Building the molecular tool kits to exploit Burkholderia bacteria as biotechnological agents

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Project description:
Background. Bacterial, fungal and worm pathogens destroy millions of tonnes of agricultural crops and require use of pesticides to control. Resistance to pesticides is emerging in the same way that we are seeing antibiotic resistance rise in clinical infections. Naturally protective microorganisms and their products have been successfully used as biopesticidal agents, and Gram-negative Burkholderia bacteria show considerable promise as novel antibiotic producers. We have been using a state-of-the-art genome mining approach to discover novel antibiotics in Burkholderia bacteria. We do this by determining their genome sequence and then searching for novel pathways capable of producing antibiotics. This has been highly successful and led to the discovery of several novel antibiotics, however, many more pathways of interest do not express antibiotics under normal laboratory conditions and therefore alternate approaches to activate them are required. Aim. The PhD will aim to express these silent gene clusters, optimally produce novel antibiotics and genetically engineer pathways to create novel products. To do this the student will build a state-of-the-art molecular and synthetic biology toolkit to enable exploitation of Burkholderia bacteria as biotechnological agents.

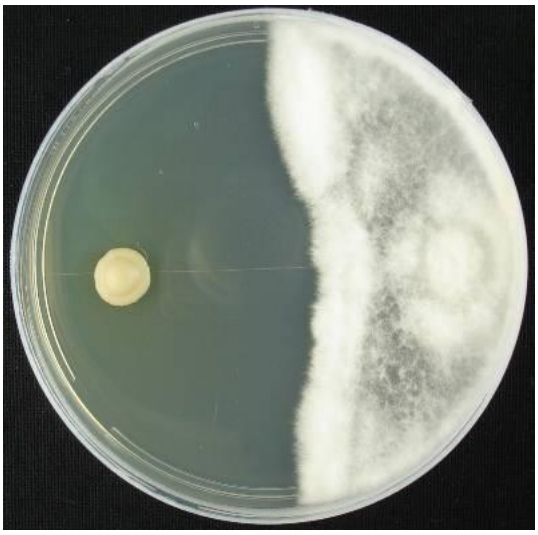
Three ways of discovering new Burkholderia antibiotics will be pursued:

(i) A CRISPR-CAS genome editing toolkit (Year 1). We have successfully employed a slow, multiple-step homing endonuclease based mutagenesis system to create unmarked mutations in Burkholderia. However, the CRISPR-CAS mutagenesis systems have revolutionised the genetic engineering of living systems. Development and application of CRISPR-CAS to manipulate known and novel Burkholderia pathways will be carried out.

(ii) A yeast pathway capture and heterologous expression toolkit (Year 2). Burkholderia natural product biosynthetic pathways are frequently large (>50 kb) and difficult to engineer. The yeast transformation associated homogous recombination system can be used capture large biosynthetic pathways and build vectors, but has not yet been applied to Burkholderia. We will apply TAR-capture and synthetic biology express novel Burkholderia antibiotic biosynthesis pathways.

(iii) A Burkholderia-fungal interaction screening toolkit (Year 3-4). Burkholderia often form close relationships with fungi (Figure 1. A Burkholderia strain inhibiting a woodland fungus). Screening laboratory collections of fungi and naturally occurring isolates will be carried out to discover novel antibiotic producing Burkholderia. Summary and training. This interdisciplinary PhD project will train students in state-of-the-art molecular

microbiology, biochemistry and antibiotic/biopesticide discovery approaches. The student will gain training in big data informatics approaches such as genome sequencing, and combining this with wet lab training to engineer, manipulate and screen for novel antibiotics.



Burkholderia fungal inhibition