

## Research Background

Each year, over seven million men and women in the United States acquire a urinary tract infection. This number is expected to increase in response to growing numbers of antibiotic resistant microbes in patients seen with UTIs (Tortera et al, 2004). UTIs are defined as bacterial infections in the bladder, kidneys, or prostate and they often present themselves as nosocomial (hospital transmitted) or as sexually transmitted diseases. The majority of UTIs are found in young females. A large number (20%) of women will have experienced an infection before the age of thirty while 50% will contract a UTI sometime in their lifespan (Engleberg et al, 2007). The higher frequency of UTIs in the female population is attributed to the urethra's location in proximity to the normal flora of the vagina as well as the size of the female urethra, which is significantly shorter than the male urethra (Hooton, 1996). This allows the bacteria to gain easier access into the urethra thus causing UTIs in higher proportions. Factors such as age, diabetes, and patients with catheters enhance the susceptibility to UTIs. The most common type of pathogens seen in urinary tract infections include the Gram negative bacteria *Escherichia coli* or *E. coli*, *Proteus mirabilis*, and *Klebsiella*, and the Gram positive bacterium *Staphylococcus saprophyticus* (Guay, 2008). This project will focus on the bacterium *S. saprophyticus*, which is also the least understood of the bacteria seen in UTIs.

The presence of bacteria in urinary tract infections has been associated with an enzyme called urease which is thought to make the environment of the urine more inhabitable. The catalytic reaction of the enzyme urease involves hydrolysis of urea into ammonium and carbonate (Amtul et al, 2002). The ammonium raises the pH and leads to a build-up of struvites, which are minerals made of ammonium, phosphate and magnesium. Ultimately, this can cause stone development in the kidneys and bladder (Gatermann et al, 1988). The goal of this research is to inhibit the enzyme urease through various chemical inhibitors. Once inhibition of the enzyme has been established, the chemicals will be tested to see if bacterial growth in urine is also inhibited.

## **Program Description**

*Staphylococcus saprophyticus* has been a recent subject of interest due to a high occurrence of antibiotic resistance to compounds such as penicillin and antibacterial sulfonamides (Mobley et al, 1995). Student researchers in the laboratory of Dr. Charles Deutch are working to develop pharmaceutical compounds that will inhibit the enzyme urease. This protein is a known virulence factor for *S. saprophyticus* and inhibition of the urease may stop bacterial growth, thus reducing the chance for developing a UTI. The foundation for the inhibition experiments come from studies on compounds introduced in previous microbiological research on the urease from the bacterium *Helicobacter pylori* (Nagata et al, 1993). This organism has been found to survive in the highly acidic environment of the stomach because of its urease activity, which makes the environment more basic. Studies of chemical inhibitors in previous student projects have used a complex enriched medium. In this project, *S. saprophyticus* will be grown in an environment of urine and so the effectiveness of the compounds can be determined under more “natural” conditions.

## **Program Objective and Methodologies**

The objective of the project will be satisfied by a multi-step process involving: 1) growth of *S. saprophyticus* in an artificial urine medium; 2) measuring the amount of the enzyme urease in these bacteria by means of a colorimetric assay; 3) studying the inhibition of urease by different classes of inhibition; and 4) analyzing the effect of these inhibitors on the growth of *S. saprophyticus* in artificial urine medium. These experiments will indicate whether the compounds used inhibit survival of *S. saprophyticus*.

To grow *S. saprophyticus* in an environment similar to real urine, several types of artificial urine medium will be created and three strains of *S. saprophyticus* will be grown in the media. The strains that will be examined are ATCC 15305, ATCC 35552, and ATCC 49907: ATCC 15305 is a strain with a known genome while ATCC 49907 and ATCC 35552 are used as controls in clinical testing systems. To determine the amount of bacterial growth, turbidity, or cloudiness of the urine media will be measured in

a spectrophotometer. Once significant growth has been established, the specific activity of the enzyme urease will be measured by using a standard curve for ammonium, the byproduct formed by the hydrolysis of urea (Mobley et al, 1995). This is a well developed protocol for measuring enzyme activity. Different chemicals will be tested to determine if they are inhibitors of the urease enzyme. The compounds of interest include hydroxamates, phosphodiamidates, and glycosides. These are compounds that were used in past studies with an enriched peptone base or P medium as opposed to artificial urine medium. The last step after determining the inhibition of the urease enzyme is to see if the compounds inhibit growth of *S. saprophyticus*. If inhibition of the bacteria is effective, then the compound involved will be studied in depth for future experiments related to antibiotic development. This may also allow us to determine if or when urease activity is essential for growth of *S. saprophyticus*.

### **Professional Benefits**

As I progress towards a future career goal in pharmacy, the research I conduct during my undergraduate campaign will teach me the proper laboratory techniques which can be applied to multiple concentrations in medical and pharmaceutical research. During this opportunity, I will benefit from working alongside professionals in the field where I can participate in thought-provoking conversations about the research that has been completed. Along with the professional benefits, I will be able to contribute to the study of a medical condition that can, in time, be prevented. A urinary tract infection is known to be a recurring condition and can lead to life threatening illnesses such as kidney failure and sepsis (blood disorder). The goal of this project is to reduce the amount of urinary tract infections so the condition does not reach a level that is beyond repair. Lastly, I will be given an opportunity to utilize the concepts I have learned in Biology and Chemistry and apply it to research that requires critical thinking and in depth practices.

## **Feasibility**

As a major in the life sciences, the core classes I have taken are essential in the understanding of this NCUIRE research opportunity. To date, I have taken and excelled in classes such as Biology I and II, Chemistry I and II, Organic Chemistry I, and Cell Biology which is a good foundation that will be carried over to the research involved in this project. During the year in which research is conducted I will also be committed to Biochemistry and Microbiology courses which will further enhance my understanding of the chemical reactions involved as well as the characteristics of *S. saprophyticus*. In addition to the work completed in these classes, I have past volunteer experience in medical research at the University of Arizona's Biomedical Collaborative in downtown Phoenix. Lab techniques that were utilized will be carried over to this project and used effectively. Lastly, I will be learning the methodologies employed for this project by completing volunteer research hours during the summer with Dr. Deutch.

## **Anticipated Outcomes**

As the project goes further into completion, it is expected that the results found in the inhibition of the urease enzyme will be presented at regional scholars meetings as well as meetings on the ASU campuses. The goal is to present our findings at that point and explain our research to other scholars. It is also expected that we are closer to finding a compound that can be used to prevent bacterial growth. If, at that time, an effective compound is discovered, the next stages will be involved with the development of an antibiotic which may require a group effort with researchers outside of the lab to do clinical testing in which we would use a lab animal model to see if bacterial inhibition has occurred with the urease enzyme inhibitor.

## **Project Timeline**

The projected timeline of this project will be completed over a period of two semesters in which each stage is conducted sequentially. In the Fall semester of 2011 it is expected that research will begin with the preparation and optimization of growth of the *S. saprophyticus* in artificial urine medium. This stage will continue until a suitable environment for the *S. saprophyticus* has been established. Once

optimal growth is determined, a whole cell colorimetric assay will be conducted to determine the presence of chemicals within the urine medium. This requires the construction of a standard curve which should take a few days to create. Once these stages are complete, the project will be into the Spring semester and it is expected that the next step is the inhibition of urease activity by an array of chemicals. At the end of the Spring semester, once inhibition of urease activity has been determined, the next step will be to inhibit growth of *S. saprophyticus* in artificial urine medium by various chemicals. Each of the four phases will take about 6 to 8 weeks to complete with a semester lasting 12 to 16 weeks.

### **Project Budget**

In order to effectively complete this project, various supplies will be utilized and the approximated costs of these supplies are as follows:

- Artificial urine medium ... \$100
- Sterilized filtration units ... \$100
- Chemicals for the urease assay and inhibitors... \$150
- 13 x 100 glass tubes for assays... \$75
- Cuvettes for the spectrophotometer ... \$75

The total project budget is approximately \$500 which covers the research supplies and included in the project is a \$2,500 stipend for student compensation.

## References

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