UREAP APPLICATION FORM

THOMPSON RIVERS UNIVERSITY
Office of Research and Graduate Studies

First Name: Benjamin

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Student ID: T00705900 Start Date of Project: 12/01/2026 (DD/MMM/YYYY)

Please complete all sections of this application form.

1. FACULTY MENTORS INFORMATION

1.1 Who is your Primary Faculty Mentor? Dr. Heidi Huttenen-Hennelly

1.2 Who is your Secondary Faculty Mentor? Dr. Eric Bottos

NOTE: Your Primary and Secondary Faculty Mentors must each complete a Faculty Mentor Support Form. Forms can be found under the attachments tab within your TRU Romeo UREAP application and on the TRU UREAP webpage under information and Forms for Faculty Mentors..

2. PROJECT DESCRIPTION

2.1 Provide an abstract of your proposed research: (maximum 1500 characters)

Antibiotic resistant bacteria and biofilms are a big problem in public health, and treatments alternative to traditional antibiotics are currently highly sought after. Biofilms specifically often show high antibiotic resistance and can cause chronic, hard-to-treat infections. Few specific antibiofilm agents are known, however antimicrobial peptides (AMPs) are promising candidates. AMPs are commonly derived from natural sources and act against bacteria with fast, broad-spectrum action. Some AMPs are established as effective antibiofilm agents but many more have not yet been tested.

This research project will assess the antibiofilm capabilities of five AMPs from two different natural sources developed by previous research done at TRU. Biofilms of Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus will be grown in specialized 96-well plates and treated with the five AMPs to assess antibiofilm action. Biofilm growth will be assessed using dyes and spectrophometric measurements.

To try and gain more insight into the mode of action of these AMPs, DNA binding assays will also be completed to learn each peptides affinity for DNA. A high affinity to DNA could indicate the peptide binds and disrupts DNA once inside a microbial cell.

The project has a proposed timeline of twelve weeks. A research report and poster presentation will also be produced to share the process and findings afterwards.

2.2 Provide a brief literature review for your proposed research: (maximum 3500 characters)

Biofilms are bacterial communities with one or many species that are adhered to a surface and are generally more resistant to antibiotics and other stressors than normal colonies. They have a protective extracellular matrix which acts as a protective barrier against mechanical and chemical threats. The

matrix is made of secreted polysaccharides, proteins and nucleic acids collectively referred to as extracellular polymeric substances (EPS) (Shree et al., 2023; Agrawal, 2023; Wood, 2009). Biofilm formation can be initiated simply by cell adherence to a surface and cell-cell signalling, both of which stimulate EPS gene expression (Shree et al., 2023; Agrawal, 2023). The growth beneath the matrix is stratified, with higher activity cells toward the surface, and more dormant cells underneath (Shree et al., 2023). The high antibiotic resistance of biofilms is due to multiple factors including fast, efficient, and widespread horizontal gene transfer (especially of antibiotic resistance genes), inefficient diffusion of antibiotics through the matrix, and faster production of antibiotic inhibitors or efflux pumps (Agrawal, 2023; Shree et al., 2023). Because of their toughness, biofilms pose problems in healthcare settings where they can grow on instruments or in the body, but not all biofilms are pathogenic and some are engineered for use in bioremediation, biopower generation or chemical synthesis (Shree et al., 2023).

The existence of antibiotic resistant pathogens (including pathogenic biofilms) is a growing problem for public health and is worsened as traditional antibiotics are constantly overprescribed. As an alternative to traditional antibiotics, many antimicrobial peptides (AMPs) derived from natural or artificial sources have been researched. AMPs are characterized by their broad-spectrum activity, fast action, and difficult development of resistance, but also their tendency for cytotoxic and hemolytic effects (Matheson et al., 2013; Podorieszach and Huttunen-Hennelly, 2010; Mercer-Brunelle, 2024). The generally proposed mode of action for AMPs is membrane disruption and then penetration into the cell, where they may bind DNA, RNA or protein and disrupt cell processes (Azad et al, 2011; Huang et al, 2010). The amount and sequence of hydrophobic and cationic amino acids are the main factors that govern both antimicrobial activity and hemolysis of a potential AMP. When designing or optimizing an AMP it is these amino acids, specifically tryptophan and lysine, which are the most relevant (Matheson et al., 2013; Podorieszach and Huttunen-Hennelly, 2010; Azad et al, 2011; Huang et al, 2010).

The effectiveness of AMPs against biofilm is less researched, and the testing process of new AMPs usually prioritises minimum inhibitory concentration (MIC) tests and hemolysis assays (Podorieszach and Huttunen-Hennelly, 2010; Mercer-Brunelle, 2024). However, it is established that many AMPs also act as biofilm inhibitory peptides (BIPs) which can target a biofilm matrix and/or biofilm genes specifically (Agrawal, 2023). Similarly to traditional AMPs, BIPs also tend to be rich in hydrophobic and cationic amino acids (Agrawal, 2023). This project will focus on testing the antibiofilm capabilities of the established AMPs macropin1 and the derivative MR (Plowe 2024) as well as indolicidin and some derivatives (Mercer-Brunelle, 2024). Nucleic acid or protein binding assays may also be conducted with these AMPs to gain insights into mode of action.

2.3 What is the hypothesis or research question for your proposed research? Include any specific objectives: (maximum 500 characters)

This project will be based on the following research questions:

Do the specified antimicrobial peptides (macropin1 and derivative MR (Plowe 2024), indolicidin and derivatives Δ6,8 and Δ9,11(Mercer-Brunelle 2024)) exhibit effective activity against gram-positive and gram-negative bacterial biofilms?

What is the DNA binding affinity of the specified AMPs and what can we infer about mode-of-action from that DNA binding affinity?

2.4 Provide a description of the research methodology/methodologies and analysis that you intend to employ in completing this research: (maximum 1500 characters)

The bacterial species Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus (all containment level 1) will be grown in ideal conditions and isolated colonies will be obtained by plate streaking. Biofilms of each species will then be grown in a specialized 96 well biofilm assay plate. The biofilm growth will be assessed by treatment with dyes and then plate reader measurement at 621 nm.

The antibiofilm assay will be conducted with AMPs macropin1 (GFGMALKLLKKVL), macropin1 derivative MR (GRGRALKLLKKVL), indolicidin (ILPWKWPWWPWRR-NH2), indolicidin Δ6,8 (ILPWKAPAWPWRR-NH2), and indolicidin Δ9,11 (ILPWKWPWAPARR-NH2) (Plowe 2024; Mercer-Brunelle 2024). The extent of biofilm inhibition or eradication by each peptide at varying concentrations will be evaluated using procedure previously done by Olsen (2024). The antibioiflm assays typically take 5-7 days.

To determine the DNA binding capabilities of the peptides, an electrophoretic mobility shift assay will be conducted using plasmid DNA. Varying amounts of each peptide will be mixed with plasmid DNA and run on a 1% agarose gel. The DNA bands will be visualized under UV light and the migration distance of control plasmid versus peptide+plasmid will be observed. A reduction in migration distance indicates high affinity of a peptide to the dsDNA.

2.5 Provide a description of how your research will significantly impact your field of study:

(maximum 1500 characters)

Antibiotic resistant bacteria can turn a minor infection into a life-threatening one, and are becoming increasingly common in the public health space. Pathogenic biofilms cause two thirds of all infections and often cause chronic symptoms that persist even after antibiotic treatment. Biofilms are also much less understood than planktonic colonies, and there are few specific and effective antibiofilm treatments (Haney et al. 2021). Furthermore, testing of antibiofilm capability is often left out in novel AMP development due to time and financial constraints (Plowe, 2024; Mercer-Brunelle, 2024).

This research could establish some of the five tested AMPs as specific antibiofilm agents. Otherwise, the results of the biofilm and DNA binding assays will still contribute to the AMP knowledge base and help give direction to future studies. DNA binding assays specifically give AMP mode-of-action insights, which can be useful when developing new AMPs. Furthermore, these results could be added to the antimicrobial peptide database (APD) (Wang & Wang, 2004), where other researchers can look when designing future studies.

2.6 Describe your plans to disseminate your research findings: (maximum 500 characters)

A report of my research will be submitted to my supervisor and co-supervisor once the project is complete. This will provide a summary of all the methodology, findings, and challenges of the research project.

I also plan to produce a poster and presentation based on this research for the TRU Undergraduate Research conference and any other research sharing opportunities at TRU.

2.7 List the references that you have citied throughout your research proposal observing the appropriate citation style for your discipline: (maximum 3500 characters)

Agrawal, R. Biofilms; 2023.

Azad, M. A.; Huttunen-Hennelly, H. E. K.; Ross Friedman, C. Bioactivity and the First Transmission Electron Microscopy Immunogold Studies of Short de Novo-Designed Antimicrobial Peptides. Antimicrob Agents Chemother 2011, 55 (5), 2137–2145. https://doi.org/10.1128/AAC.01148-10.

Huang, Y.; Huang, J.; Chen, Y. Alpha-Helical Cationic Antimicrobial Peptides: Relationships of Structure and Function. protein. cell. 2010, 1 (2), 143–152. https://doi.org/10.1007/s13238-010-0004-3.

Matheson, S.; Cheeptham, N.; Huttunen-Hennelly, H. Investigating the Effects of Hydrophobicity and Charge on the Therapeutic Ability of the Antimicrobial Histatin 8 Peptide for Potential Use in Oral Applications. International Journal of Biology 2013, 5 (2), p85. https://doi.org/10.5539/ijb.v5n2p85.

Mercer-Brunelle, A. Determining Antimicrobial Susceptibility and Hemolytic Activity of Indolicidin Derivatives, Thompson Rivers University, 2024.

Olsen, T. Determination of Antimicrobial, Antibiofilm, and Hemolytic Activity of Aurein-1.2 Derivatives, 2024.

Plowe, E. Optimizing Antimicrobial Susceptibility and Hemolytic Activity in Macropin Derivatives, 2024.

Podorieszach, A. P.; Huttunen-Hennelly, H. E. K. The Effects of Tryptophan and Hydrophobicity on the Structure and Bioactivity of Novel Indolicidin Derivatives with Promising Pharmaceutical Potential. Org. Biomol. Chem. 2010, 8 (7), 1679–1687. https://doi.org/10.1039/B921248E.

Shree, P.; Singh, C. K.; Sodhi, K. K.; Surya, J. N.; Singh, D. K. Biofilms: Understanding the Structure and Contribution towards Bacterial Resistance in Antibiotics. Medicine in Microecology 2023, 16, 100084. https://doi.org/10.1016/j.medmic.2023.100084.

Wang, Z.; Wang, G. APD: The Antimicrobial Peptide Database. Nucleic Acids Res 2004, 32 (suppl_1), D590–D592. https://doi.org/10.1093/nar/gkh025.

Wood, T. K. Insights on Escherichia Coli Biofilm Formation and Inhibition from Whole-Transcriptome Profiling. Environ Microbiol 2009, 11 (1), 1–15. https://doi.org/10.1111/j.1462-2920.2008.01768.x.

3. PROJECT TIMELINE WITH BENCHMARKS

3.1 Provide a timeline for your project that includes key benchmarks: (maximum 1000 characters)

Week 1: Get laboratory orientation and training.

Week 2: Prepare media and establish initial bacterial cultures.

Week 3: Begin biofilm assays. (5-7 days each)

Weeks 4-7: Continue biofilm assays. Including inevitable troubleshooting.

Weeks 8-9: Finish biofilm assays. Complete the DNA binding assay.

Week 10-11: Synthesize all results into a research report and poster presentation.

Week 12: Finishing touches and report submission.

NOTE: Please refer to the UREAP Help Guide for a project timeline example. Students must demonstrate a willingness to engage in 12 weeks or equivalent of sustained research per the Terms of Reference.

4. OPERATING GRANT BUDGET PROPOSAL

4.1 The UREAP award offers up to $1000 toward direct research expenses. These expenses must be preapproved by the UREAP committee in the adjudication phase. Use the provided template under the Attachments tab in the TRU Romeo UREAP application to complete your budget proposal. Copy amount from the TOTAL AMOUNT line of the budget here. Total Amount: $ 1000

4.2 Additional budget information: (maximum 500 characters)

General lab supplies for this type of research are required and include growth media, petri dishes, pipet tips, agarose (for gel electrophoresis), and other general supplies. Specifically for this project, biofilm assay plates are required, which are expensive.

The total cost of these supplies is approximately $1000.

I have no plans to attend a conference so no cost is incurred there.

5. CONTRIBUTION TO ACADEMIC/PROFESSIONAL GOALS

5.1 Describe how this project will contribute to your academic and/or professional goals:

(maximum 1000 characters)

The primary takeaway of this research project for me will be experience doing research in a proper lab environment. My primary goal after my undergraduate degree is a Master's degree and then a career doing research. I feel that this opportunity will let me start doing what I would like to be doing for the next portion of my life.

This project has the whole research process: starting from a broad topic and then background research in the literature, developing a research question, developing methodology, conducting the research, working through the inevitable challenges, and then effectively reporting the results. Going through this

process before completion of my undergrad would be invaluable in terms of supplementing my normal courses, but also when I begin looking into the next chapters of my career.

Secondly, working on such a relevant topic means I could make real contributions to the scientific body of knowledge, making the opportunity even more special for me.

NOTE: Include your role in conceiving of the project, your role in the implementation of the project, and your overall academic objectives – explaining how this project will help to advance those objectives.