In vitro Investigation of Hydrogen Peroxide Production and Susceptibility of Oral Streptococci Species and Respiratory Commensal Bacteria in Simulated Respiratory Lining Fluid

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Description of Project:

Background: The human respiratory tract is home to multiple bacterial species interacting with each other as well as with the host cells. Many of these resident bacteria will produce chemical agents, such as hydrogen peroxide (H_2O_2), to inhibit the growth of other microbes. While a by-product of aerobic respiration, H_2O_2 can be produced and utilized to damage cellular macromolecules and inactivate bacterial enzymes. Moreover, H_2O_2 can serve as a signaling molecule to modulate gene activity, a feature that can be exploited by commensal bacteria, pathogenic bacteria, and even host cells. This is most evident in the oral cavity and nasopharynx where *Streptococci* species produce certain H_2O_2 concentrations to kill bacteria but sublethal concentrations in mixed-species communities. This is of interest as the respiratory microbiome is replenished, in part, by bacteria from the oral cavity. These transitioning microbes could have been exposed to H_2O_2 from interactions with Streptococci species; and re-calibrated their gene expression and cellular physiology in response. They may also have developed a tolerance to certain levels of H_2O_2 stress. Also noteworthy is that the only Streptococci species resident to the lower respiratory tract is *S. pneumoniae*. Thus, H_2O_2, whether its production, exposure, and/or tolerance, could be instrumental in the establishment and maintenance of microbial niches further within the respiratory microenvironment.

Previous in vitro studies have either centered on select Streptococci species, or co-culture systems, and their H2O2 production in nutrient-rich environments. Yet, the microenvironment of the respiratory tract sequesters nutrients from the lumen; more specifically, the respiratory tract lining fluid (RTLF) is nearly devoid of all nutrients and potential growth factors. Thus, a remaining challenge is to decipher how this change in microenvironment impacts the responses and survival mechanisms employed by commensal and pathogenic bacteria under H2O2 stress. This project aims to provide insight into the following questions:

[1] Do Streptococci species as well as respiratory commensal bacteria produce H2O2 in the presence of RTLF?
[2] When Streptococci species and respiratory commensal bacteria are co-cultured, is there an increase in H2O2 production?
[3] Does RTLF affect respiratory commensal bacteria's ability to respond to H2O2 stress?

Experimental Approach: Streptococci commensal strains (S. mutans, S sanguinis) and respiratory strains of lung commensal bacteria (*S. pneumoniae, H. influenzae, M. catarrhalis,* and *S. aureus*) will be utilized in a planktonic state, from liquid cultures grown to mid-exponential phase, for experiments. Gamble's solution will be utilized as simulated RTLF (sRTLF). H_2O_2 production in sRTLF will be assessed directly during bacterial growth (OD600 absorbance) in liquid cultures as well as with co-cultures on agar plates (CFU counts, zones of inhibition). Colony morphology will also be assessed. H_2O_2 susceptibility of individual bacteria and co-cultures in sRTLF will be assessed directly during bacterial growth (OD600 absorbance) in liquid cultures; metabolic activity will be determined by MTT assay. The percentages of specific bacterium within co-cultures will be assessed by excising colonies from agar plates and plating on selective media plates for cfu counts. Colony morphology will also be assessed. Both DNA (culture supernatants, bacterial colonies) and protein (from culture supernatants) will be collected and stored for future experiments and analysis.

Potential Pitfalls and Alternate Strategies: Some of the strains to be utilized grow faster than the others; this advantage could influence interactions and the mechanism(s) to be studied. Will utilize different bacterial ratios to compensate for this growth difference. While this project is viewing H_2O_2 production as a signaling molecule and survival mechanism, it could also be viewed as a virulence factor. Laboratory strains may have H_2O_2 production capabilities removed or repressed. May purchase alternative strains to verify H_2O_2 production observed. Cultivation of new strains could take one to two weeks but would be happening co-currently with experiments. Commensal bacteria may use other molecules to influence growth of other microbes, but these molecules will have to be addressed in future experiments. Furthermore, this project is not modeling all the microbial interactions that occur *in vivo*; may be missing a key interaction that could be addressed in future experiments.

Measureable goals and Objectives:

[1] Ascertain H_2O_2 production levels for Streptococci species in sRTLF
[2] Ascertain H_2O_2 production levels for lung commensal bacteria in sRTLF
[3] Ascertain H_2O_2 susceptibility for Streptococci species in sRTLF

[4] Ascertain H_2O_2 susceptibility for lung commensal bacteria in sRTLF
[5] Ascertain H_2O_2 production levels for Streptococci species in co-cultures with lung commensal bacteria
[6] Ascertain H_2O_2 production levels for lung commensal bacteria in co-cultures with Streptococci species
[7] Ascertain bacterial percentage of individual bacteria from co-cultures
[8] Collect and store protein samples
[9] Present data via faculty development grant presentation series or biology department meeting

Project Timeframe:

[1] Cultivation of bacteria	1 week
[2] Evaluation of H_2O_2 production	3-4 weeks
[3] Evaluation of H_2O_2 Susceptibility	3-4 weeks
[4] Determination of bacterium percentages in co-cultures	2-3 weeks

Note that some of these experiments will be run co-currently.

How project enhances teaching and research at Lincoln University:

This project overlaps with the goals of the institution as well as the biology department. This project is a continuation of my overall research interest in developing an *in vitro* ALI polymicrobial culture as well as polymicrobial-epithelial cell co-culture systems that better model the *in vivo* respiratory tract environment. It would provide important predictive information regarding a key component of processes that occur at mucosal surfaces and the immune response to pathogenic bacteria. This project would be expanded into additional research opportunities for Lincoln students, utilizing any of the grant, internship mechanisms at their disposal. For instance, a previous faculty development grant directly lead to a new project for a student that was funded via Title III. The results of this project would also serve as preliminary data for grants.

This project could be the basis for a new project for students that need to complete an independent research project as required for a biology major. Moreover, students enrolled in the research courses BIO413 and BIO414 as a prerequisite need to have completed a research project or actively involved with one. There would also be an opportunity for expansion into a project for a post-bachelorette candidate. I strive to improve the laboratory experiences of Lincoln students, with emphasis on clinical applications. For instance, the commensal bacterial strains utilized for a previous faculty development grant were utilized in lab exercises for BIO250L, BIO401L, and BIO402L. I can incorporate aspects of this project's research design into future laboratory exercises of current labs or a new lab-focused course. I could use the project's results to develop a case study for data analysis.

Measure of Success of Project:

Success of this proposal can be measured in a couple of manners. Ideally, obtaining significant data for each of the objectives. Generation of the report as well as a poster and/or presentation.

Sharing of Project's Outcomes:

The findings of this proposal will be prepared as both an oral and poster presentation that will be part of the faculty development grant presentation series or research symposium. This presentation can also be presented as part of the biology department seminar series or monthly meeting. The findings of this proposal could be presented, in the form of poster, at the American Society for Microbiology (ASM) Conference for Undergraduate Educators (ASMCUE) in the fall of 2024. Moreover, the results of this project are complementary to a current ongoing project, and the combined results could be presented by the undergraduate researcher at Annual Biomedical Research Conference for Minoritized Scientists (ABRCMS) conference in the fall of 2024.

Budget:

[1] Research materials and supplies	$1,200
[2] Summer Salary (2 months @ $3,375/month)	$6,750
TOTAL	**$7,950**