Developing a Markerless Deletion System in *Haemophilus parainfluenzae*

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Haemophilus parainfluenzae is a facultatively anaerobic Gram negative bacteria found in abundance in human supragingival plaque. Microbiome sequence data indicates a positive correlation between the presence of *H. parainfluenzae* and *Streptococcus* spp. including hydrogen peroxide-producing *S. mitis*. However, our coculture experiments have shown that when in high abundance hydrogen peroxide produced by *S. mitis* can kill *H. parainfluenzae*. In most aerobic bacteria, catalase is the primary enzyme that is responsible for protection from hydrogen peroxide. Whilst *H. parainfluenzae* encodes and expresses catalase, its contribution to peroxide detoxification is minimal. We have demonstrated that single gene deletions for catalase and other known peroxide-detoxification genes do not render *H. parainfluenzae* sensitive to hydrogen peroxide. Thus, the likelihood of a single gene product being responsible for the breakdown of peroxide in *H. parainfluenzae* is unlikely. Our hypothesis is that *H. parainfluenzae* hydrogen peroxide resistance is a redundant system utilizing multiple gene products. To test this hypothesis a strain lacking multiple genes would need to be constructed. Our current methods have relied on allelic replacement with antibiotic resistant cassettes. However, due to the limited number of cassettes that function in *H. parainfluenzae*, the use of allelic exchange for this purpose is implausible. This work focuses on development and testing of a sucrose (SacB) counter-selection system that will enable the construction of markerless or “clean” deletions which circumvents the issues that arise from antibiotic cassette usage and facilitates rapid construction of multiple gene knockouts within a single strain.

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