Inducing Antibiotic Sensitivity in Staphylococcus Saprophyticus

Siobhan Gifford, Mariaeva Siourouni, Elena DeVore
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Abstract

Antibiotic-resistant microbes are pathogens that have developed the ability to withstand drugs with increasing resistance which poses a significant threat to modern medicine. The purpose of this experiment is to determine if the presence of chalcone can cause *S. saprophyticus* to become sensitive to the antibiotic novobiocin, to which it is innately resistant. The procedure involved plating samples of *S. saprophyticus* and *S. aureus* on the antibiotic novobiocin with and without the addition of incubation in 2'-Hydroxychalcone. The results show that the addition of chalcone to the bacteria did not affect the antibiotic resistance of *S. saprophyticus* in the presence of Novobiocin.

Introduction

The increasing prevalence of antibiotic-resistant bacteria poses a unique threat to modern medicine and complicates everything from cancer treatments to everyday infections. According to the Centers for Disease Control and Prevention (CDC), more than 2.8 million antimicrobial-resistant infections occur annually in the United States and more than 35,000 deaths result from those infections. *Staphylococcus saprophyticus* is a Gram-positive bacterium that is innately resistant to the coumarin antibiotic novobiocin, which makes it the perfect variable to use in this experiment. Chalcones are compounds that have been shown to inhibit efflux pumps, β-lactamase enzymes, and the formation of biofilms in Gram-positive and Gram-negative bacteria. By affecting bacteria in these ways, chalcones have the potential to induce sensitivity in antibiotic-resistant microbes. To counteract this experiment, *Staphylococcus aureus* is being used in tandem as a control group, S. aureus does not have any resistance to the antibiotic novobiocin. The design of this experiment was derived from the research carried out in the CHEM&163 class on how chalcones affect Sortase A inhibition. The purpose of the experiment was to determine if the addition of 2'-Hydroxychalcones could induce antibiotic sensitivity in the bacteria *S. saprophyticus.*

Methods

Materials & Location

This experiment was carried out in lab room 108 at Whatcom Community College using materials provided by the college, including two TSA agar plates, four Novobiocin agar plates, broth cultures of *S. aureus* and *S. saprophyticus*, and 2'-Hydroxychalcone in a DMSO solution. Micropipettes were used to mix our chalcone stock solution and deposit bacteria samples onto agar plates. Sterile glass beads were used to evenly spread the bacteria samples on the agar plates.

Procedure

In the procedure for this experiment, we weighed out the appropriate amount of 2'-Hydroxychalcone to make 0.666 mL (as calculated to the amount of chalcone needed per bacteria sample) of a 10 mM solution and vortexed to mix. The chalcone stock solution was then combined with bacteria samples of *S. aureus* and *S. saprophyticus* and incubated at room temperature for 30 minutes. After incubation, sterile pipettes were used to transfer 0.200 mL samples of *S. aureus* and *S. saprophyticus* onto two TSA agar plates, respectively, and two novobiocin agar plates, respectively. Sterile pipettes were then also used to deposit 0.200 mL samples of *S. aureus* and *S. saprophyticus* that were not incubated with chalcone stock onto two novobiocin agar plates, respectively. Sterile glass beads were then used in each of the agar plates to evenly distribute bacteria. Finally, the agar plates were wrapped in Parafilm and stored in the incubator at 28°C for 48 hours. After the incubation period, the plates were observed for growth, and results were documented to compare with what was expected.

Bacterial Growth Under Experimental Conditions

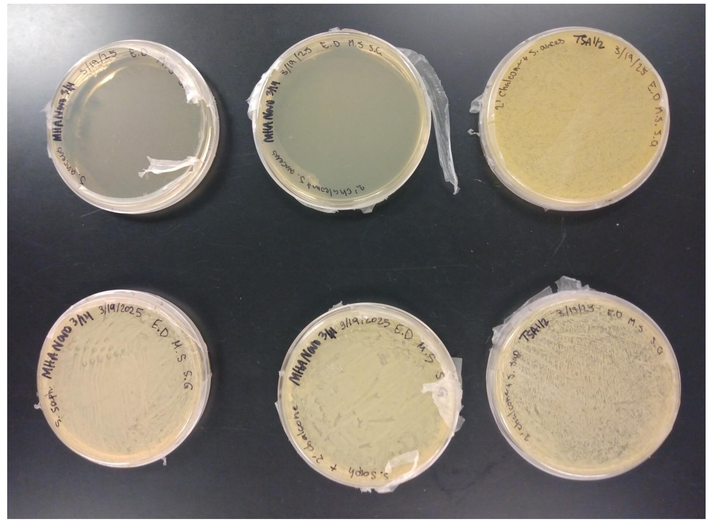

Figure 1. From left to right, bacterial growth of *S. saprophyticus* and *S. aureus* plated on novobiocin without 2'-Hydroxychalcone, novobiocin with 2'-Hydroxychalcone, and TSA agar with 2'-Hydroxychalcone without novobiocin.

2'-Hydroxychalcone Stock Solution

Figure 2. 2'-Hydroxychalcone stock solution

Discussion

The results of this experiment did not match the expected outcome, which was that the *S. saprophyticus* and chalcone stock incubated on novobiocin agar plates would result in decreased bacterial growth or no growth at all. After incubation, the agar plate containing novobiocin, and the chalcone-incubated *S. saprophyticus* showed bacterial growth too numerous to count and *S. aureus* showed no growth under the same conditions.

The positive control plates of bacteria incubated with chalcone and plated without antibiotic novobiocin present yielded the expected results of growth too numerous to count in *S. saprophyticus* and *S. aureus*. The negative control plates of bacteria plated on novobiocin without chalcone present also yielded the expected results of growth too numerous to count in S. saprophyticus and no growth in S. aureus.

These findings did not support our hypothesis that 2'-Hydroxychalcone would enhance antibiotic sensitivity in *S. saprophyticus*. However, several limitations in the experiment, including time and resource constraints, could have influenced the results. Future studies could be improved by expanding the sample size, testing a broader range of antibiotic-resistant bacteria, using different concentrations or compositions of chalcone stock, varying incubation conditions, and exploring different antibiotic concentrations. These modifications would help provide more definitive conclusions.

2'-Hydroxychalcone

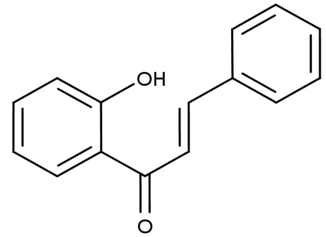

Figure 3. 2'-Hydroxychalcone Line-bond structure

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