Porphyromonas gingivalis OMV production

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INTRODUCTION
Porphyromonas gingivalis is a gram-negative anaerobe known for its pathogenesis in periodontal disease. Its pathogenesis relies on a broad range of virulence factors. Studies have suggested the P. gingivalis outer membrane vesicles (OMVs) enhance biofilm formation. Alarmon apGpp is thought to regulate OMV production. Biofilm formation also known as dental plaque is responsible for the destruction of periodontal tissue.

Figure 1. Development of periodontal disease from dental plaque accumulation. Figure from web source: https://www.frontiersin.org/articles/10.3389/fmicb.2018.00053/full

OBJECTIVE
The purpose of this study was to look at the relationship between the stringent response which is mediated by the alarmon apGpp and the expression of relA and rshB which help synthesize ppGpp by creating a mutant with these genes. (Figure 2)

Figure 2. This research model suggests that ppGpp synthesis by relA and rshB promote OMV production. Their research found that mutant rshB had decreased OMV production, but increased biofilm formation. Figure from web source: https://doi.org/10.1038/s41522-020-0115-4

METHODS AND MATERIALS
Figure 3. Construction of suicide vector using puc19, which upon successful transformation of rshB gene would be introduced into Pg to delete the rshB genes. Figure from web source: https://www.addgene.org/50080/

Table 1.

Next step was to cut out purified bands of plasmid and perform ligation reaction of the pcr product: relA upstream/ermF/relA downstream to transform suicide vector

Table 2. depicts our troubleshooting after unsuccessful transformation, where we bought new ligase. Reaction 3 & 4 vs. 5 & 6 appear problematic.

Conclusion
While we conducted various troubleshooting methods of the transformation of puc19 with rshB we were unable to determine the cause. We did however determine that puc19 transformation isn’t as straightforward as we thought. Due to time constraints, we were unable to make a relA construct. However, with the current development of ppGpp association with a decrease in OMV production it would be valuable to continue this research. I hypothesize that insert might be expressing a toxic protein

REFERENCES

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