Run through 2x to audience while making changes before writing on notecards.
High light notecards with different colors for slides

Slide 1

Good afternoon ladies and gentlemen, today I am presenting my research on novel compounds in the inhibition of biofilm production. I would like to start off by saying the only reason I had the opportunity to do this research was because of the BOMM research program overseen by Dr. Linda Hensel and Dr. David Goode. They also had several wonderful teaching assistants to provide additional help. Lastly, I would not have been able to complete my research without my lab partners, Emily Bell and Alex Matsunaga.

Slide 2

The research of novel compound in biofilm production is important to stop the spread of antibiotic resistance, which is a global epidemic. Big Pharmaceutical companies are not working on a solution for antibiotic resistance, they continue to develop me-too drugs, which are slight variations of currently existing medication. Most pharmaceutical companies put more money into advertising drugs than the actual research and development of new drugs, which can be seen in the figure on the screen, in orange is the portion of budget spent on marketing and blue is research and development for 2019. According to the AARP, 24 billion dollars of the average pharmaceutical companies advertising budget goes towards marketing to doctors. Because of the pharmaceutical companies' influences, doctors over prescribe and patients misuse broad-spectrum antibiotics causing them to become resistant. Antibiotic resistance occurs when not all bacteria is killed, and the survivors are given the chance to mutate and reproduce. This mutation prevents our current broad-spectrum antibiotics from being effective. Also, Broad spectrum

antibiotic weaken your immune system, because they damage or kill, not only harmful bacteria, but your good normal flora. In this research, we are trying to combat antibiotic resistance by testing drugs that are competitive inhibitors, that way we are not killing bacteria or preventing bacteria growth. By not harming the bacteria, it is not given the opportunity to become resistant to our drug.

Slide 3

To understand how this research works you need to know what quorum sensing and signal transduction are, because this is how bacteria become harmful to begin with. Previous research from Dr. Bassler has proven that quorum sensing is the process of bacterial communication between the same species and other prokaryotes. This communication occurs when bacteria secrete a small amount of chemicals into their environment, which allows other bacteria with signal receptors to interpret the signals that were secreted. Once there are enough bacteria secreting and receiving chemicals then, the signal starts to induce a response. Therefore, when we reach a certain bacteria density, we can get a response by activating the genes for virulence factors. This can be seen in the picture when comparing two bacteria cell densities. The receptor, the yellow packman, is being filled by chemical signal, the red piece, in high density but not low density. Therefore, the gene for high density becomes active, indicated by the arrow above gene, and in low density the red cross over the gene indicates that’s its inactive.

Slide 4

Virulence factors occur because there is no immune system response to a nonpathogenic also known as nonharmful bacterium. The nonpathogenic bacteria divide multiple times until it reaches a high density. This is when quorum sensing occurs. A receptor has an active site which reacts with the chemicals secreted by other bacteria. This binding causes a ripple effect of activations of structures within the cell, such as proteins, this process is referred to as amplification. As amplification causes this intercellular change, bacteria will eventually lead to gene expressions of virulence factors, which means the bacteria is now harmful. This is shown in the picture, the ball at the very top is the signaling molecule in the receptor, which is causing amplification, which eventually reaches gene transcription. Once it becomes virulent, there will be too many bacteria for the immune system to fight off. A type virulence factor is biofilm formation, which we want to avoid in medicine.

Slide 5

To inhibit biofilm formation, we want to use a novel compound as a competitive inhibitor. A novel compound is simply an untested chemical entity. We want this compound to bind to the receptor before the signaling molecule from the bacteria can. This will stop the bacteria from ever becoming virulent, in this case prevent biofilm formation, because it won't be able to change intercellularly or amplify. The picture shows the drug represented by the green circular cross attaching to the receptor molecule, which stops the pathway. Our drugs will be able to stop antibiotics resistance using this method, because the goal of the drug is to NOT be bactericidal meaning they won't kill bacteria or bacteriostatic which means they won't prevent bacteria

growth. Currently, broad spectrum antibiotics prevent quorum sensing by being bactericidal and bacteriostatic.

Slide 6

The drugs we made were composed of 1 amino acid and 1 carboxylic acid through dehydration synthesis and a purification process. Based on the 2018 BOMM program results, as you can see in the picture, there were lots of promising novel compounds made with Tyrosine, which is why we chose tyrosine. We then chose tryptophan, because it has the same structure as tyrosine with the exception of an additional pentane ring with nitrogen and lack of a hydroxyl group on the benzene which can be seen by the purple highlighted areas. For the rest of this presentation Tryptophan will be referred to as Trp and Tyrosine will be referred to at Tyr.

Slide 7

The carboxylic acids that we chose were numbers (49) 4-Methoxybenzoic acid, (50) 3-Methoxybenzoic acid, and (51) 2-Methoxybenzoic acid. We chose our carboxylic acids, because we wanted to see if there was a difference in the placement of the ether group on the benzene ring, which can be seen by the pink numbering. Our hypothesis is that if we test these novel compounds, we will be able to find drugs that have biofilm inhibiting potential.

Slide 8

We ran crystal violet, our preliminary test on drugs Trp 49, Tyr 50, Trp 50, Tyr 51, and Trp 51 with the bacteria *B. subtilis, E. coli, P. aeruginosa, S. aureus, and S. mutans.* If the drugs show the ability to cause biofilm inhibition in a certain drug, then we did secondary testing. The

secondary test, included the assays as seen, which were used to identify composition biofilm and to verify that the drugs are not bacteriostatic or bactericidal. The procedures for these tests were provided by Dr. Goode and Dr. Hensel in the Intro to Biology 2 Lab Packet.

Slide 9

Our crystal violet results shows our control, DMSO, had a mean greater than 0.06, (you can see this in the first column on the graph) this shows that the drug is inhibiting biofilm growth. The crystal violet assay results revealed that Trp-49, Trp-51, and Tyr-51 were significant values in *S. mutans* and Tyr-51 was significant in *P. aeruginosa.* (These columns are identified by asterisks above them). All the columns with asterisk have a p-value less than 0.05. To get these graphs we used ANOVA, All Pairs, Tukey-Kraner HSD test using JMP. Mean + or - standard deviation, N=8. Unfortunately, when we did a second round of testing our data was insignificant, because the DMSO values were under 0.06.

Slide 10

In our secondary results, the Congo Red plates stayed red in color, which indicates that the biofilm is polysaccharide based. In the Nutrient Agar test results, there were no signs of growth differences which means the drugs were not bactericidal.

Slide 11

The planktonic assay can be seen on the left side of the screen and disk diffusion on the right. The planktonic assay measures the amount of bacteria density in the media. As you can see the control and tested drug have relatively the same bacterial density, which means the drug is not

bacteriostatic. However, we did not have any *S. Mutans* left to run our other drugs through this test. The disk diffusion assay measures the inhibition of bacterial growth. In the pictures there are no rings shown due to condensation on the plate, however when held up to the light there was a ring of no bacterial growth around AMP, which is a broad-spectrum antibiotic. There were no rings around any of our drugs, which means it was not bacteriostatic or bactericidal.

Slide 12

Biofilm formation depends on 3 main factors: species of bacteria, environment of bacteria, and media used. We used the same media and environment for all the bacteria; however, we did change the species. Although we started the experiment testing 5 different bacteria, these two bacterial species showed biofilm inhibition potential with the drugs chosen. In the gram staining results, you can identify the types of bacteria being used and verify that there was no cross contamination. *P. aeruginosa* and *S. mutans* were the most affected by the drugs we have chosen to test. In the picture, you can see that one is purple and the other is pink. This is because of the cell make-up of the bacterial species; the pink rod-shaped bacteria are gram negative meaning it has a small amount peptidoglycan in-between two plasma membranes. The S. Mutans which are the purple round shaped bacteria are gram positive meaning they have a thick outer layer of peptidoglycan on outside of one plasma membrane. Because these two bacteria have two different structures in their outer layers, the receptors often have an even greater difference in composition. Since they are different, not only are the quorum sensing compounds different when they communicate within their species, but the drugs will also react differently between species active sites in the receptors.

Slide 13

Trp-49 had the highest inhibition rate at 66% in *S. Mutans* while the other two drugs, Tyr-51 and Trp-51, had about 25-30% in *S. Mutans*. We believe the higher rate in S. Mutans is due to the positioning of the ether on the fourth carbon, and the addition of the pentane ring with nitrogen. The location of these groups can change the efficiency to bonding to the active site of the receptor, because hydrogen bonding and other reactions occur faster at certain positioning. The group positioning is the main reason why carboxylic acid 49 might be chosen over 51, and why 50 couldn't inhibit biofilm at a significant level at all. The location of the carboxylic acid differences are highlighted in pink, while differences of amino acids are in green. When looking between the Tyr and Trp, you can see that hydrogen bonding occurs in several sites for both compounds, however the main potential hydrogen bonding difference are between the amine group in the pentane ring and the hydroxyl group on the benzene ring. The amine group on the pentane ring in Tyr drugs are more willing to react, because it is an antiaromatic compound, which wants to become more stable. Trp drugs are less willing to react at the hydroxyl group even if the oxygen is more electronegative, because it is attached to a very stable aromatic ring. For P. aeruginosa Tyr-51 was able to have an inhibition rate of 27% due to the positioning of the ether as previously mentioned and the symmetry between the Tyr drug's aromatic rings and opposite facing ethers making the drug more nonpolar than all the other drugs.

In conclusion we cannot reject our hypothesis, because we did have biofilm inhibition with some of our drugs that we decided to test using the crystal violet assays. Our secondary results verified that our drugs were not bacteriostatic or bactericidal. Therefore, we were showing that the novel

compounds were acting as competitive inhibitors, unlike the broad-spectrum antibiotics which just kill bacteria and prevent bacteria growth.

Slide 14

In the future, we would like to retest the current drugs, along with drug Tyr-49. By doing this we will be able to make more precise judgement on the ability of inhibiting biofilm and what groups of the novel compounds were reacting with the active site. There was not a significant DMSO value on our second test run and an error occurred when making drug Tyr-49, which is why the retesting is necessary. I would also like to test the same carboxylic acid groups but use phenylalanine, known as Phe, as the amino acid. Phe has the same molecular structure as Tyr except for Phe lacks a hydroxyl group on the benzene. The pictures have the differences highlighted in pink, although the picture of Phe is the amino acid without the carboxylic acid attached. This will allow us to know more precisely whether the hydroxyl group on the benzene ring matters, whether being anti-aromatic or aromatic matters, and whether the arrangement of the carboxylic acid's matter. When we do find the right medications, we may finally be able to get a grasp on the battle of antibiotic resistance, instead of sitting on the sidelines like big pharmaceutical companies currently are.

Are there any questions?

(about 14 min long)