BASIS Flagstaff

Senior Project Proposal
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I. Title of Project: "CHUG"-ing the methicillin-susceptible Staph (MSSA) Biofilms Away

II. Contact Information:

- BASIS Advisor's Name: Dr. Tom T
- Internship Location (Site Name & City/State/Country):

  Northern Arizona University/Flagstaff/Arizona/United States
- Off-Site Advisor's Name: Dr. Andy K
  - His/Her Title: Assistant Professor, Biochemistry, at NAU

III. Statement of Purpose:

Recently, more and more pathogens have been becoming antibiotic-resistant such as Candida albicans (C. albicans, Streptococcus mutans (S. mutans) species, and methicillin-resistant staph (MRSA)/methicillin-susceptible staph (MSSA/S. aureus). Thus, it is necessary to turn to alternative solutions to cure certain diseases or eliminate these harmful pathogens. One solution that my lab investigates is the usage of ionic liquids/deep eutectic solvents (IL/DES). Ionic liquids are molten salts that are known to treat specific antibiotic-resistant pathogens and my lab aims to use the IL that consists of choline, germinate, urea, and arginine (CHUG+Arg). The overall goal of our project is to develop IL formulations that are effective against microbial biofilms of oral pathogens and that can contribute to oral health overall. A component of this goal is to find which concentration of the ILs is most effective at eliminating the pathogen. Our strategy is to also incorporate different molar equivalents (proportions) of arginine (a molecule reported to affect positive oral health outcomes) into the IL/DES.

IV. Background:

I have always loved all aspects of biology. I love learning something new about it each day and how it surrounds us. When I was very young, I would go out to the garden and investigate the different organisms that I saw. I would look at the plants, the bugs, and even my sisters to answer the random but pressing questions that would keep me up at night.

This curiosity is what propels me to question everything to this day and try to find answers in the world of chemistry and biology. Through the science courses at school, I

was introduced to the microscopic world of tiny living organisms and the certain systems and structures that make up humans as well as the pathogens that can harm us. Even after taking AP Chemistry and AP Biology, a lot of my questions were left unanswered.

Fortunately, in sophomore year, this is when I met Dr. Koppisch from a STEM outreach program at NAU. I was placed in his biochemistry lab and immediately I could tell that I would be staying in his lab for a while. That year, I conducted research under Dr. Koppisch's supervision and later presented the research in the form of a scientific poster at a national scientific conference. I was immersed in the topic that he guided me through. It was about finding the minimum concentration needed of an ionic liquid to eradicate Candida albicans biofilms. The procedure was long and I would often spend over 40 hours a week during the summer in the lab, but I loved every second of it. Through this experience, I was able to learn how to operate all the lab equipment and handle dangerous materials. Along with the project, Dr. Koppisch had me complete a literature review which prepared me for the project and allowed me to do extensive investigation and reading into ionic liquids and the pathogens that we used.

The following year (junior year), I decided to stay in Dr. Koppisch's lab and conduct more research under his guidance. Unlike the previous year, we decided to utilize a different ionic liquid with a different pathogen called Streptococcus mutans. This project had a similar but lengthier procedure. It required more concentration and skill than the previous project, but I was able to build off my previous knowledge and complete the project swiftly.

V. Prior Research:

The antibiofilm effects of some ionic liquids have been described previously. One of the most studied IL/DES is choline geranate or CAGE. CAGE is known to have a strong antimicrobial activity against biofilms of many bacteria but is generally unharmful to human cells(12).

Many studies have shown that the metabolite arginine can reduce the virulence associated with some pathogenic microbes associated with dental health. Researchers Brinta Chakraborty and Robert A. Burne contributed to the knowledge about the effect of Arginine (ARG) on Streptococcus mutans (S. mutans) in their article "Effects of Arginine on Streptococcus mutans Growth, Virulence Gene Expression, and Stress Tolerance". The purpose of their study was to observe how exogenously provided l-arginine affects the growth, virulence properties, and tolerance of environmental stresses of S. mutans. The methods they used to conduct this project were applying 1.5% ARG on S. mutans in stressful environments, a lower pH of 5.5, compared to S. mutans that were not exposed. What they found was that the S. mutans cells in the presence of ARG showed slower growth until they reached the mid-exponential phase. Through previous studies cited throughout their article, it was demonstrated that S. mutans metabolized ARG through the arginine deiminase pathway (ADS) and released ammonia and alkali by-products which neutralize the acidic environment decreasing the virulence effects of S. mutans. They concluded that ARG affects the expression of virulence attributes and bacteriocin

production. These authors performed their work on non-biofilms of a single strain but stated that future steps would be to utilize multispecies biofilms, and eventually animal models, and human clinical studies to better assess the mechanisms of action of arginine-containing oral health care products(1).

It has been known that alkali production by oral bacteria in dental environments has been linked to protection against dental caries, and arginine metabolism is also known to be associated with microbial alkali production. A study conducted by X Huang, R. A. M. Exterkate, and J. M. Cate, etc aimed to examine the relationship between pH-rise phenomena and caries susceptibility from clinical observations, as well as to conduct studies on alkali-producing bacteria in vitro models in their article "Factors Associated with Alkali Production from Arginine in Dental Biofilms". The study assessed various parameters of ammonium that are known to be produced through the metabolism of ARG in dental biofilms(2). Biofilms are collections of microorganisms that adhere to biotic and abiotic surfaces. The methods of their study consisted of obtaining polymicrobial biofilms from saliva as the inoculum - the initial amount of a microbial culture that is presented into new media in order to propagate the culture (or in this case, propagate the biofilm) - for in vitro models, and they measured the pH and ammonium levels to quantitate the bacterial reactions. The results were that the presence of sucrose hampered the production of ARG. They also found that the rate of alkali production reached a maximum around a pH of 5.5 and biofilms produced ammonium from polypeptides and proteins in the medium as well(10).

MSSA is a common bacterial species that causes musculoskeletal infection (MSKI). The pathogen is continuously evolving as a result of gene transfer and dynamic mutations in the bacterial genome conferring virulence factors and antibiotic resistance(4). *MSSA* is responsible for around 1%–7% of meningitis (up to 19% in healthcare-associated meningitis). Patients who develop antibiotic resistance have worse clinical conditions and many lead to death(5). *MSSA* and MRSA remain significant pathogens in the NICU, particularly for extremely premature infants and term infants undergoing surgery(6). An *MSSA* infection in another area of the body is frequently the precursor to *MSSA* bacteremia. Many staph infections begin as skin infections. Some common symptoms of *MSSA* are cellulitis, impetigo, abscesses, folliculitis, and Staphylococcal scalded-skin syndrome. A bloodstream infection by *MSSA* results in staph bacteremia. Staph bacteria can transfer between individuals and can withstand extreme environments. *MSSA* can also be contrived from intravascular catheters, urinary catheters, feeding tubes, dialysis tubing, and breathing tubes(13).

VI. Significance:

Dental health impacts people regardless of age or ethnicity(3). It has been seen that common treatments such as fluoride can be toxic if consumed in high doses. One solution presented has been using ionic liquids. Ionic liquids (ILS) and Deep eutectic solvents (DES) are made up of organic molten salts. They show effectiveness in eradicating certain bacterial and fungal biofilm pathogens -such as *S. mutans* and *MSSA*- that initiate and worsen dental carries. They have also been found to be non-toxic to humans and the environment(10).

The race against pathogens becoming more and more resistant to antibiotic

treatment has been a pressing and urgent issue for communities across the world. This literature review will discuss the interaction and use of ILs and DES in treating the odontoid pathogenic bacterium *S. mutans* and *MSSA*(11).

From this project, I hope to support my lab's mission and contribute to the fight against antibiotic-resistant pathogens. I hope that the findings bring insight into the virality of the new CHUG+ARG IL and whether the use of this IL should be expanded to other similar pathogens. My professor may use the findings of this project to share to the IL/DES community.

VII. Description:

We will conduct laboratory research in a biochemistry wet lab located at NAU as well as library and laboratory research while completing this project. In the lab, we will perform trials of experiments to collect the data we need. The final product that I produce will consist of a PowerPoint presentation of the research process and findings.

VIII. Methodology:

My methodology consists of performing a standard Minimum Biofilm Eradication Concentration (MBEC) procedure followed by an enumeration process.

MBEC procedure:

- I will create an isolation streak by inoculating a strain of MSSA and incubating it in 5 mL of growth media overnight.
- I will then take 5µL of inoculated MSSA and place it in 5 mL of fresh media to be incubated for 2-5 hours.
- I will place 200µL of incubated media in a 96-well plate with a water ring and place a 96-peg lid on top. I will incubate it overnight.
- I will take the incubated peg lid and rinse it in a 96-well plate with fresh media to leave overnight in the incubator.
- I will create a serial dilution of ionic liquids created and dispensed into a 96-well plate with fresh media, a negative control (10%bleach), and a positive control (growth media).
- After rinsing the incubated peg lid, I will then place it in the serial diluted ionic liquid plate. Then, I will let that incubate for 24 hours.
- After rinsing the peg lid in fresh media, I will place it in a sonicator to use high-frequency waves to agitate the biofilms to drop into the fresh media.
- I will then replace the peg lid from the plate with a regular lid and incubate for 24 hours.
- Finally, I will place the plate in an automated plate reader and collect the raw data.

Enumeration Procedure:

- Immediately after the MBEC procedure, I will perform the enumeration procedure.
- 20µl of incubated biofilm media will be taken from three rows of sonicated plates and will be dispensed in the top row of the new plate with fresh media.
- One sample will be taken from each biological replicate plate at maximum, middle, and minimum IL concentrations.
- A sample of the wanted concentration will be added to a 96-well plate with fresh media.
- 10-fold dilution will be performed on each sample to achieve a minimum dilution of 10^6.
- Plated on BHIB agar plates in A,B,C formation, incubated, and parafilmed plates for 48 hours. Finally, I will count S. mutans colonies and record the data.

IX. Problems:

Many things can go wrong in procedures that require precision such as this project needs. Contamination is one of the biggest problems that I may encounter throughout the procedure and it is necessary to maintain a sterile environment within the lab. It is also important to be extra cautious when handling hazardous materials. I have gone through a lab safety class to ensure my safety and that I maintain a sterile environment in the lab and workspace. Another problem that we may encounter is not being able to collect all necessary data in time. These procedures take time and precision and may not be able to finish by the time of presentation.
An additional problem may be that the data does not back up our original hypothesis. It is completely possible that the ionic liquid that we are utilizing is not effective against MSSA and does not respond as it does with S. mutans.

X. Budget:

All expenses will be covered by a grant from the lab by NAU. Any extra expenses may include commute expenses to NAU that will be covered personally.

XI. Annotated Bibliography:

[1]Brinta Chakraborty, Robert A. Burne and Shuang-Jiang Liu. Effects of Arginine on Streptococcus mutans Growth, Virulence Gene Expression, and Stress Tolerance.July 17, 2017. https://journals.asm.org/doi/10.1128/AEM.00496-17

[2]Huang X, Exterkate RA, ten Cate JM. Factors associated with alkali production from arginine in dental biofilms. J Dent Res. 2012 Dec;91(12):1130-4. doi: 10.1177/0022034512461652.

Epub 2012 Sep 24. PMID: 23010718.https://pubmed.ncbi.nlm.nih.gov/23010718/

[3]Nascimento M. M. (2018). Potential Uses of Arginine in Dentistry. Advances in dental research, 29(1), 98–103. https://doi.org/10.1177/0022034517735294

[4]Moore-Lotridge, S.N., Bennett, M.R., Moran, C.P., Schoenecker, J.G., Thomsen, I.P. (2022). MRSA and Virulent MSSA Infections. In: Belthur, M.V., Ranade, A.S., Herman, M.J., Fernandes, J.A. (eds) Pediatric Musculoskeletal Infections. Springer, Cham. https://doi.org/10.1007/978-3-030-95794-0_6

This paper goes into depth about S. aureus' historical journey in acquiring antibiotic resistance and variation to virulence factors. Staphylococcus aureus accounted for ~65% of all culture-positive infections at the time of consultation by orthopedic providers across the United States.

[5]Antonello, Roberta Maria, Riccardi, Niccolò. How we deal with Staphylococcus aureus (MSSA, MRSA) central nervous system infections. Front. Biosci. (Schol Ed) 2022, 14(1), 1. https://doi.org/10.31083/j.fbs1401001

The purpose of this study was to compare MSSA and MRSA against fluoroquinolone group and vancomycin antibiotics. SA is responsible for around 1%-7% of meningitis. This study also goes into detail on practical suggestions for diagnosis, prevention, management, and treatment of *S. mutans* central nervous system (CNS) infections.

[6]Ratnaraja, N. V., & Hawkey, P. M. (2014). Current challenges in treating MRSA: What are the options? Expert Review of Anti-Infective Therapy, 6(5), 601–618. https://doi.org/10.1586/14787210.6.5.601

This review looks at the challenges facing the worldwide community with the increasing problem of methicillin resistance in *S. mutans.* It also delves into the epidemiology and natural history of community-associated methicillin-resistant Staphylococcus aureus and the challenge of control, options for treatment, and the number of new agents.

[7]Carey, A., Duchon, J., Della-Latta, P. et al. The epidemiology of methicillin-susceptible and methicillin-resistant Staphylococcus aureus in a neonatal intensive care unit, 2000–2007. J Perinatol 30, 135–139 (2010). https://doi.org/10.1038/jp.2009.119\

This study assesses the epidemiology of methicillin-susceptible Staphylococcus aureus (MSSA) and methicillin-resistant S. aureus (MRSA) infections in a neonatal intensive care unit (NICU). The overall rate of S. aureus infections was approximately 15–30 per 1000 patient-admissions MSSA and MRSA remain significant pathogens in the NICU, particularly for extremely premature infants and term infants undergoing surgery.

[8]Gould, I. (2006). Costs of hospital-acquired methicillin-resistant Staphylococcus aureus (MRSA)

and its control. International Journal of Antimicrobial Agents, 28(5), 379-384. https://doi.org/10.1016/j.ijantimicag.2006.09.001

Conventional control efforts can be successful, although they are often perceived as expensive MRSA is now a huge burden in addition to MSSA for most healthcare institutions around the world and is by far the most significant antibiotic-resistant hospital-acquired pathogen we have ever encountered. New strains have begun to emerge in the community that are not only more virulent than hospital-acquired (HA)-MRSA but are spreading widely, even into hospitals This study highlights the costs of MRSA and whether, even at this late stage, it is worth the high costs of widespread attempts at control.

[9]Enright, M. C. (2003). The evolution of a resistant pathogen – the case of MRSA. Current Opinion in Pharmacology, 3(5), 474-479.https://doi.org/10.1016/S1471-4892 (03)00109-7

This paper discusses the evolution and growing problems of *S. mutans* and *S. aureus*. Expansion to the hospital environment has been facilitated by horizontal transfer of the methicillin-resistance gene, resulting in the scourge of modern hospitals. Major *MRSA* clones have emerged in only five lineages, one of which has developed resistance to vancomycin, the antibiotic of last resort for treating *MRSA*. Most life-threatening cases of S. aureus disease are hospital-acquired and are associated, in many cases, with indwelling vascular devices or catheters. Two years after the introduction of penicillin in 1944, the first resistant isolate was recovered. Vancomycin is currently the antibiotic of choice (and frequently of last resort) in treating MRSA infections and, unsurprisingly, resistance has recently developed to this agent. In 2002, vancomycin-resistant Staphylococcus aureus (VRSA) isolates were discovered in the United and there is currently no antibiotic class that is uniformly effective against S. aureus.

[10]Greene, J. R., Merrett, K. L., Heyert, A. J., Simmons, L. F., Migliori, C. M., Vogt, K. C., Castro, R. S., Phillips, P. D., Baker, J. L., Lindberg, G. E., Fox, D. T., Del Sesto, R. E., & Koppisch, A. T. (2019). Scope and efficacy of the broad-spectrum topical antiseptic choline geranate. PloS one, 14(9), e0222211. https://doi.org/10.1371/journal.pone.0222211

This paper discusses the antibacterial properties of CAGE IL against a variety of pathogens. CAGE was observed to eradicate in vitro biofilms at concentrations as low as 3.56 mM (0.156% v:v) in as little as 2 hours, which represents both an improved potency and rate of biofilm eradication relative to that reported for most common standard-of-care topical antiseptics in current use. Insight into the mechanism of action of CAGE was provided with molecular modeling studies alongside in vitro antibiofilm assays.

[11]Centers for Disease Control and Prevention. (2002, September). Biofilms: Microbial life on surfaces - volume 8, number 9-September 2002 - emerging infectious diseases journal - CDC. Centers for Disease Control and Prevention. https://wwwnc.cdc.gov/eid/article/8/9/02-0063_article https://doi.org/10.3201/eid0809.020063

[12]McCormack MG, Smith AJ, Akram AN, Jackson M, Robertson D, Edwards G. Staphylococcus aureus and the oral cavity: an overlooked source of carriage and infection? Am J Infect Control. 2015 Jan;43(1):35-7. doi: 10.1016/j.ajic.2014.09.015. PMID: 25564121.

[13]WebMD Editorial. (n.d.). MSSA bacteremia: What is it, what causes it, and how is it treated?. WebMD. https://www.webmd.com/skin-problems-and-treatments/what-is-mssa-bacteremia

This website describes the symptoms, process, development, and treatment of MSSA bacterioma.