StaphiScope

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Abstract

Superbugs, or antibiotic resistant microorganisms, are microbes that have become resistant to traditional treatments. These types of infections are difficult to diagnose, treat, and eradicate, making the healing process time consuming and resource intensive. The native quorum-sensing unit from *S. aureus* (the Agr system), will be introduced into a non-pathogenic strain of *E. coli*. The *E. coli* will then effectively have the ability to eavesdrop on the activity of the pathogenic organism and emit an indication of the magnitude of the infection in the form of RFP. Using sensitivity tuners the system can be designed such that the response will occur at an exact level, when the size of the population poses a threat to the host. Upon a positive result from a diagnosis, further tests could be done to specify whether MRSA (methicillin-resistant *S. aureus*) or MSSA (Methicillin-sensitive *S. aureus*) are present.

Purpose

Curent Methods

• **Plating:** consecutive plating on Mannitol Salt Agar, Blood Agar, and antibiotic plates.

• **RT-PCR:** the use of primers, which will recognize a sequence particular for MRSA and amplify it, producing a visible fluorescence-detectable result during the amplification stage

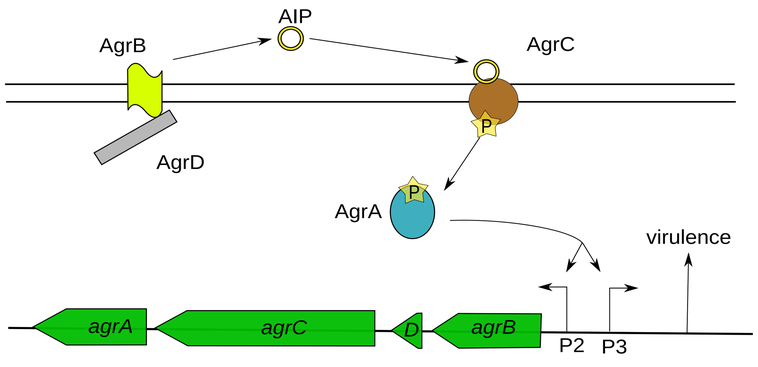
StaphiScope

• Functional construct will supplement pre-existing methods by cutting down on time and resources required for the identification of S. aureus specificity.

• MRSA specificity will be tested using either plating or PCR.

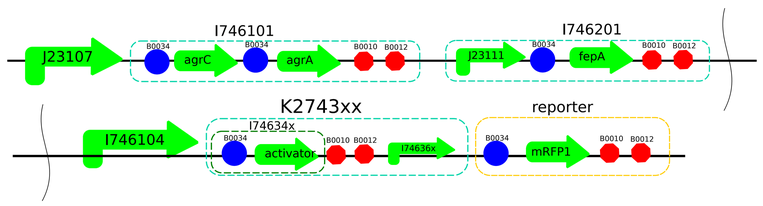
Agr system

The AIP signaling molecule (from same cell or from another cell producing the peptide) binds AgrC (a histidine kinase). This, in turn, allows for phosphotransfer to AgrA and subsequently, through activation of transcription, more AIP is produced, amplifying the signal. This feedback loop allows the bacteria to be aware of the concentration of its own strain in the infected area, subsequently leading to a “collective decision” such as expressing virulence.



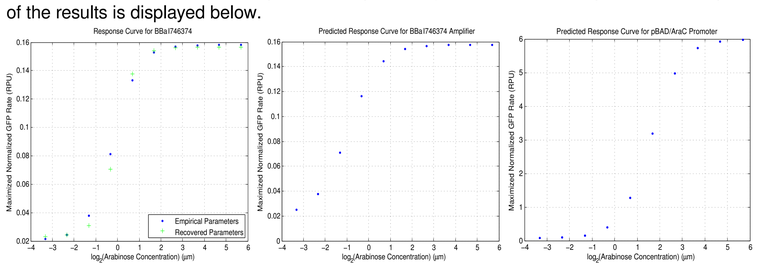
Construction

The primary design of the construct consisted of AIP sensor infrastructure BBa_I746101 + BBa_I746104, permeability device BBa_I746201, amplifier, and reporter. Amplifier would have been selected from BBa_K2743xx series based on the investigations by the modeling team. Red fluorescent protein gene BBa_E1010 from BioBrick I13507 was be used as reporter.
An additional design involved the addition of a generator part. This allowed *E. coli* to produce its own AIP, as a means for testing the response without worrying about permeability into the periplasm. This design was completed but could not be tested due to time constraints. As well, no amplifier was added in the final construct.



Modeling

StaphiScope will make use of one of twelve different "amplifier" subsystems of Cambridge's sensitivity tuners. The choice of amplifier used in Staphiscope will determine its sensitivity. To ensure our system has the optimal sensitivity, numerical characterization of each amplifier is sought independent of promoter.
The response curve of each amplifier can be modeled by a Hill function, requiring four parameters for full characterization. A Hill function is also suitable to model the response of the pBAD/AraC promoter as a function of arabinose concentration. A third Hill function is used to model the full system of promoter + amplifier.
An attempt was recently made to numerically extract the amplifier parameters from empirical data of Cambridge's sensitivity tuners. The idea exploits the fact that each of the twelve sensitivity tuners characterized by Cambridge uses the same promoter, which is assumed to behave identically (have the same Hill parameters) independent of which amplifier is attached. We also make the simplifying assumption that the parameter n = 2 (a typical value) for each amplifier. The number of unknown parameters is then 4 + 12*3 = 40, while the number of known parameters is 12*4 = 48.
To extract parameters based on this extra information, MATLAB’s nonlinear regression and optimization toolboxes were used. Specifically, a search was done over the parameter space of the pBAD/AraC promoter. For each set of promoter parameters chosen, nonlinear regression was performed on each amplifier to match the joint system of nested Hill functions (promoter + amplifier) to the empirical curve found by Cambridge. To minimize the total error, optimization over the promoter parameters was then performed. The result of this effort is a numerical characterization of the pBAD/AraC promoter, as well as each amplifier. An example of the results is displayed below.



Outreach

ESQ Partnership

ESQ (Engineering Science Quest) is a day camp hosted at the University of Waterloo that brings hundreds of curious young minds to Waterloo each year to learn more about science and engineering. We held two different weekly activities for campers of ages 8-9 and 12-14. The activity for children of 8 to 9 years of age involved extracting their own DNA from their cheek cells. The second activity, which involved children 12 to 14 years of age was named “Do We Really Need to Wash Our Hands.” In this activity, the children were asked to swab their own hands (using a sterilized cotton swab) and plate the resulting swab on solid media. They would then wash their hands/use hand sanitizer and swab and plate again. The plates would be left to incubate at 37°C overnight, and the next day, the resulting growth would be presented to the children.

Ontario iGEM Meeting

The second annual OGEM (iGEM Teams of Ontario) Meeting was held at McMaster University in Hamilton on June 15th, 2010. Members of iGEM teams from University of Waterloo, University of Western Ontario and University of Toronto gathered to discuss the future of a regional synthetic biology community, as well as a regional conference. The day turned over to discussions which centered around creating more communication and support between teams. In addition, the gathering was also an opportunity for teams to get to know one another before heading down to MIT. The meeting was a great success and the second of more regional gatherings to come.
As the meeting was held during the annual CSM (Canadian Society for Microbiologists) conference, members were able to attend a series of lectures given by valuable members of the field. As well, iGEM Ontario members were given the opportunity to speak about their projects and promote the idea of iGEM during a poster presentation held along with researchers in the field of microbiology.
In the future we hope to see this organization (independent of an individual iGEM team) become an important resource to Ontario iGEM teams and for educating the general public about synthetic biology. We are currently working on an iGEM Ontario website.

Public Lecture: Building Life

On June 23, Waterloo iGEM adviser, Dr. Trevor Charles, held an open public lecture aiming at discussing synthetic biology, its means and aims. During the lecture, the purpose of synthetic biology, its ethical and safety implications well as many other current topics were discussed. At the end, a facilitated panel discussion featuring Andre Masella (Waterloo iGEM team), Dr. Kathryn Plaisance (bio-ethicist), as well as Dr. Maria Trainer (Council of Canadian Academics) answered some of the public’s pending questions regarding synthetic biology. The lecture was a great success, with a large number of attendees of various scientific background. It was part of a series of lectures organized by the University of Waterloo's Department of Biology, in attempt to increase public awareness of current scientific issues.

Human Practices

As a group we are most interested in the course of development of synthetic biology in industry; the goal of our project is to try and decipher the path that synthetic biology will forge as it expands in the business world.
Our attempt to answer this question has begun with a comprehensive inquiry into important factors affecting the diffusion of synthetic biology. This inquiry is intended for use by multiple audiences; particularly scientists and members of business in relevant industries.
This analysis looks at both extrinsic and intrinsic factors relating to the development of synthetic biology as a whole. In particular intrinsic factors such as the development of synthetic biology in specific industries (biofuels, pharmaceuticals, and bioremediation) is examined in depth. Important extrinsic factors such as the impact of patenting and open source are also analyzed.

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