Visualisation of the effects of antimicrobial agents on bacterial cells

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Introduction

Due to widespread and well reported problems with antibiotic resistance, it is becoming increasingly important to understand precisely how antibiotics interact with bacterial cells in order to develop new antibiotics that bacteria are less likely to develop resistance against.

The aims and objectives of this project were to determine the lowest concentration of two antimicrobial agents, Nisin and Polymyxin B, that have an effect on bacteria by determining their Minimum Inhibitory Concentration (MIC) against two bacteria. Polymyxin B is produced by the bacterium *Bacillus polymyxa* and is used mostly to treat infections by Gram negative bacteria that are resistant to treatment by other antibiotics. Polymyxin B destroys cells by making them more permeable. Nisin is produced by the bacterium *Lactococcus lactis* and has a broad-spectrum of activity, particularly against Gram positive food spoilage organisms.

To test the effectiveness of Polymyxin B and Nisin, *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative) were chosen for this study due to their very different cell wall morphologies. Once the MICs were determined, the project moved onto the visualisation of the interactions between those antimicrobial agents and the bacteria, at concentrations already determined via the MIC experiments. These interactions were visualised using a Scanning Electron Microscope (SEM).

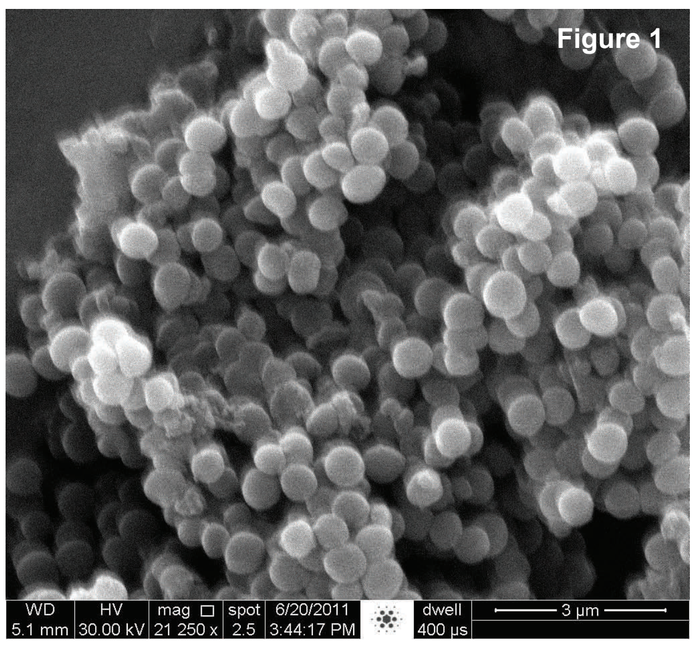
Methods and Materials

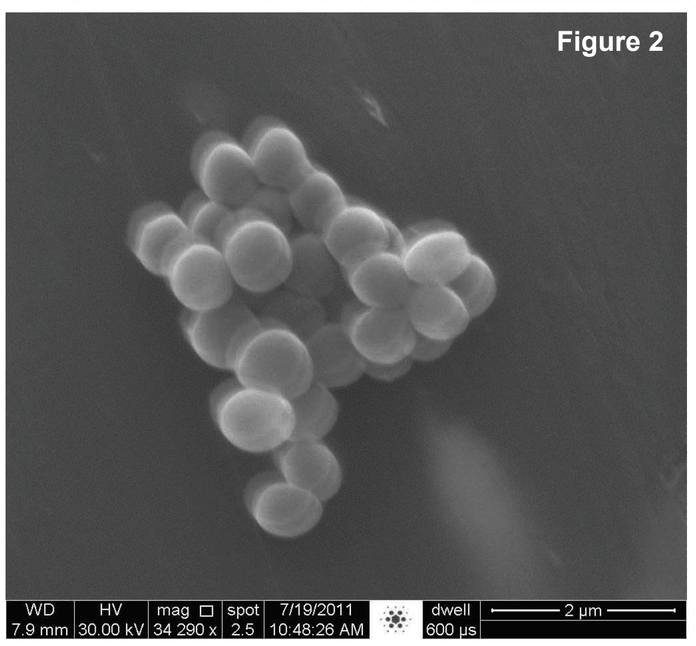
MIC determinations

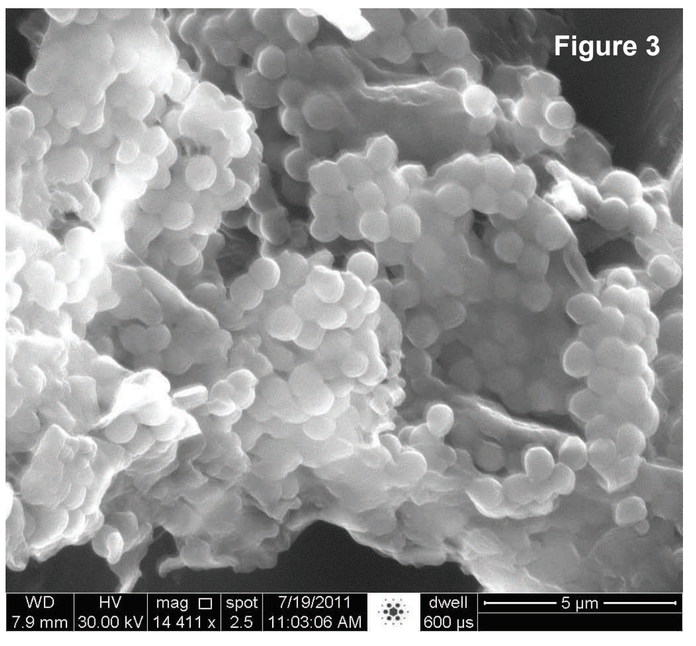
Serial dilutions of Polymyxin B and Nisin solutions in sterile distilled water were prepared at the following concentrations: 64 mg/ml, 32 mg/ml, 16 mg/ml, 8 mg/ml, 4 mg/ml, 2 mg/ml, and 1 mg/ml. These antimicrobial agents were then further diluted by pouring into nutrient agar plates. Overnight cultures of *S. aureus* and *E. coli* were then also diluted in nutrient broth to produce a range of concentrations of bacterial suspensions: 10^{0} (original culture), 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} and a control containing no bacteria. A multipoint inoculator was then used to add the different concentrations of bacteria to the agar plates which already contained the antimicrobial agents. The plates were incubated at 37˚C overnight before recording growth or no growth to allow determination of the MIC the next day.

SEM visualisation

Concentrations close to the previously determined MICs were chosen and appropriate broth cultures containing antimicrobial agents were prepared. Samples were then fixed onto SEM stubs before coating and visualisation.







Results

MIC determinations

- The more concentrated the Polymyxin B and Nisin, less *S. aureus* and *E. coli* colonies grew.
- The more diluted the bacterial suspensions, the less colonies grew.
- The 10^{-6} and 10^{-7} dilutions of *S. aureus* showed no growth on any plates.
- The full range of Polymyxin B concentrations demonstrated activity against *E. coli* (1-128µg/ml).
- 64 and 128µg/ml of Polymyxin B showed no growth at all on any of the results for *S.aureus*.
- The MIC of Polymyxin B against *S.aureus* is 64 µg/ml.

SEM visualisation

- Figure 1: Control SEM image of *S. aureus* with no antimicrobial agent, coated in carbon.
- Figure 2: SEM image of *S. aureus* treated with 2µg/ml of Polymyxin B, coated in gold palladium, magnification x34290.
- Figure 3: SEM image of *S. aureus* treated with 2µg/ml of Polymyxin B, coated in gold palladium, magnification x14411.

Conclusion

- The images produced showed some clear images of both organisms.
- The results gained proved that the higher the concentration of antimicrobial agent, the lower the growth of bacteria.
- Also, the lower the dilution of bacteria, the more of an effect the antimicrobial agents have on them.
- More work on the effects of Nisin on *S. aureus* and *E. coli* can be undertaken in the future along with the effects of Polymyxin B and Nisin on other bacteria.

References

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