This is provided as an example proposal.
it is important that you follow the current guidelines.

The mentor letter has been removed.

The John J. Kopchick Molecular and Cellular Biology/Translational Biomedical Sciences Undergraduate Student Support Fund
Cover Page Academic Year 2022-2023

NAME OF APPLICANT: Riley Eileen Zielinski

E-MAIL ADDRESS: rz650818@ohio.edu
DEPARTMENT: Biological Sciences
EXPECTED GRADUATION DATE (Month and Year): May 2023
TITLE OF PROJECT: Exploring the Effect of Physiologically Relevant Stress Conditions on Bacterial RNA Cytotoxicity

FACULTY MENTOR INFORMATION:
NAME: Dr. Ronan Carroll
E-MAIL ADDRESS: carrollr3@ohio.edu
DEPARTMENT: Biological Sciences

NUMBER OF YEARS: 8 IN THE ☑ MCB OR ☐ TBS PROGRAM

BUDGET: Total Request $1,500
(May not exceed $1,500)

STATEMENT OF HOW THE RESEARCH IS RELEVANT TO TRANSLATIONAL BIOMEDICAL SCIENCES* (500 character limit)

Staphylococcus aureus is a bacterium that has evolved resistance to many antibiotics, making treatment of infections difficult. Determining how *S. aureus* causes cell death in host organisms is of particular interest to biomedical research, as interrupting this pathway could be a major target for treatment. This study will further investigate our recent discovery that *S. aureus* RNA has the ability to cause cell death, focusing on the potential for bacterial growth conditions to affect rates of RNA cytotoxicity.

*For the purposes of this program, translational biomedical sciences is defined as the translation of basic research effectively into enhanced healthcare outcomes for the entire population in fields such as biomedical research, bioengineering, drug development, informatics, communications, health policy and planning.

IRB AND IACUC APPROVAL:
To ensure that the University is in compliance with all federal regulations, complete the checklist below. *Note: your proposal can be approved prior to IRB or IACUC approval (put "pending" or "to be submitted" instead of approval number), but funding will be withheld until notification of approval or exemption.*

Yes	***No***	***Office of Research Compliance***	***Policy #***
	X	Human Subjects in Research (including surveys, interviews, educational interventions): Institutional Review Board (IRB) Approval #: Expiration Date:	19.052
	X	Animal Species: Institutional Animal Care & Use Committee (IACUC) Approval #: Expiration Date:	19.049

SIGNATURES

Applicant's Signature		**Faculty Mentor's Signature**	
Signature	[signature]	Signature	[signature]
Name	Riley Zielinski	Name	Ronan Carroll

Optional: ☑ Yes ☐ No

If selected for funding, I give permission to the Office of the Vice President for Research and Creative Activity to use my proposal as an example during training and workshop exercises.

Biographical Sketch

- Applicant Name: Riley Eileen Zielinski
- GPA: 3.896
- Educational Training:
  - Ohio University, Honors Tutorial College Biological Sciences, Anthropology minor, Chemistry minor
    - Senior (Expected Graduation May 2023), Current GPA 3.896
  - Athens High School, Honor's Diploma, The Plains, Ohio
    - Graduated 2019 with 3.88 GPA
- Publications and Presentations:
  - Briaud P, Frey A, Marino EC, Bastock RA, Zielinski RE, Wiemels RE, Keogh RA, Murphy ER, Shaw LN, Carroll RK. 2021. Temperature influences the composition and cytotoxicity of extracellular vesicles in Staphylococcus aureus. mSphere 6:e00676-21. https://doi.org/10.1128/mSphere.00676-21
  - Riley E. Zielinski, Rachel L. Zapf, Emily C. Marino, Emily G. Sudnick and Ronan K. Carroll 2022. Investigating the toxicity of Staphylococcus aureus "naked RNA." Poster at The Ohio University Student Research and Creative Activity Expo.
    - 2nd Place: 2022 John Kopchick EXPO Poster Award
- Relevant Coursework
  - BIOS 3205- Cellular and Microbiology Techniques
  - BIOS 3220- General Microbiology
  - CHEM 3050/3060- Organic Chemistry I & II
  - BIOS 4900- Model Systems of Microbiology
- Honors and Awards
  - Raymond C. Cook Endowed Scholar, 2019-Present
  - Dr. John R. Johnson Endowed Scholar, 2020
  - Hiram/Florence Wilson Scholarship Recipient, 2020
- Current, Pending, and Previous Funding
  - Previous: 2022, DAAD RISE Internship Recipient, In vitro fitness of Rwandan P. falciparum field isolates harboring K13 mutations
  - Current: 2021 John J. Kopchick Molecular and Cellular Biology (MCB)/Translational Biomedical Sciences (TBS) Undergraduate Student Support Fund for Investigating the Toxicity of Staphylococcus aureus "Naked RNA"
  - Current: 2021 Provost Undergraduate Research Fund for Investigating the Toxicity of Staphylococcus aureus "Naked RNA"

After graduating with a B.S. in Biological Sciences, I plan on pursuing a Fulbright in biomedical research in Germany, where I will work on a project currently seeking to update treatment recommendations for malaria. Afterwards, I will be beginning my Ph.D. education back in the United States in microbiology or pathobiology, both fields related to this proposal. The completion of this project will aid in my goals by allowing me to independently manage a research project, as well as practice hands-on skills that will be useful in my future career in research.

Current or Previous Funding

- Current: 2021-2022 John J. Kopchick Molecular and Cellular Biology (MCB)/Translational Biomedical Sciences (TBS) Undergraduate Student Support Fund for Investigating the Toxicity of Staphylococcus aureus “Naked RNA”
    - The research conducted through this award was foundational to this proposal and is presented as preliminary data (Fig 1). Experimentation began in November of 2021 and data analysis for this project was completed in early September 2022. The funding obtained was used to demonstrate the interspecies similarities in cytotoxicity of bacterial pathogen-derived RNA. The current proposal seeks to expand upon this result and analyze the impact of bacterial growth conditions on this observed cytotoxicity.
- Current: 2021 Provost Undergraduate Research Fund for Investigating the Toxicity of Staphylococcus aureus “Naked RNA”
    - This award was for the same project outlined above, to cover remaining costs of materials over the Kopchick budget allotment. Having access to all of the listed materials was crucial to executing the project as designed.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Carroll, Ronan

eRA COMMONS USER NAME (credential, e.g., agency login): ronan.carroll

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Trinity College Dublin, Dublin	BA	06/1999	Microbiology
Trinity College Dublin, Dublin	PHD	07/2004	Microbiology
University of Illinois at Chicago, Chicago, IL	Postdoctoral Fellow	08/2008	Microbiology
The Methodist Hospital Research Institute, Houston, TX	Postdoctoral Fellow	02/2010	Microbiology
Trinity College Dublin, Dublin	Postdoctoral Fellow	10/2010	Microbiology
University of South Florida, Tampa, FL	Postdoctoral Fellow	08/2014	Microbiology

A. Personal Statement

Throughout my career, my overarching interest has been in bacterial genetics, pathogenesis, and gene regulation. As an early career scientist, I worked on a number of different bacterial species including *Salmonella enterica*, *Streptococcus pyogenes*, and *Bartonella henselae*. Research in my lab at Ohio University, where I have been an independent investigator for 8 years, is focused on the bacterial pathogen *Staphylococcus aureus*, a leading cause of nosocomial and community acquired infections in the United States. My research group investigates a variety of diverse topics related to the molecular pathogenesis of *S. aureus*. One central theme for the research in my lab is atypical RNA molecules (i.e. not mRNA, tRNA, or rRNA). My group also studies temperature dependent regulation in *S. aureus*, *S. aureus* extracellular vesicle production, and the role of peptidyl-prolyl cis/trans isomerases (PPIases) in *S. aureus* protein secretion.

This proposal will continue our investigation into *S. aureus* secreted RNA. We have previously demonstrated that *S. aureus* can secrete RNA both inside and outside of EVs, and one central research focus of my lab is understanding the biological impacts of secreted RNA.

1. Briaud P, Frey A, Marino EC, Bastock RA, Zielinski RE, Wiemels RE, Keogh RA, Murphy ER, Shaw LN, Carroll RK. Temperature Influences the Composition and Cytotoxicity of Extracellular Vesicles in *Staphylococcus aureus*. mSphere. 2021 Oct 27;6(5):e0067621. PubMed Central PMCID: PMC8510519.
2. Briaud P, Carroll RK. Extracellular Vesicle Biogenesis and Functions in Gram-Positive Bacteria. Infect Immun. 2020 Nov 16;88(12) PubMed Central PMCID: PMC7671900.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2020 -	Associate Professor, Ohio University, Athens, OH
2014 - 2020	Assistant Professor, Ohio University, Athens, OH
2010 - 2010	Postdoctoral Research Fellow, Trinity College Dublin, Dublin
2010 - 2014	Postdoctoral Scholar, University of South Florida, Tapma, FL
2008 - 2010	Postdoctoral Fellow, The Methodist Hospital Research Institute, Houston, TX
2005 - 2008	Postdoctoral Scholar, University of Illinois at Chicago, Chicago, IL

Honors

2020	Outstanding Faculty Research, Scholarship, and Creative Activity Award, Ohio University
2017	Excellence in Teaching Award, The National Society of Leadership and Success
2012	Research Award, USF Postdoctoral Scholars Association
2003	Marie Curie Scholarship , European Union
2002	Travel Award, European Molecular Biology Organization

C. Contribution to Science

1. Temperature based gene regulation in *Staphylococcus aureus*

   Many pathogenic bacteria use temperature as a signal to regulate expression of virulence genes once they have entered the human host. During its transition from commensal to pathogen *S. aureus* undergoes a small but significant change in temperature from 34˚C to 37˚C. I have investigated how this change in temperature alters the transcriptional and proteomic landscape of the bacteria and shown that *S. aureus* is subject to a large degree of post-transcriptional temperature dependent gene regulation. RNA molecules appear to be at the heart of this regulation with sRNAs, thermoswitches and RNA thermometers all playing a role. My group has also examined the impact of temperature on the production and composition of Extracellular Vesicles (EVs) in *S. aureus*. Interestingly this work has shown that EVs produced at 34˚C contain significantly more RNA than EVs at 37˚C, again suggesting an important role for this molecule in the adaptation to different temperatures.

   a. Briaud P, Frey A, Marino EC, Bastock RA, Zielinski RE, Wiemels RE, Keogh RA, Murphy ER, Shaw LN, Carroll RK. Temperature Influences the Composition and Cytotoxicity of Extracellular Vesicles in *Staphylococcus aureus*. mSphere. 2021 Oct 27;6(5):e0067621. PubMed Central PMCID: PMC8510519.
   b. Bastock RA, Marino EC, Wiemels RE, Holzschu DL, Keogh RA, Zapf RL, Murphy ER, Carroll RK. *Staphylococcus aureus* Responds to Physiologically Relevant Temperature Changes by Altering Its Global Transcript and Protein Profile. mSphere. 2021 Mar 17;6(2) PubMed Central PMCID: PMC8546721.
   c. Briaud P, Carroll RK. Extracellular Vesicle Biogenesis and Functions in Gram-Positive Bacteria. Infect Immun. 2020 Nov 16;88(12) PubMed Central PMCID: PMC7671900.
   d. Hussein H, Fris ME, Salem AH, Wiemels RE, Bastock RA, Righetti F, Burke CA, Narberhaus F, Carroll RK, Hassan NS, Mohamed SA, Fahmy AS, Murphy ER. An unconventional RNA-based thermosensor within the 5' UTR of *Staphylococcus aureus* cidA. PLoS One. 2019;14(4):e0214521. PubMed Central PMCID: PMC6443170.

2. Regulating with RNA in *S. aureus*

   Regulatory RNAs (also called small RNAs or sRNAs) greatly outnumber regulatory proteins in the *S. aureus* cell, yet they remain poorly understood and severely understudied. One factor that has hampered the study of sRNAs in *S. aureus* has been the lack of a clear nomenclature system and their absence from genome annotation files. To facilitate improved study of sRNAs in *S. aureus* I performed a comprehensive re-annotation of the *S. aureus* genome to identify and annotate all known sRNAs. We can now perform RNAseq experiments and, using the updated genome file as a reference, search for previously undiscovered sRNA genes, and monitor global sRNA gene expression. This has opened the door to multiple lines of investigation in my lab centered around the role of RNA molecules in gene regulation. We have identified a trans acting sRNA (Teg41) that controls production of the cytolytic αPSM peptide(s) and also investigated cis acting thermoswitches that control translation of *S. aureus* genes in response to temperature.

   a. Sorensen HM, Keogh RA, Wittekind MA, Caillet AR, Wiemels RE, Laner EA, Carroll RK. Reading between the Lines: Utilizing RNA-Seq Data for Global Analysis of sRNAs in *Staphylococcus aureus*. mSphere. 2020 Jul 29;5(4) PubMed Central PMCID: PMC7392542.

b. Hussein H, Fris ME, Salem AH, Wiemels RE, Bastock RA, Righetti F, Burke CA, Narberhaus F, Carroll RK, Hassan NS, Mohamed SA, Fahmy AS, Murphy ER. An unconventional RNA-based thermosensor within the 5' UTR of *Staphylococcus aureus* cidA. PLoS One. 2019;14(4):e0214521. PubMed Central PMCID: PMC6443170.

c. Zapf RL, Wiemels RE, Keogh RA, Holzschu DL, Howell KM, Trzeciak E, Caillet AR, King KA, Selhorst SA, Naldrett MJ, Bose JL, Carroll RK. The Small RNA Teg41 Regulates Expression of the Alpha Phenol-Soluble Modulins and Is Required for Virulence in *Staphylococcus aureus*. mBio. 2019 Feb 5;10(1) PubMed Central PMCID: PMC6428751.

d. Carroll RK, Weiss A, Broach WH, Wiemels RE, Mogen AB, Rice KC, Shaw LN. Genome-wide Annotation, Identification, and Global Transcriptomic Analysis of Regulatory or Small RNA Gene Expression in *Staphylococcus aureus*. mBio. 2016 Feb 9;7(1):e01990-15. PubMed Central PMCID: PMC4752604.

3. Peptidyl prolyl cis/trans isomerases (PPIases) contribute to virulence in *S. aureus*

PPIase enzymes are best studied in Gram-positive bacteria for their role in folding proteins after they are secreted from the cell, however they can also have additional roles and functions. My research focuses on two *S. aureus* PPIases, PrsA and PpiB. PrsA is a cell-membrane anchored lipoprotein that appears to influence *S. aureus* pathogenesis by folding secreted proteins. PpiB also influences secretion and activity of *S. aureus* virulence factors, however it does so from inside the bacterial cell. Recent results suggests that PpiB may be influencing secretion in *S. aureus* either through interactions with Sec system components or by playing a role in the release of extracellular vesicles.

a. Keogh RA, Zapf RL, Frey A, Marino EC, Null GG, Wiemels RE, Holzschu DL, Shaw LN, Carroll RK. *Staphylococcus aureus* Trigger Factor Is Involved in Biofilm Formation and Cooperates with the Chaperone PpiB. J Bacteriol. 2021 Mar 8;203(7) PubMed Central PMCID: PMC8088519.

b. Keogh RA, Zapf RL, Trzeciak E, Null GG, Wiemels RE, Carroll RK. Novel Regulation of Alpha-Toxin and the Phenol-Soluble Modulins by Peptidyl-Prolyl *cis/trans* Isomerase Enzymes in *Staphylococcus aureus*. Toxins (Basel). 2019 Jun 16;11(6) PubMed Central PMCID: PMC6628628.

c. Keogh RA, Zapf RL, Wiemels RE, Wittekind MA, Carroll RK. The Intracellular Cyclophilin PpiB Contributes to the Virulence of *Staphylococcus aureus* Independently of Its Peptidyl-Prolyl *cis/trans* Isomerase Activity. Infect Immun. 2018 Nov;86(11) PubMed Central PMCID: PMC6204709.

d. Wiemels RE, Cech SM, Meyer NM, Burke CA, Weiss A, Parks AR, Shaw LN, Carroll RK. An Intracellular Peptidyl-Prolyl cis/trans Isomerase Is Required for Folding and Activity of the *Staphylococcus aureus* Secreted Virulence Factor Nuclease. J Bacteriol. 2017 Jan 1;199(1) PubMed Central PMCID: PMC5165095.

4. Role of peptidase enzymes in *Staphylococcus aureus* virulence

The *Staphylococcus aureus* genome contains genes encoding approx. 147 protease and peptidase enzymes. The role of most of these is unknown. Secreted proteases are well known virulence factors in *S. aureus* and in a series of research articles I demonstrated that intracellular and cell-wall associated peptidases can also influence disease causation in this important human pathogen. The research concentrated on an intracellular aminopeptidase (LAP or PepZ) and a cell-wall associated carboxypeptidase (CtpA). In a series of in vitro biochemical activity assays I determined the substrate preference profile for LAP, pH and divalent metal conditions required for optimal activity, and demonstrated that LAP possesses cysteinylglycinase activity in addition to broad spectrum peptidase activity. I demonstrated that the substrate preference and biological activity of LAP can be reprogrammed by alterations in pH and divalent metal conditions.

a. Carroll RK, Robison TM, Rivera FE, Davenport JE, Jonsson IM, Florczyk D, Tarkowski A, Potempa J, Koziel J, Shaw LN. Identification of an intracellular M17 family leucine aminopeptidase that is required for virulence in *Staphylococcus aureus*. Microbes Infect. 2012 Sep;14(11):989-99. PubMed Central PMCID: PMC3426635.

b. Carroll RK, Veillard F, Gagne DT, Lindenmuth JM, Poreba M, Drag M, Potempa J, Shaw LN. The *Staphylococcus aureus* leucine aminopeptidase is localized to the bacterial cytosol and demonstrates a broad substrate range that extends beyond leucine. Biol Chem. 2013 Jun;394(6):791-803. PubMed Central PMCID: PMC3744234.
c. Carroll RK, Rivera FE, Cavaco CK, Johnson GM, Martin D, Shaw LN. The lone S41 family C-terminal processing protease in *Staphylococcus aureus* is localized to the cell wall and contributes to virulence. Microbiology (Reading). 2014 Aug;160(Pt 8):1737-1748. PubMed Central PMCID: PMC4117222.

5. Mutations in the transcription regulator RopB alter Streptococcus pyogenes virulence

Over a 15 year period, 344 clinical isolates of invasive group A Streptococcus (GAS - Streptococcus pyogenes) were collected from patients in the Canadian province of Ontario. Detailed genomic analysis, including whole genome sequencing, was carried out on these strains. While analyzing this data I discovered that the transcription regulator RopB (Rgg) has an unusually high rate of single nucleotide polymorphisms (SNPs). These SNPs consistently resulted in amino acid changes to the RopB protein sequence. I investigated the biological consequence of these changes and demonstrated that overwhelmingly these amino acid changes result in decreased virulence. The decrease in virulence was linked to decreased expression of the secreted protease SpeB, a known target of RopB. The decrease in SpeB coincided with increased extracellular levels of numerous S. pyogenes secreted virulence proteins thus representing a novel form of “post-secretion” regulation. This work demonstrated the large impact that subtle changes on the bacterial genome can have on disease progression.

a. Beres SB, Carroll RK, Shea PR, Sitkiewicz I, Martinez-Gutierrez JC, Low DE, McGeer A, Willey BM, Green K, Tyrrell GJ, Goldman TD, Feldgarden M, Birren BW, Fofanov Y, Boos J, Wheaton WD, Honisch C, Musser JM. Molecular complexity of successive bacterial epidemics deconvoluted by comparative pathogenomics. Proc Natl Acad Sci U S A. 2010 Mar 2;107(9):4371-6. PubMed Central PMCID: PMC2840111.
b. Carroll RK, Shelburne SA 3rd, Olsen RJ, Suber B, Sahasrabhojane P, Kumaraswami M, Beres SB, Shea PR, Flores AR, Musser JM. Naturally occurring single amino acid replacements in a regulatory protein alter streptococcal gene expression and virulence in mice. J Clin Invest. 2011 May;121(5):1956-68. PubMed Central PMCID: PMC3083769.
c. Carroll RK, Musser JM. From transcription to activation: how group A streptococcus, the flesh-eating pathogen, regulates SpeB cysteine protease production. Mol Microbiol. 2011 Aug;81(3):588-601. PubMed PMID: 21707787.
d. Carroll RK, Beres SB, Sitkiewicz I, Peterson L, Matsunami RK, Engler DA, Flores AR, Sumby P, Musser JM. Evolution of diversity in epidemics revealed by analysis of the human bacterial pathogen group A Streptococcus. Epidemics. 2011 Sep;3(3-4):159-70. PubMed PMID: 22094339.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/ronan.carroll.1/bibliography/public/

Project Narrative

Objectives and Scope

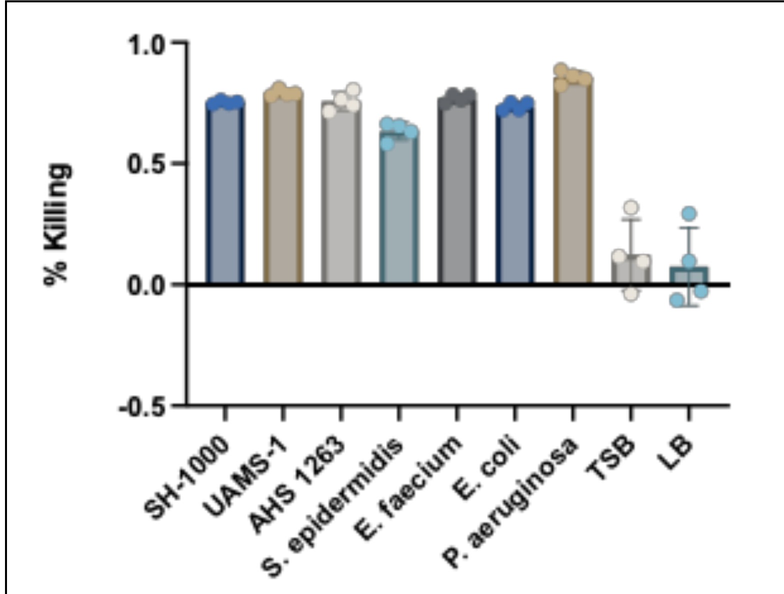

Fig. 1: Percent cell death associated with the addition of 20ng/ul RNA isolated from various pathogens, as well as sterile broth to control for elements of the RNA preparation procedure. The first three bars represent different *S. aureus* strains.

Staphylococcus aureus is a common cause of hospital-acquired bacterial infection, often with characteristic substantial tissue damage (1). Recent work in our laboratory has investigated an interesting phenomenon whereby pathogen-derived RNA causes human cell death (Fig. 1). Initial experiments, using different concentrations of *S. aureus* RNA, determined there is a dose-dependent effect. The majority of cultured THP-1 cells die following exposure to *S. aureus* RNA in the range of 20-50 ng/μl. Follow up experiments, using RNA isolated from a diverse range of bacterial pathogens and human cells revealed that RNA from all bacterial species tested induced comparable cytotoxicity (Fig. 1). RNA isolated from human cells was not cytotoxic suggesting cytotoxicity is not an endogenous property of RNA, rather it is unique to RNA from prokaryotes. The RNA used in these assays was collected from bacteria grown in optimal conditions, which are rarely found in the body. To elaborate on these results, the effect of stressful conditions during bacterial growth will be tested. We hypothesize that RNA from stressed bacterial cells will demonstrate increased cytotoxicity. This reflects the scenario found

in the infection environment whereby infecting bacteria, under physiological stress, modify their RNA so that once released into the environment (either by lysis of the cell, or via some export mechanism) this RNA can destroy immune cells. The modification could be increasing the abundance of specific transcripts that are cytolytic, or a biochemical modification of the RNA itself (this will be investigated in the future). While there are a variety of stresses encountered by *S. aureus* during infection, for the purposes of this proposal I will concentrate on two specific examples, pH and temperature.

To infect a host, bacteria must be able to survive at a range of pH conditions. The skin and endocytic vesicles are common locations of acidic conditions that bacteria must survive to effectively colonize a host (2). While basic conditions in the body are relatively rare, the ability to persist in fluctuating pH conditions is relevant to infections. Therefore, understanding how bacteria modulate their ability to induce cytotoxicity in response to conditions of varying pH is important. Another stress a pathogen will encounter during infection is excessive heat, or pyrexia. Pyrexia is a response of the body to infection because high heat is able to impair bacterial growth while usually doing minimal damage to the much larger host (3). Bacteria all have ideal temperature ranges. *S. aureus* prefers to grow at temperatures around 37 °C, and struggles to grow at temperatures around or above 42 °C (4). A fever of 40°C is considered high but medically significant, and thus this temperature will be used to heat shock a culture of *S. aureus* cells.

After the cells are shocked (with either acid or temperature), RNA will be isolated and THP-1 derived macrophages will be exposed to that RNA at the same dilutions as used previously (Fig. 1), to explore if cells experiencing stress produce differentially cytotoxic RNA.

Materials and Methods

RNA purification/RNeasy preps

Bacterial pellets will be resuspended in 600 μl RLT buffer (Qiagen). Samples will be transferred to 2 ml tubes containing glass beads and bacterial cells lysed by beadbeating. After a brief centrifugation step the lysates will be added to 900 μl ethanol and then passed through an RNeasy purification column. Samples will then be washed and eluted according to the manufacturer's protocol.

Cell Treatment

THP-1 cells will be grown for 2 weeks, to allow sufficient cell multiplication for experimentation. Half of existing media will be removed and replaced with fresh media every 72 h, to maintain the desired environment for cell growth. 2 days before the experiment, cells will be seeded into 24-well plates and phorbol 12-myristate-13-acetate (PMA) added to induce differentiation into macrophages and adherence to the bottom of the wells. Cells will then be exposed to RNA samples diluted in cell culture medium. An MTT assay will be performed to assess cell viability after 4 h exposure to RNA samples. Cells will be washed twice with PBS after RNA exposure. 300 μl PBS and 30 μl MTT reagent will be added to each well and placed back in the incubator for 4 h. After this step, 0.01 M HCl SDS solution will be used to lyse the cells, and they will be left in the incubator overnight and processed with a plate reader the following day.

Acid/Base Stress Assay

S. aureus cultures will be allowed to grow until mid-exponential phase, at which time they will be pelleted and washed with phosphate buffered saline (PBS). They will then be suspended in a

glycine solution adjusted to a pH of 2 or 12, for the acid and base shock respectively. After 3 h of exposure, the cells will be pelleted and washed again with PBS, and their RNA will be extracted and used in the procedure described above for exposing the cells to pathogen-derived RNA.

Heat Shock Assay

Similar to the above procedure, to induce heat shock *S. aureus* will be grown to mid-exponential phase and washed with PBS. In this experiment, however, the cells will be resuspended in a neutral glycine solution, then exposed to a temperature approximating pyrexia (40°C). The cells will be washed again and RNA will be collected. THP-1 derived macrophages will be exposed to this RNA using the established protocol.

Significance

A mounting problem with the treatment of *S. aureus* infections is that repeat infection is entirely possible and often likely. This stems from the fact that the human immune system has reduced capacity to remember *S. aureus* infection, unlike other pathogens (e.g. the SARS-CoV-2 virus). Through some unknown mechanism, *S. aureus* is interacting with the host immune system and interfering in immunological memory formation. Exploration of this pathway, which could involve the extracellular RNA included in this proposal, could allow for better treatments of repeat *S. aureus* infection.

Intellectual Property

Implication of extracellular RNA in disease severity and cell death would allow for potential new therapeutic treatments target at this RNA or the cellular response to it.

Bibliography

1. Handler, M. Z., & Schwartz, R. A. (2014). Staphylococcal scalded skin syndrome: diagnosis and management in children and adults. *Journal of the European Academy of Dermatology and Venereology*, *28*(11), 1418–1423. https://doi.org/10.1111/JDV.12541
2. Fang, F. C., Frawley, E. R., Tapscott, T., & Vázquez-Torres, A. (2016). Bacterial Stress Responses during Host Infection. In Cell Host and Microbe (Vol. 20, Issue 2, pp. 133–143). Cell Press. https://doi.org/10.1016/j.chom.2016.07.009
3. Niven, D. J., & Laupland, K. B. (2016). Pyrexia: Aetiology in the ICU. In Critical Care (Vol. 20, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s13054-016-1406-2
4. Missiakas, D. M., & Schneewind, O. (n.d.). *Growth and Laboratory Maintenance of Staphylococcus aureus*. https://doi.org/10.1002/9780471729259.mc09c01s28

Budget

Material	**Price**	**Supplier**	**Justification**
RPMI	185	Fisher	Media facilitating growth of THP-1 cells
RNeasy kit (50 preps)	384	Qiagen	To extract and purify RNA from bacteria
Fetal Bovine Serum	627	VWR	Required component of media for THP-1 cell growth
MTT cell viability assay	334	Fisher	To assay for cell survival after RNA exposure
Total	1530	***remaining $30 to be supplied by Carroll lab**	