Using Polymicrobial Interactions to identify possible novel targets in Staphylococcus, Bacillus, and Candida

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Abstract

Microbes compete for the same limited nutrients, space, and resources; therefore, they show competitive relationships. Our laboratory has previously shown that *Alcaligenes* inhibits the growth of *Staphylococcus*, a Gram-positive bacterium, and *Candida*, a fungi, which are both substantial causes of human infections. We are interested in determining the genetic factors in *Alcaligenes that* are responsible for killing these competitors. Transposon mutagenesis was used to interrupt gene segments by introducing a foreign piece of DNA into the *Alcaligenes* genome. By creating these mutants of Alcaligenes, we were able to screen these against *Staphylococcus* to find those that can no longer inhibit. The absence of zones of inhibition indicated that we successfully interrupted the genetic element in *Alcaligenes* that kills *Staphylococcus*. The genome of the mutants were isolated and the area disrupted was sequenced. In one mutant, we discovered that the gene being interrupted was a MFS transporter. This is an important transporter in bacteria for virulence, metabolism, and quorum sensing. Results from this study may help us find new targets for *Staphylococcus aureus* infections.

Introduction

Antibiotics have been used to fight infections and save countless number of lives since the 1920's. It is essential to use them correctly and only when necessary as inappropriate use can increase the chance of developing antibiotic-resistant bacteria. Antibiotic resistance is an increasing problem as many communicable diseases are gaining resistance to current treatments. Diseases like these as well as other common infectious diseases gaining resistance threatens our ability to treat infected people. MRSA or Methicillin Resistance *Staphylococcus aureus* is a disease that is notorious for becoming resistant to antibiotics such as penicillin and erythromycin. *Staphylococcus aureus* is spread through both infected individuals and contaminated surfaces and is commonly seen throughout the hospital setting. *Candida albicans* is a fungi that causes cutaneous, mucosal, and systemic infections and has gained resistance specifically to antifungal treatments. It is spread by direct or indirect contact with contaminated people or objects. Lastly, *Bacillus subtilis* are generally non-pathogenic, however, have been seen to cause instances of food poisoning. This bacterium has been shown to have qualities of antibiotic resistance. Competitive polymicrobial interactions can be described as microbes all competing for the same limited nutrients, space, and resources. *Alcaligenes faecalis* is a gram-negative, rod-shaped bacterium that is a part of the Alcaligenaceae family. This bacterium is commonly found in soil and does not cause much harm to humans. Our laboratory has previously shown that *Alcaligenes faecalis* has inhibitory qualities toward *Staphylococcus*, *Bacillus*, and *Candida*, however the mechanism for inhibition is unknown. Presently, we have developed a transposon mutant library of *Alcaligenes* to aid in the determination of this mechanism. This genome-wide screening of *Alcaligenes* may show promise in identifying new targets for therapeutic interventions for *Staphylococcus, Bacillus*, and *Candida* infections, particularly those that are drug resistant.

Results – Figure 1

Figure 1: *Alcaligenes faecalis* spots on a *Staphylococcus aureus* lawn or a *Candida albicans* lawn. Incubated 24h at 37°C.

Results – Figure 2

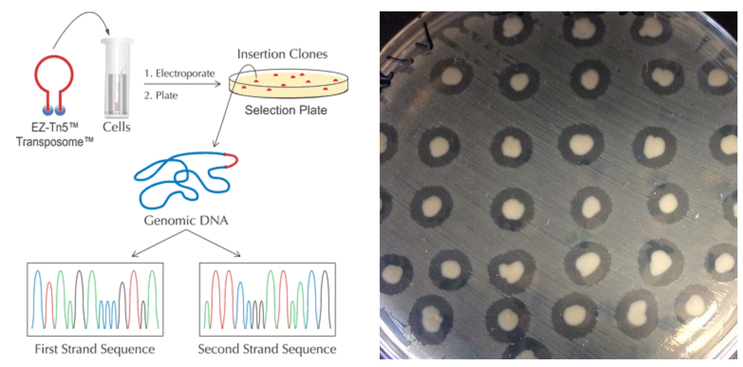

Figure 2: Method of delivering a transposon to *Alcaligenes faecalis* genome to create mutants and a representative photo of screening the *Alcaligenes faecalis* mutants for the loss-of-function phenotype

Results – Figure 3

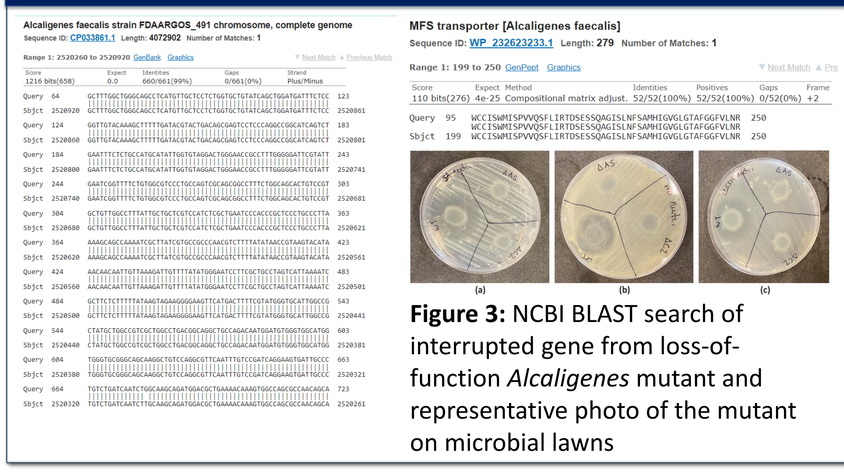

Figure 3: NCBI BLAST search of interrupted gene from loss-of-function *Alcaligenes* mutant and representative photo of the mutant on microbial lawns

Results – Summary

- *Alcaligenes faecalis* is a potent inhibitor of *Staphylococcus aureus*, *Candida albicans*, and other Gram-positive and Gram-negative bacteria (Figure 1)
- The EZ-Tn5 transposon delivery system is effective in producing numerous independent *Alcaligenes faecalis* mutants (Figure 2)
- Using transposon mutagenesis, *Alcaligenes faecalis* can be screened rapidly for the loss-of-function phenotype. These mutants are of interest to elucidate the genetic components used by *Alcaligenes* in the pathway to defeat competing microbes (Figure 2)
- One such loss-of-function *Alcaligenes* mutant identified, the gene responsible for Major Facilitator Superfamily transporter was interrupted. This mutant was no longer able to inhibit *Staphylococcus aureus* when tested (Figure 3)

Discussion

After sequencing the genome of the mutant, we were able to see that the gene that was being interrupted was MFS transporter. The MFS transporter or major facilitator superfamily transporter is known to facilitate movement of small solutes across the cell membrane via active transport. These largest and most diverse superfamily transporters operate in means of uniport, symport, or antiport mechanisms and have been identified as drug targets. These transporters are important in bacteria for virulence, metabolism, and quorum sensing. Not only are these transporters recognized as being important in bacteria, but they have also been seen in humans. It has been seen that when these transporters have defects, they cause diseases such as cancers or metabolic diseases. The MFS transporters, specifically the members of the drug/H(+) antiporter family, have been seen as a contributor of multidrug resistance in yeast C. *albicans*. In order for it to be a major contributor to multidrug resistance, it contains a mechanism known as active efflux which causes bacterial resistance to antibiotics. This means that as the drug is entering the bacteria, the MFS transporter is actively pumping out the drug and allowing for the bacterium to gain resistance. With this MFS transporter being interrupted in our mutant, this allows for Alcaligenes *faecalis* to inhibit Staphylococcus *aureus*. Looking over at the data, we can see that our mutant Alcaligenes *faecalis* is able to inhibit Staphylococcus *aureus* more effectively than our wildtype Alcaligenes *faecalis*. This data shows three different trials of dilutions of the co-cultures and how many colonies of Staphylococcus *aureus* grow at the dilution of 10^5. With the results of this data, it is important to note that with the interruption of this MFS transporter, there is likely a substance being carried on it that causes this multidrug resistance. Further research needs to be done in order to determine what materials this MFS transporter is facilitating.

References

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