Pair of Genes That Fit Just Right: Identifying Advantageous Genes in *Burkholdaria thailandensis That Confer a Fitness Advantage When Amplified*

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GOAL

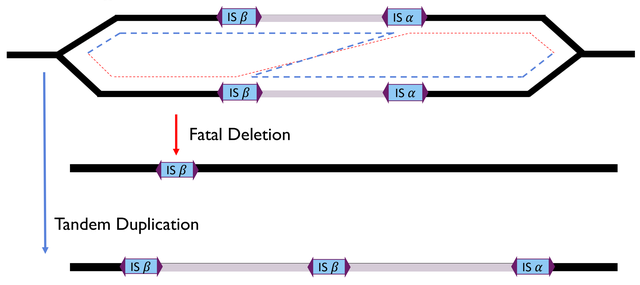
Investigate the 157 genes duplicated in *B. thailandensis* in biofilm conditions.

BACKGROUND

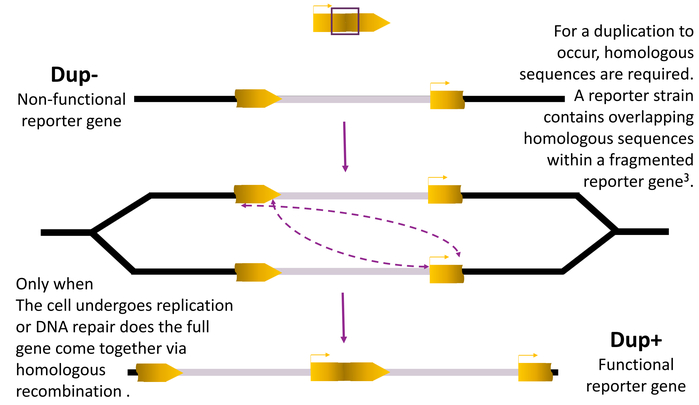
DNA sequences can be duplicated or deleted, resulting in a different copy number of coding sequences, which influences protein abundance, and often creates variable phenotypes[1,2]. DNA duplications and deletions occur at orders of magnitude more frequently than point mutations[1,2].

Burkholderia thailandensis E264 is capable of duplicating a 208.6 kb region of DNA that contains 157 genes. This duplication is transient and can be selected under different conditions. It also occurs via RecA dependent homologous recombination[3].

Homologous Recombination Model:



Mechanism to study homologous Recombination Frequency:

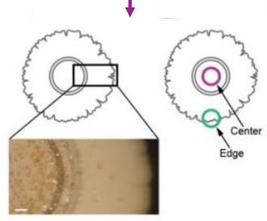


Methods, Approaches, and Current Research

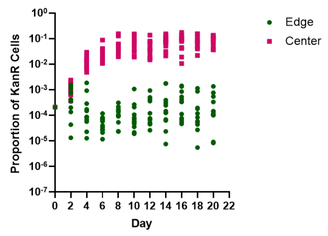
Methods: Selecting for Cells with a Duplication

Colony Biofilms

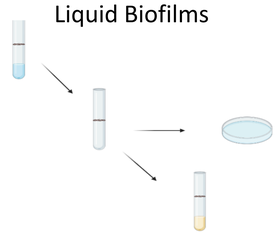
An overnight culture is normalized to an optical density of 0.2. Then, 40 ul of the sample is spotted onto an LSLB plate. The spotted sample is left to grow for 5-7 days at room temperature.



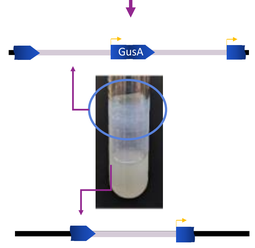
Cells are collected from the center of the biofilm, resuspended in PBS, diluted, and plated on Kanamycin and LSLB.



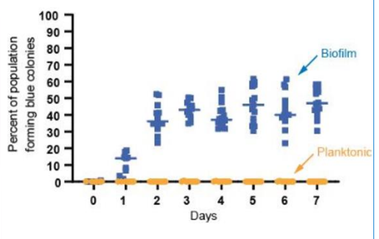
Liquid Biofilms



An overnight culture is normalized to an optical density of 0.2 and calibrated to a 3 ml liquid overnight. For 5-7 days media is removed and replaced with fresh media without disturbing the biofilm.



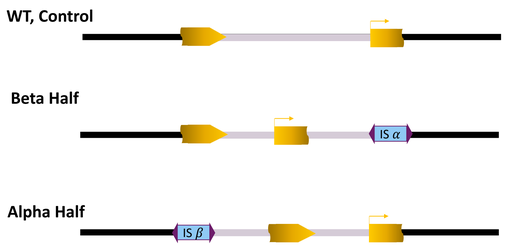
Cells are collected from the biofilm on the culture tube. These cells are resuspended, diluted, and plated on X-gluc.



Hypothesis

One or more Genes when duplicated provide an advantage to *Burkholderia thailandensis* in biofilm conditions.

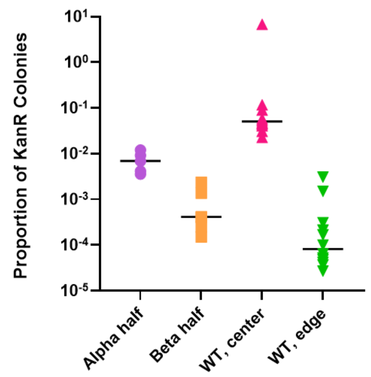
Divide and Conquer



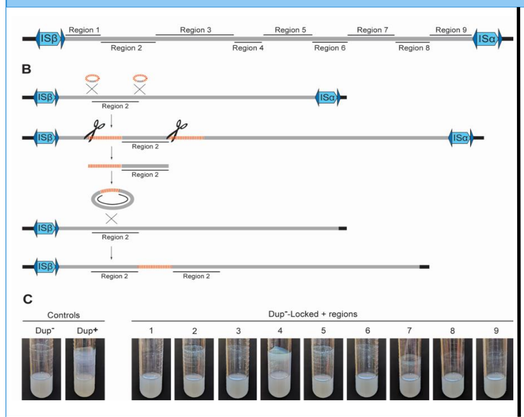
Each strain was grown as a colony biofilm. The proportion of kanamycin resistant colonies is calculated by comparing the same dilution on plates with and without antibiotics. The wild type colony biofilm is used to produce an upper and lower comparative bound.

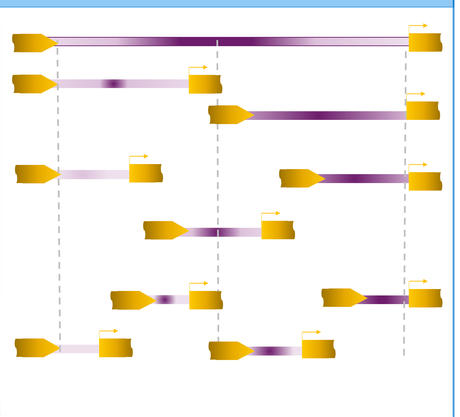
Neither half duplicated as much as the WT center, nor as little as the WT edge. This suggests that there could be advantageous genes in both halves.

By shifting the location of the homologous sequences small portions will be duplicated if amplification of this subset of genes is advantageous.



Current Research: Dual Approaches





References and Acknowledgements

1. Sandegren, L. & Andersson, D. I. Bacterial gene amplification: implications for the evolution of antibiotic resistance. Nat. Rev. Microbiol. 7, 578–588 (2009).
2. Andersson, D. I. & Hughes, D. Gene amplification and adaptive evolution in bacteria. Annu. Rev. Genet. 43, 167–195 (2009).
3. Lillian C Lowrey, Leslie A Kent, Bridgett M Rios, Angelica B Ocasio, Peggy A Cotter (2023) An IS-mediated, RecA-dependent, bet-hedging strategy in Burkholderia thailandensis eLife 12:e84327

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