Description of Project:

Background: A number of human bacterial pathogens are able to cause infection in a variety of locations within the human body due to their adaptability; possessing multiple mechanisms of resistance; and/or their ability to produce a biofilm. The most notably of such microbes are the ESKAPE pathogens (*E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* species), so named for the frequency at which they can "escape" antibiotic and other antibacterial treatments. As such, these microbes are frequently responsible for hospital-associated and opportunistic infections [1]. The tendency of ESKAPE pathogens to be or become tolerant, and eventually resistant, to antibiotics has made development of novel antibacterial therapies of the utmost importance. A potential source of new antimicrobials are soil microbes that produce such compounds as secondary metabolites [2]. Environmental conditions, such as soil compaction, pollution, and nutrient imbalances, create intense competition within these microbial communities, promoting the evolution and synthesis of the antimicrobial metabolites as a survival strategy. While harnessing soil bacteria for novel antimicrobials is not new, the screening process can be slow and meticulous due to the difficulty in culturing soil bacteria in the lab using traditional methods. Moreover, these bacteria likely only produce their antimicrobial metabolites in the presence of other bacteria. Nevertheless, a colleague at Mercer University School of Medicine has recently isolated an unknown soil bacterium that could be cultured in bacterial media and displayed antibacterial activity against *S. aureus*. This is an exciting prospect as this bacterium, herein dubbed "JC isolate 1," could have broader antibacterial potential. In an effort to foster this collaboration and leverage prior experience with ESKAPE pathogens, **this proposal aims to characterize the interactions between JC isolate 1 and select ESKAPE pathogens**. Moreover, the microenvironments of the oral cavity and upper respiratory tract will be considered; the associated anatomical structures are continuously exposed to potential pathogens via inhaled air as well as in food and water. However, nutrients are sequestered from the lining fluids of these structures, creating a less ideal environment. Nevertheless, preventing the establishment of pathogens in these regions is paramount. **I hypothesize that JC isolate 1, with either direct interaction with ESKAPE pathogens or indirect interaction with their secreted compounds, will present antibacterial activity against some of the ESKAPE pathogens.** The results of this project could reveal a promising antibacterial agent to be further explored.
Experimental Approach: Laboratory strains of pathogenic bacteria (*S. aureus*; *K. pneumoniae; P. aeruginosa; A. baumannii*), as well as JC Isolate 1 will be cultivated. Gamble's solution will be utilized as simulated respiratory tract lining fluid (sRTLF); simulated saliva (SS) will be purchased. Growth of the bacterial strains in simulated fluids will be confirmed by overnight growth in various concentrations of the simulated fluids (sRTLF, SS) and measurement of absorbance (OD600); complemented by plating and CFU counts. Growth kinetics over time will be measured by absorbance as well. For direct interactions, JC Isolate 1 will be co-cultured with an ESKAPE pathogen on agar plates and zones of inhibition measured. Additionally, overnight cultures of JC Isolate 1 and an ESKAPE pathogen will be mixed 1:1 and, after incubation, plated and ESKAPE pathogen colonies (cfus) counted and morphology assessed. For indirect interactions, bacteria-conditioned simulated fluids will be generated; planktonic cultures of the bacterial strains will be grown in the simulated fluids before the cultures are centrifuged, and supernatant collected and filtered. For all conditioned simulated fluids conditions, lack of viable microbes will be confirmed by plating. DNA and Protein concentrations of conditioned simulated fluids will be determined by Nanodrop and Bradford Protein assay, respectively. For all experiments, the conditioned simulated fluids will be diluted 1:1 with fresh simulated fluid. To ascertain the impact of conditioned simulated fluids on bacterial growth, planktonic cultures will be exposed to the conditioned simulated fluids for 24 h and growth determined by absorbance and cfu counts; total biofilm biomass and biofilm activity state will also be assessed by the crystal violet staining method and quantification of bacterial DNA, respectively.

Measurable Goals and Objectives:

[1] Ascertain growth of JC isolate 1 in SS and sRTLF
[2] Generate long-term glycerol stocks of JC isolate 1
[3] Assess impact of co-culturing with JC isolate 1 on ESKAPE pathogens' growth and colony morphology
[4] Generate conditioned SS and sRTLF for each bacterial strain
[5] Assess impact of exposure to conditioned SS and sRTLF on all bacterial strains, in terms of (a) growth; (b) biofilm biomass; and (c) biofilm activity

Project timeframe:

Project will be conducted throughout the summer of 2026.

[1] Cultivation of JC isolate 1	1 week
[2] Generation of long-term glycerol stocks of bacterial strains	1 week
[3] Co-culture experiments and data analysis	3 – 4 weeks
[4] Generation of conditioned SS and sRTLF	1 – 2 weeks
[5] Conditioned media experiments and data analysis	4 – 5 weeks

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

How project enhances teaching and research at Lincoln University:

This project aligns with the personal research goals of Dr. Saunders, the goals of the biology department, and the goals of the institution. One of Dr. Saunders' research goals here at Lincoln is focused on developing and utilizing *in vitro* models to characterize the microbial interactions that occur at the body's mucosal surfaces, and influence health and diseased states. This project extends that interest into a more clinical application, potentially identifying novel antibacterial agent(s). While providing insights and data for future projects and grants, the current proposal would strengthen the relationship with the Mercer University School of Medicine, potentially leading to future collaborations, grant proposals, and even a memoranda of understanding that would aid Lincoln students seeking medical or graduate programs to apply to. Considering the University's biology majors, the students have to complete an independent research project; this project may also be co-current with enrollment in the research courses, BIO4013 and BIO4014. This project could be expanded into projects for said students during the academic year or summer, as evident by previous faculty development grants Dr. Saunders has received resulted in research opportunities for Lincoln students. Each of those students have presented at either the Lincoln science fair or research symposium; a LSAMP research symposium; the Annual Biomedical Research Conference for Minoritized Scientists (ABRCMS); and the Association for Dental Safety (ADS) 2025 Annual Conference. Further, considering biology students, the findings of this proposal would improve their laboratory experiences, particularly those with clinical or research aspirations. Dr. Saunders has utilized materials from previous grants in lab exercises for BIO2050L, BIO4001L, and BIO4002L.

Measure of Success of Project:

Success of this proposal can be measured several ways. One, receiving and establishing JS isolate 1 for future use here at Lincoln; the conditioned simulated fluids generated will also be utilized in future experiments. For another, ideally, obtaining significant data for all outlined experiments. And, finally, producing the project report as well as a poster and/or presentation; ideally, presenting at a national or regional conference.

Sharing of Project's Outcomes:

The findings of this proposal will be prepared as both an oral and poster presentation for the faculty research symposium. The findings of this proposal could also be submitted for presentation at the annual meeting of the American Society for Microbiology (ASM) in the summer of 2026. Lab exercises developed from this project could be prepared for presentation at either the annual meeting for the Society for the Advancement of Biology Education Research (SABER) in the summer of 2026, or the American Society for Microbiology (ASM) Conference for Undergraduate Educators (ASMCUE) in the fall of 2026.

References:

[1] De Rodas FG, Corcione S, Pagani N, and Di Perri G. From ESKAPE to ESCAPE, from KPC to CCC. *Clin Infect Dis*. 60(8): 1289-90. April 2015.

[2] Dinglasan, J.L.N., Otani, H., Doering, D.T., Udwary D, and Mouncey NJ. Microbial secondary metabolites: advancements to accelerate discovery towards application. *Nat Rev Microbiol* 23: 338–354. January 2025.