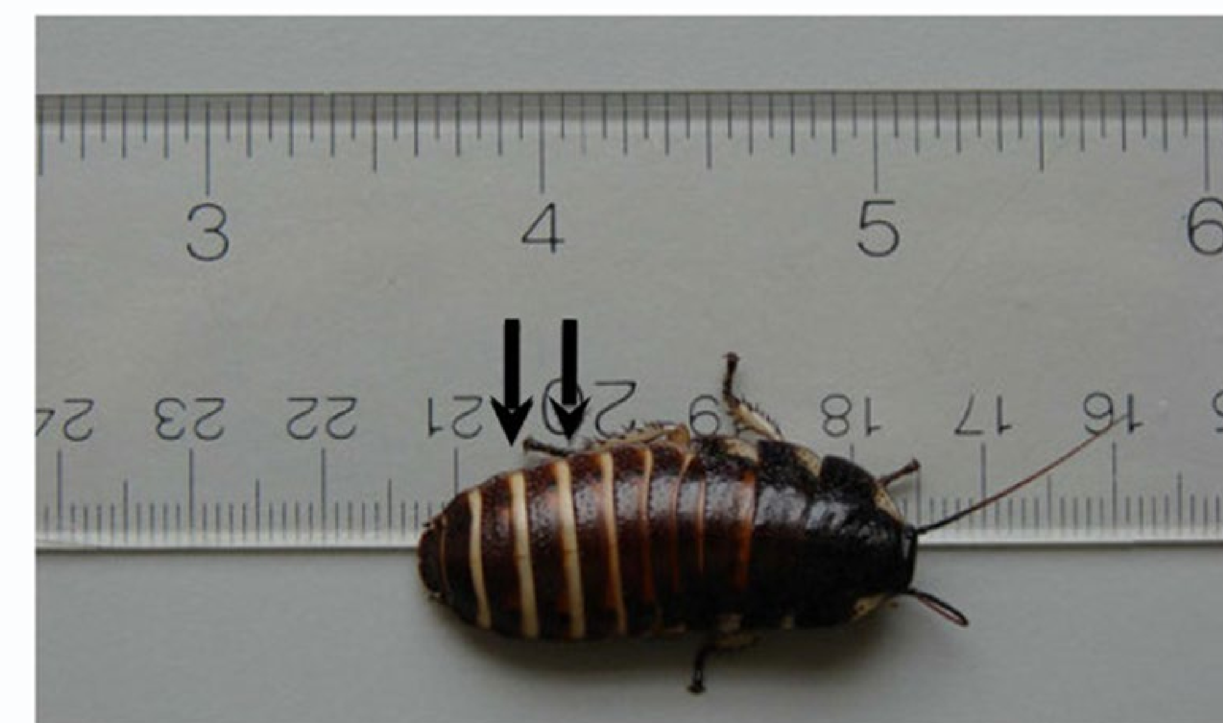

Figure 3. Madagascar Hissing Cockroach

Results

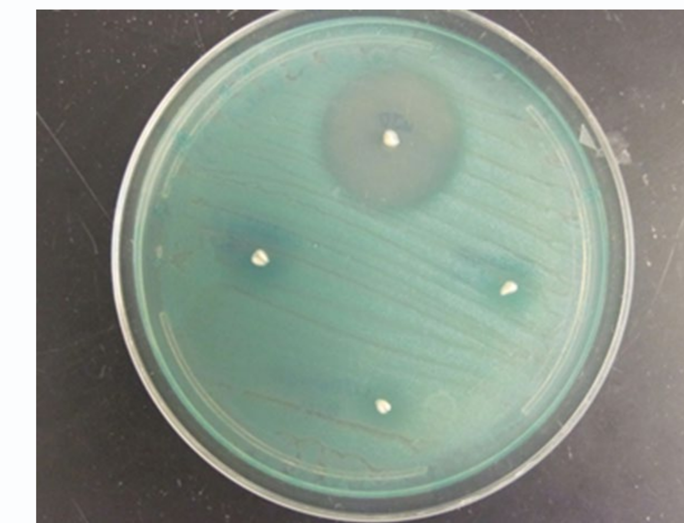

Figure 4. JW270 produced a zone of inhibition when co-cultured on a lawn of *Neobacillus bataviensis*, but not JW270 *ΔhmqD*

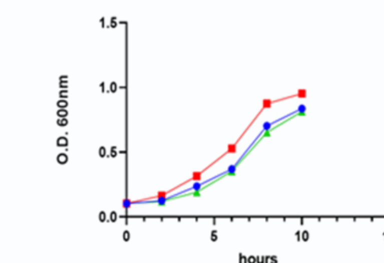

Figure 5. B. pseudomallei in vitro growth curves in rich media

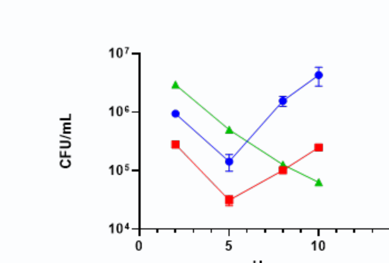

Figure 6. Kanamycin protection assay to determine *B. pseudomallei* survival and replication within RAW 264.7 macrophages

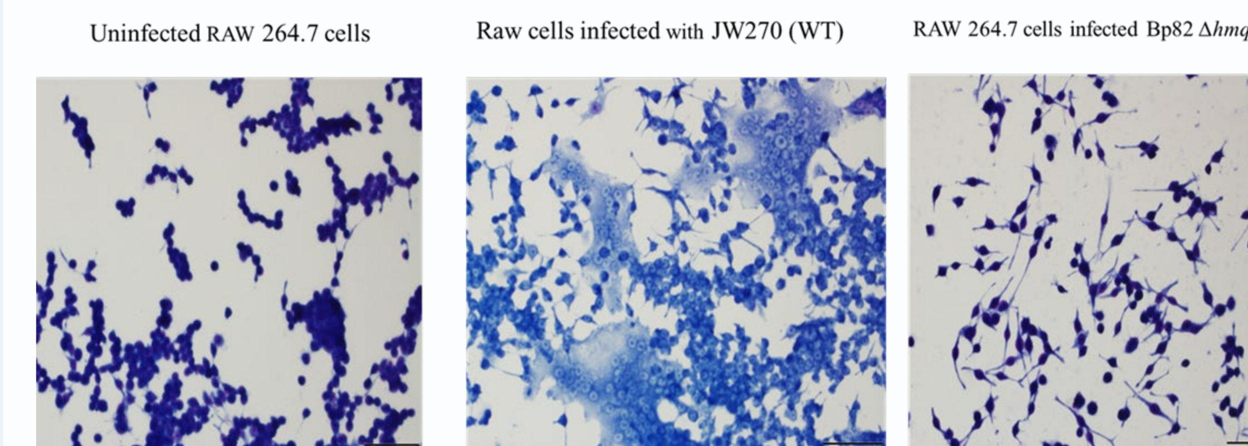

Figure 7. RAW 264.7 cells infected with JW270 and JW270 *ΔhmqD* at 12 hours

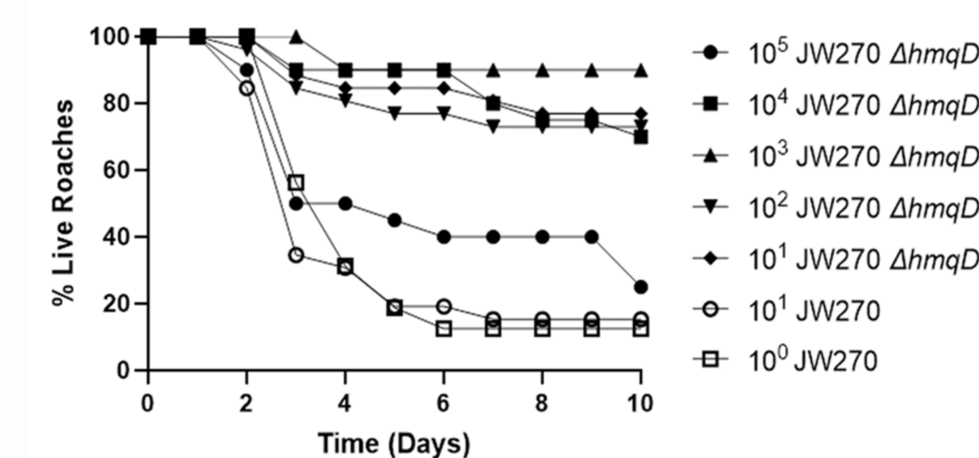

Figure 8. JW270 *ΔhmqD* is highly attenuated in Madagascar hissing cockroaches

SUMMARY

1. JW270 *ΔhmqD* did not possess antimicrobial activity or exhibit a growth defect in Luria-Bertani broth, but it was defective for survival and replication in RAW267.4 cells.
2. JW270 formed multinuclear giant cells after 12 hours of infection of RAW 267.4 cells, but JW270 *ΔhmqD* did not induce any multinuclear giant cell formation.
3. When virulence was assessed by infection of MHCs, the 50% lethal does of JW270*ΔhmqD* was ~8,000 times higher than JW270.
4. Our data suggests that the 4-hydroxyl-3-methyl-2-alkenylquinoline biosynthesis enzyme HmqD is involved in competitive fitness in the environment and pathogenesis in the host.

References

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2. Coulon PML, Groleau MC, Déziel E, 2019. Potential of the *Burkholderia cepacia* complex to produce 4-hydroxy-3-methyl-2-alkyquinolines. Front Cell Infect Microbiol 9:33.
3. Piochon M, Coulon PML, Caulet A, Groleau MC, Déziel E, Gauthier C, 2020. Synthesis and antimicrobial activity of *Burkholderia*-related 4 hydroxy-3-methyl-2-alkenylquinoline (HMAQs) and their N-oxide counterparts. J Nat Prod 83:2145-2154.

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POINTS OF CONTACT

Sherry Mou
sherry.mou2.civ@health.mil