**Introduction**

Tetracyclines are commonly used to treat infections caused by numerous microorganisms such as Chlamydial and Mycoplasma pneumoniae in addition to combating moderate-severe acne. These antibiotics act through binding to the 16S rRNA of the 305 ribosomal subunit, preventing the amino-acyl tRNA from binding to the A site of the ribosome and thus inhibiting protein synthesis. Tetracyclines originally exhibited a wide range of antibiotic action against both gram-negative and gram-positive clinically relevant bacteria, with each successive optimization of the core scaffold resulting in improved activity.

However, due in part to their vast popularity and lateral bacterial gene transfer, the rate of tetracycline resistance has increased dramatically, corresponding with a dissemination in common resistance mechanisms. These include drug efflux pump activity and molecular target protection, which have been frequently investigated in bacteria as possible targets for combating widespread drug resistance. Our goal in this work is to focus on a less investigated mechanism utilizing enzymes to modify tetracyclines into inactive or unstable derivatives and thus limiting their clinical efficacy.

**Bacteriodes Targets and Susceptibility Screening**

Bacteriodes dorei and Bacteriodes fragilis are opportunistic pathogenic bacteria which take advantage of a weakened immune system to infect tissues and organs surrounding the human gut. Previous studies have found B. fragilis to be the most commonly isolated Bacteriodesae in anaerobic infections. These strains are part of the expansive human gut microbiome that is known to play essential roles in host physiology and human disease in addition to holding an accessible reservoir of antibiotic resistance. From initial susceptibility screening tests of 10 different anaerobic strains, B. dorei and B. fragilis were determined to hold resistance against most of the tetracyclines tested (Table 1).

**Results & Discussion**

Antibiotic activity was determined via two-fold drug dilutions in 100 μl reinforced clostridium medium (RCM) in a 96-well plate (in triplicate). Each well was inoculated with 50 μl of strain inoculum and inoculated in 37°C for 20 hours before ODs were read via microplate reader.

Metabolism time courses were prepared for the following antibiotics:

- **Tetracycline**: 50 μM treatment in 5 mL media
- **Minocycline**: 12.5 μM treatment in 5 mL media
- **Doxycycline**: 25 μM treatment in 5 mL media
- **Chlortetracycline**: 25 μM treatment in 5 mL media

**Mechanism of Tetracycline Destructases**

Previously, genes encoding tetracycline resistance through destructase enzymes have been sequenced in B. fragilis and expressed in E. coli. As a result, the enzymes TET(X) and TET(Y)-TET(S) were determined to mediate the hydroxylation of Tetracycline into the inactive derivative. However, the destructase enzymes uncovered are flavin adenine dinucleotide (FAD)-dependent monooxygenases, signifying the need for O₂ during the hydroxylation reaction which is not available in the anaerobic environment of the human gut. Furthermore, Bioinformatic analysis via BLAST shows the tet(X) gene in B. fragilis 3_1_12 used in our tests sharing high identity (99.7%) with previously characterized B. fragilis tet(X) via E.coli transformation, while only sharing 44.44% identity with the tested B. dorei tet(x). This highlights high variability within the tetracycline destructase gene that could effect resistant activity in anaerobic strains.

**Conclusion**

The hydroxylated tetracycline metabolite product was formed after a 4-hour tetracycline treatment in anaerobic conditions, signifying the possibility of either resistance activity by a novel enzyme or possible tet(x) variants that can function in these conditions. This metabolism product is also seen in B. dorei, which has a significantly different tet(x) genetic profile than B. fragilis. Longer time courses performed saw increased nemonyzate degradation of the antibiotic and the possible hydroxylated metabolite. Other tetracycline antibiotics tested did not induce this resistance response, although further screening is needed in different anaerobic strains. Further work is also needed on the potential bioactivities of the tetracycline metabolite and its possible impact on the relative inhibition of bacteria growth.

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