Food Preservative and Antibiotic Cross-Resistance in *Escherichia coli*

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Introduction

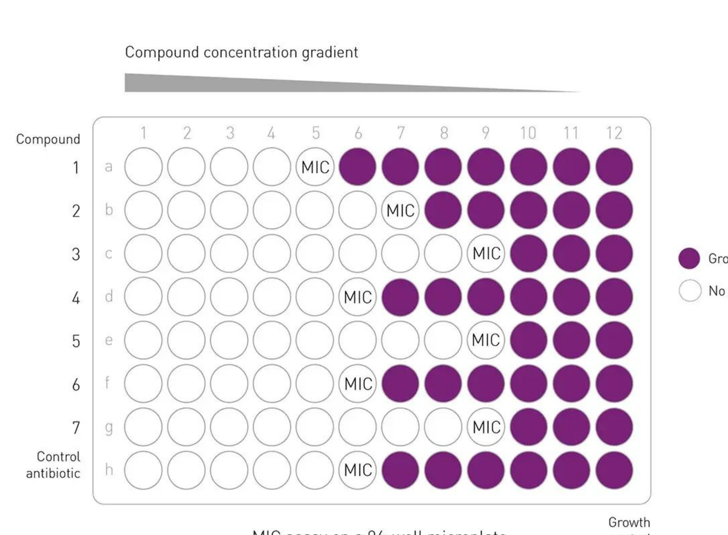
It is well known that overuse of antibiotics can select for bacterial strains with antibiotic resistance. Resistance has been shown to be due to mechanisms like increased efflux activity, or mutation of the antibiotic target. This project studies whether other biocides, like the food preservative diacetyl, can also select for antibiotic resistance, a phenomenon called cross resistance. The mechanism behind adaptation to one biocide may allow for the bacteria to resist different types of biocides.[1]

Diacetyl, known for its butter-like flavor, is found in products such as cheese, wine, and beer, as it is a natural byproduct of fermentation.[2] Diacetyl is categorized as a biocide because of its ability to denature proteins and damage cellular membranes.[3] Mutations that allow improved survivability in the presence of diacetyl may also cause reduced sensitivity to antibiotics, and reveal new mechanisms by which bacteria adapt to environmental stressors and resist the effects of antibiotics.

Methods

Escherichia coli strain ATCC 25922 was sequentially exposed to increasing concentrations of diacetyl. Twenty-four individual strains able to grow in the presence of 0.3M diacetyl were selected, and tested for cross resistance to antibiotics. Minimal inhibitory concentration (MIC) assays were used to determine the lowest concentration of antibiotic that will prevent bacterial growth.[4]

However, if both wild type and adapted strains expressed the same MIC, the sub-MIC (highest concentration of antibiotic the strain can grow in) was evaluated for growth differences.



Results

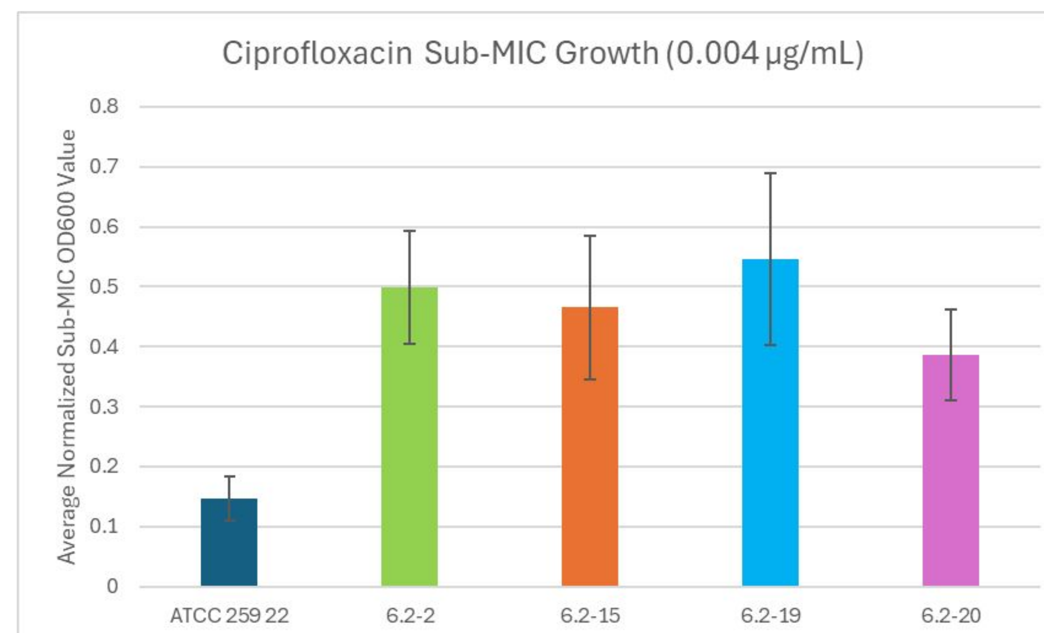


Figure 1. Identification of ciprofloxacin cross resistant strains. Spectrophotometric analysis (600 nm) of *E. coli* strains exposed to 0.004 µg/mL ciprofloxacin. For each strain, growth data (OD600) in 0.004 µg/mL ciprofloxacin was normalized to growth without antibiotic. ATCC 25922 (n=4), 6.2-15 (n=3), remaining adapted strains (n=2).

Both the wildtype and diacetyl-adapted strains were inhibited by 0.008 µg/mL ciprofloxacin (MIC). Comparison of growth in the sub-MIC concentration, 0.004 µg/mL, showed that 4 adapted strains grew better (Fig. 1).

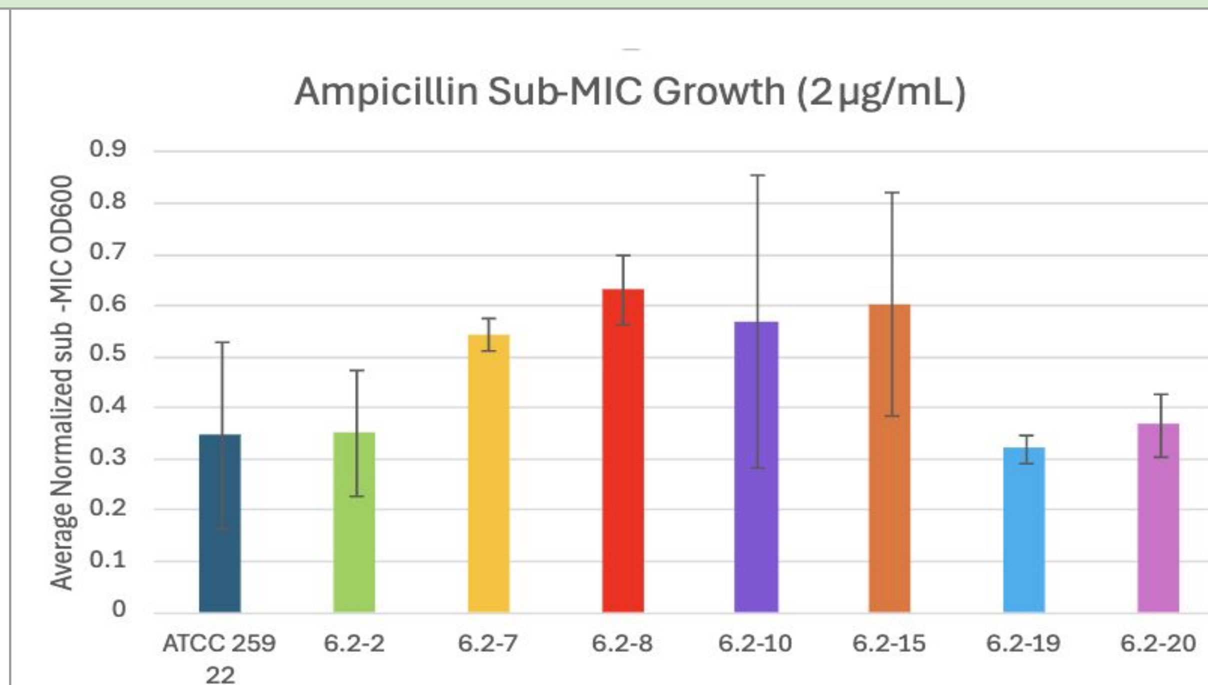


Figure 2. Identification of ampicillin cross resistant strains. Spectrophotometric analysis (600 nm) of *E. coli* strains exposed to 2 µg/mL ampicillin. For each strain, growth data (OD600) in 2 µg/mL ampicillin was normalized to growth without antibiotic. ATCC 25922 and 6.2-2 (n=5), 6.2-19 (n=4), 6.2-20 (n=3), remaining adapted strains (n=2).

Both the wildtype and diacetyl-adapted strains were inhibited by 4 µg/mL ampicillin. Comparing growth in the sub-MIC of 2 µg/mL showed that only 6.2-7, 6.2-8 and potentially 6.2-10 and 6.2-15, grew better than wildtype (Fig. 2).

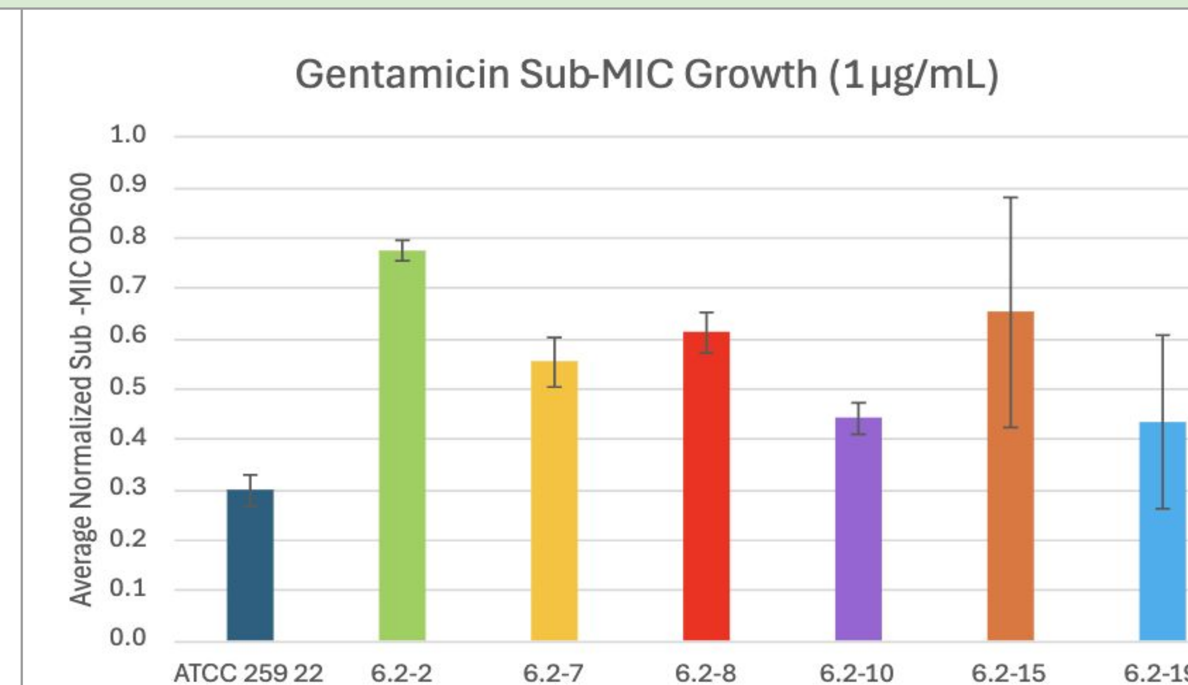


Figure 3. Identification of gentamicin cross resistant strains. Spectrophotometric analysis (600 nm) of *E. coli* strains exposed to 1 µg/mL gentamicin. For each strain, growth data (OD600) in 1 µg/mL gentamicin was normalized to growth without antibiotic. ATCC 25922 (n=6) and adapted strains (n=2).

Both the wildtype and diacetyl-adapted strains were inhibited by 2 µg/mL gentamicin. Comparison of growth in the sub-MIC of 1 µg/mL showed that five adapted strains, and potentially 6.2-19, grew better than wildtype (Fig. 3).

Conclusions

These results support the idea that some level of cross-resistance can occur between food preservatives like diacetyl and antibiotics. Not all 24 strains have been tested with all antibiotics shown here, but a majority of them demonstrated less sensitivity to at least one antibiotic. It should be noted that none of the adapted strains had an increased MIC when tested against the antibiotics suggesting that the mutations allowing growth in 0.3M diacetyl only impart a slight selective growth advantage upon exposure to antibiotics.

Strain 6.2-15 showed less sensitivity to all three antibiotics tested; 6.2-2, 6.2-7, 6.2-8 and 6.2-19 showed less sensitivity to two of the three antibiotics. These multi-drug resistance phenotypes may be attributed to mutations that have broad effects such as increased expression of efflux pumps.

Moving forward, diacetyl adapted strains will be sequenced. Discovered mutations will guide us in our steps moving forward in determining the mechanisms of cross-resistance.

References

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