Apr 25th, 9:00 AM - 11:00 AM

Transposon Mutagenesis Identification of Polymicrobial Interaction Mechanisms Between Prokaryotic and Eukaryotic Microorganisms

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Abstract

Antibiotic resistance occurs when bacteria change in response to selective pressures induced by antibiotics, which has become a major concern worldwide and one of the biggest threats to global health. Antibiotic resistance can occur naturally, but the misuse and overuse of antibiotics is accelerating the process. One way to combat this process is to understand the different relationships between microbes, also known as polymicrobial interactions. Bacteria can interact with one another synergistically or antagonistically and understanding the mechanisms behind these interactions can lead to the discovery of new therapeutics or targets to fight and kill pathogenic microbes. The rarely pathogenic Gram-negative bacterium, *Alcaligenes faecalis*, has previously been shown in our lab as playing an important role in potentially fighting antibiotic and antifungal resistance due to its competitiveness during polymicrobial interaction. Our research has found that *A. faecalis* kills *Bacillus* spp., *Staphylococcus aureus*, and *Candida albicans*. This is a unique characteristic as these targets encompass both prokaryotic (bacteria) and eukaryotic (fungi) microbes. These three species are known to cause numerous infections in humans and have increased cases of antibiotic and antifungal resistance. In the present study, we investigated the genetic elements *A. faecalis* utilizes to inhibit growth when interacting with *S. aureus, C. albicans, B. megaterium, and B. subtilis*. Transposon mutagenesis was performed to create a genetic library of *A. faecalis* loss-of-function mutants. These strains were then screened against all three microorganisms to determine which mutants no longer inhibited growth. The mutants that lacked zones-of-inhibition were sequenced to determine the gene that had been interrupted. BLAST analysis of these sequences identified a MFS transporter, a 2FE-2S iron sulfur binding protein, a mechanosensitive ion channel, and a glucose-6-phosphate isomerase as instrumental in this inhibitory mechanism. Results from this research study can be used to further study polymicrobial interactions and potentially discover new therapeutics to combat antimicrobial resistance.

Introduction

Antibiotics have been used for many years to either kill or inhibit the growth of bacteria. Although antibiotics have been beneficial in curing common infections, the misuse and overuse of these antimicrobial substances has led to many microbes developing resistance to antibiotics. A way to combat this drug resistance is to use and understand the mechanisms behind polymicrobial interactions. Polymicrobial interactions occur when one microorganism promotes or inhibits another's growth. This interaction occurs between *Alcaligenes faecalis* and *Candida albicans*, *Staphylococcus* species, and *Bacillus* species. *Candida albicans* is an opportunistic fungal pathogen in humans that has the ability to colonize much of the human tissue and organ system causing serious, invasive infections. *Staphylococcus aureus* is a gram-positive, opportunistic bacterium that has developed a strain known as Methicillin-resistant *Staphylococcus aureus* (MRSA), which is responsible for several difficult-to-treat infections due to its resistance to antibiotics. The *Bacillus* species are gram-positive, rod-shaped bacterium and foodborne pathogens that produces toxins causing gastrointestinal illnesses. Our laboratory has found that *Alcaligenes faecalis* inhibits the growth of the *Candida* species, *Staphylococcus* species, and *Bacillus* species. *A. faecalis* is a gram-negative, rod-shaped bacterium commonly found in soil, water, and environments associated with humans. With this information in mind, it is important to determine the exact mechanism in which *A. faecalis* inhibits these organisms through polymicrobial interactions in order to discover new therapeutics or targets to treat bacterial and fungal infections.

Figure 1

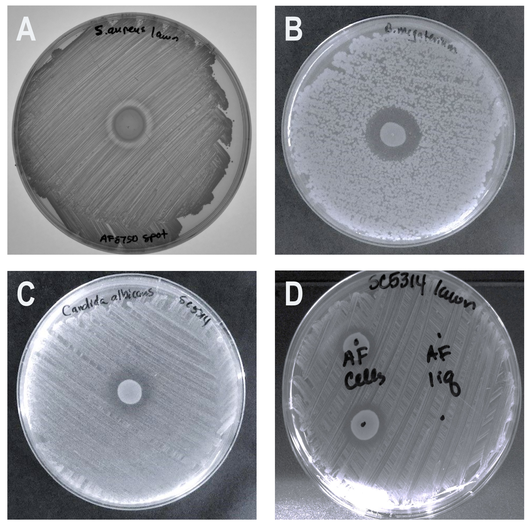

Figure 1: *Alcaligenes faecalis* produces zones of inhibition (ZOI) against both Prokaryotic & Eukaryotic microbes. Overnight cultures of *A. faecalis* were spotted on microbial lawns: (A) *Staphylococcus aureus*, (B) *Bacillus megaterium*, (C) *Candida albicans* and incubated for 24h at 37ºC and monitored for Zones of Inhibition. (D) *A. faecalis* cells (left) or *A. faecalis* cell-free spent media (right) were spotted on *C. albicans* lawns, incubated for 24h at 37ºC and monitored for Zones of Inhibition

Figure 2

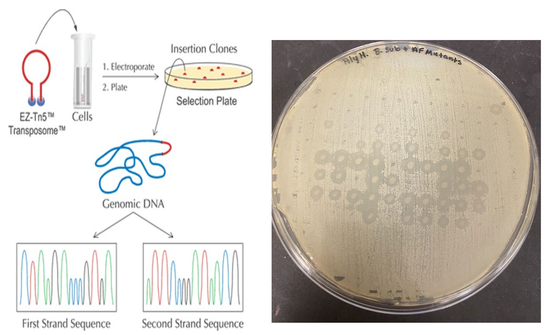

Figure 2: Generation of a transposon loss-of-function *A. faecalis* library. Illustration of the method of delivering the EZ-Tn5 transposon to *A. faecalis* genome to create a library of random transposon mutants (left) and a representative photo of screening the *A. faecalis* library mutants for the loss-of-function phenotype on competing *B. Subtilis l*awns. Circles denote the loss-of-function phenotype (right).

Figure 3

Mutant ID	Interrupted Gene	Gene Function in Cell
AM25 & SA1	MFS Transporter	Superfamily of secondary active transporters
AM230	Mechanosensitive ion channel	Multimeric integral membrane proteins
CAHB	Di-iron oxygenase	Metabolic pathways
CH2	Gluco-6-phosphate	Substrate transport
AM29 & AM142	FAD	Important for metabolic reactions

Figure 3: List of loss-of-function transposon insertion *A. faecalis* mutants and the genetic element disrupted as identified from sequencing and NCBI BLAST analysis.

Figure 4

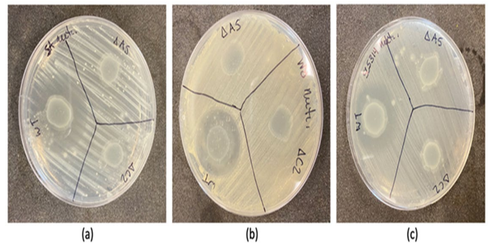

Figure 4: Representative photo of *A. faecalis* mutant on microbial lawns showing loss of function. Overnight cultures of *A. faecalis* wild-type or mutants were spotted onto *Staphylococcus (4A), Bacillus (4B), or Candida (4C)* lawns, incubated overnight at 37ºC, and monitored for lack of ZOI.

Figure 5

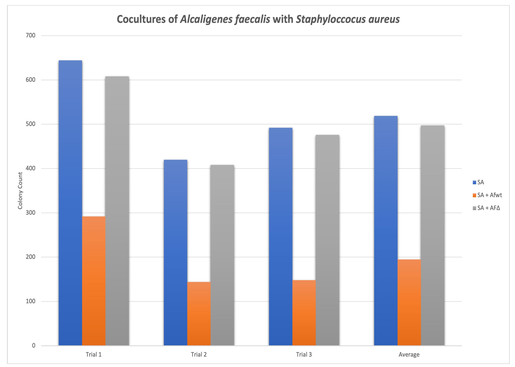

Figure 5: Cocultures of *A. faecalis* with *S. aureus*. *S. aureus* alone as a control (blue), *S. aureus* co-cultured with *A. faecalis* wild-type (orange), or *A. faecalis*$_{\Delta}$ were grown overnight at 37ºC with shaking, plated on selective media, and colony forming units (CFU) were enumerated.

Results

- Only the presence of *A. faecalis* cells create zones of inhibition against *Staphylococcus*, *Bacillus*, & *Candida species*, whereas cell-free spent media from *A. faecalis* cannot **(Figure 1)**.
- The EZ-Tn5 transposon delivery system is effective in producing numerous independent *A. faecalis* mutants **(Figure 2)**.
- NCBI BLAST analysis of identified loss-of-function mutnats has identified 5 different interrupted genes with two genes (MFS & FAD having been identified multiple times **(Figure 3)**.
- The *A. faecalis* mutant does not inhibit the growth of *Staphylococcus, Bacillus, or Candida* while the wild-type *A. faecalis* shows zone of inhibition **(Figure 4)**.
- *S. aureus* growth is inhibited by *A. faecalis* wild-type, but *A. faecalis* loss-of-function mutants produce *S. aureus* growth to similar levels of the control of *S. aureus* grown alone **(Figure 5)**.

Future Direction

- Determine the exact mechanism in which *A. faecalis* inhibits the growth of other microorganisms.
- Create true loss-of-function knockouts of genes in *A. faecalis*
- Use a model system, such as C. elegans, to test this interaction in a living in-vivo environment
- Identify therapeutic targets for pathogenic infections to potentially help with future antibiotic regiments.

Acknowledgements

- The Department of Health Sciences
- ETSU Research & Sponsored Programs (Major RDC Grant to Fox)
- ETSU Molecular Biology Core Facility for sequencing services

References

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