

***In Vitro* Investigation of Bacterial Growth and Functionality
in Simulated Respiratory Tract Lining Fluid**

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Project Background:

The COVID-19 pandemic and the resulting strain of health systems worldwide has reinforced the need to develop and improve *in vitro* modeling systems that reflect the *in vivo* respiratory environment during normal and diseased states. Responsible for gas exchange, the organs of the human respiratory tract are continuously exposed to pathogens, allergens, and toxicants from the external environment. However, recent studies have demonstrated a community of microbes reside within the respiratory tract; and that this resident microbiota are involved in lung health as well as disease outcomes [1]. Altered respiratory microbiome homeostasis has been found in patients with a number of respiratory conditions, including asthma, pneumonia, cystic fibrosis (CF), idiopathic pulmonary fibrosis (IPF), and chronic obstructive pulmonary disease (COPD). The unique ecological niche created by the lung microenvironment for microbes is shaped by the differences in epithelial composition along the respiratory tract as well as the lining fluid that covers the epithelial layer. Notably, it is the respiratory tract lining fluid (RTLFL) that a microbe likely deposits into when they arrive in the respiratory tract. And, it is within the RTLFL that microbes interact with other microbes, either by direct cell-to-cell contact or indirectly with released microbial products. These interactions could influence bacterial growth and/or behavior within the respiratory microenvironment as well as subsequent interactions with respiratory epithelial or immune cells and antimicrobial drugs or antibiotics. Moreover, acute infectious or non-infectious insults can result in both the lining fluid and epithelial barrier being disrupted, affecting the respiratory microbiome.

Most *in vitro* studies on polymicrobial interactions have not been conducted in the context of the respiratory microenvironment. Studies that have focused on microbial respiratory infections, addressed individual bacterial species, and the microbial-host cell interactions, using *in vitro* air-liquid interface (ALI) systems that do not reproduce the lining fluid dynamics seen *in vivo*. Analysis of bronchoalveolar lavage fluid (BALF) from healthy and diseased patients is a common practice, but extracting, concentrating, and sterilizing BALF for *in vitro* research has not been demonstrated to be a viable option. Several formulations for simulated lung lining fluid have been developed but have utilized primarily for inhalation bioavailability and particulate bioaccessibility/bioactivity assays. **With this proposal, I aim to ascertain the growth and functionality of respiratory commensal microorganisms in simulated RTLFL.** This project will provide insight into the bacterial sensing and decision-making that occurs during the initial colonization of the respiratory tract as well as guide incorporation of polymicrobial cultures into *in vitro* epithelial cell systems.

Experimental Approach:

Respiratory strains of lung commensal bacteria (*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *S. aureus*) will be acquired, and cultivated. Experiments will utilize planktonic bacteria, from liquid cultures grown to mid-exponential phase. To simulate RTLFL, Gamble's solution will be supplemented with antioxidants and mucin proteins. Known concentrations of each bacterial strain will incubated in RTLFL diluted to different concentrations in the recommended bacterial media for each strain. Cell density and growth rate will be determined by spectrophotometry. Cell viability will be determined by plating onto nutrient agar and CFU counting; colony morphology will also be assessed. Metabolic activity will be determined by MTT assay. Biofilm production rate will be determine by crystal violet assay. Biofilm metabolic activity will be determined by AlamarBlue assay. Both DNA and protein (from culture supernatants) will be collected and stored for future experiments and analysis.

Measureable Goals and Objectives:

- [1] Purchase and cultivate respiratory strains of lung commensal bacteria
- [2] Generate long-term glycerol stocks of bacterial strains
- [3] Ascertain a range of RTLFL concentrations that:
 - [a] supports growth of each bacterial strain

[b] inhibits biofilm production or activity

[4] Correlate metabolic activity to cell viability and biofilm production

[5] Collect and store DNA and protein samples

[6] Present data at biology department meeting

Project timeframe:

Bacterial cultivation and generation of long-term glycerol stocks	1 week
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Bacterial – RTLF incubation experiments and analysis	6 – 8 weeks
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How project enhances teaching and research at Lincoln University:

This project overlaps with the goals of the institution as well as the biology department in several manners. The microorganisms, once cultured and long-term stocks made, will be utilized in existing lab exercises in BIO250 and BIO401. These particular microbial strains are not currently utilized in these labs, and offer additional opportunities for students to work safely with clinically-relevant microbes that possess unique characteristics. Moreover, the select microbes in this project will be a part of new lab exercises that will be developed for BIO250, BIO401, and BIO402. With many of the students enrolled in these noted courses having clinical or research aspirations, it is important to provide intensive laboratory experiences that prepare them for what they will encounter in their professional/graduate studies and careers.

One of the primary aims of the research I aim to conduct at Lincoln is to establish *in vitro* ALI polymicrobial culture as well as polymicrobial-epithelial cell co-culture systems. This project will provide necessary information to develop such systems that will address the problems and shortfalls of existing models. Project results will also serve as preliminary data for National Science Foundation grants. This project would also serve as a research opportunity for Lincoln students interested in careers in health sciences and biological research; and, as I build upon the results from the project, allow me to offer students additional research opportunities in my lab.

References:

[1] Ivernizzi R, Lloyd CM, Molyneaux. Respiratory microbiome and epithelial interactions shape immunity in the lungs. *Immunology*. 160(2):171-182. June 2020.

Budget:

[1] Research materials and supplies	\$1,500
[2] Summer Salary (2 months @ \$3,075/month)	\$6,150