Learning to Ker: Safety and Ethics in High School Synthetic Biology: Breaking Down Keratin and "Ker"ing

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The Our Lady of the Snows Catholic Academy iGEM team is a dynamic synthetic biology group made of high school students from grades 10 through 12. On the team there are brains, athletes, princesses, and basket cases all working towards one goal: expressing Keratinases in *Escherichia coli* JM109 and DH5α. Keratinases have the ability to reduce the 8.5 billion tons of keratin waste produced by the poultry industry every year, along with the keratin waste in wastewater treatment industries in the form of hair. The hair and feathers degraded by the keratinases could even be recycled into a high-quality protein product that can be used to make fertilizer and animal feed. While one focus is to complete this project, the true goal is to provide high school students with a well-rounded education beyond the classroom. This diverse scientific education is not limited to working inside the lab or on the technical aspects of the project. It extends to all areas of science, including safety and ethical considerations, as well as the acknowledgement of different religious and economic perspectives in scientific endeavours. Over the past three years, the Our Lady of the Snows iGEM and synthetic biology team has been learning how to build a lab that is economically feasible, through Do-It-Yourself projects and secondhand equipment. The team has tackled safety issues related to sharing lab space with many students in a high school. Students have also considered the project from a faith-based perspective and addressed other ethical concerns related to young student scientists and genetic engineering.

Keywords: Synthetic biology, safety, education, iGEM, keratinase.

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Watch a video introduction by the authors at
https://youtu.be/D0by8-uiEAo

In 2003, the "International Genetically Engineered Machines Competition", or as it is more commonly known, iGEM, was founded with only five university teams in participation (iGEM, d.t). As we approach the 2016 competition, it has now expanded to over 300 teams, having grown to be the largest synthetic biology competition in the world. It was created in order to inspire students to use synthetic biology to modify bacteria into living "machines" that would solve real world problems, allowing for thousands of students, teachers, and professionals to make astonishing developments and share their discoveries on an internationally recognized platform.

When iGEM was founded, the competition only allowed for undergraduate students to participate. However, in 2011, the competition gained international attention at the collegiate level, allowing for high school students to participate. In late 2013, Our Lady of the Snows Catholic Academy from Canmore, Alberta, Canada started an iGEM team with no previous experience and a regular high school lab. Today, three years later, we are participating in our third competition season. In our short time as an iGEM team, we have seen a lot of development, gained an abundance of knowledge, and have tackled some of the most important iGEM-related issues. These issues concern safety, ethics, lab development, and our largest goal as a Catholic high school, which is finding a balance between synthetic biology and faith. Studying synthetic biology in high school has allowed us to take part in a different kind of learning; a very hands-on approach, which is vastly different from the memorization-based learning that students are typically subject to in North America. iGEM has given us the opportunity to take our education into our own hands, and as a result, we have gained knowledge and experience that goes far beyond anything we could have learned in a classroom.

Our Project

Over the past two seasons, the goal of our project has been to produce two kinds of keratinase enzymes, Keratinase A (KerA) and Keratinase US (KerUS). The genes for both proteins are found naturally in the Bacillus genera and produce enzymes that break down keratin, one of the strongest naturally existing proteins. Keratinase is one of the few enzymes capable of breaking these strong bonds that hold keratin together.

The degradation of keratin protein is important, as it is generally found in both hair and feathers, which contribute to a huge waste problem. Feathers alone yield approximately 8.5 billion tons of waste each year ("New Solution Found to Disposal of Feathers" 2009). Hair waste mainly affects wastewater treatment facilities. This is detrimental because hair clogs the pipes and inhibits pump functions, causing water treatment to slow down or halt completely. Feathers are a main contributor to the waste problem within the poultry industry, as feather waste builds up due to the lack of ethical disposal methods. Currently, there are four main solutions addressing the keratin problem, including incineration, burial, and transporting elsewhere. As well, another option is to mechanically break down the waste, which produces a low-quality keratin product, often used for fertilizers and animal feed (Satyanarayana 2014). Our project aims to break down the keratin protein using a safer, more viable method that creates a more useful protein product (Arvisais et al. 2016). By breaking the keratin down into its amino acids, it can be used in higher quality protein products.

As we've made progress on our project, iGEM has allowed us to do so much more than just learn about synthetic biology and receive valuable lab experience. We have become knowledgeable about safety measures and how to maintain an ethically responsible project, because we "KER" (care).

There are many concerns that arise when people hear that high school students are participating in synthetic biology. Most concerns that people have specifically relate to ethics and safety; these can be addressed by educating people about the field of synthetic biology and reiterating that we, high school students, are doing synthetic biology to pursue knowledge. A concern that people often have is that we cannot pursue this field of research safely in a high school setting. We take into consideration the concerns that people have about our participation in synthetic biology as we acquire opportunities in numerous fields of science and further our education as a whole.

Safety

Being a team of high school students, lab safety measures are of great importance. We not only need to take our personal safety into account, but also the safety of those around us. We need to be especially cautious, as our lab is situated in the prep room of our school's science classroom. This means that we share the space with students, teachers, and staff on a daily basis. Our school neighbours another school that shares our lab space, thus leaving us with the responsibility of maintaining safety and cleanliness for both schools. In order to ensure the safety of members of both school communities and iGEM team, we use sterile techniques, plan safe project implementation, and use our Do-It-Yourself (DIY) equipment safely.

We practice aseptic techniques in the lab by keeping all of our equipment sterilized using an autoclave, and during protocols we sterilize with bleach and/or flame, both before and after each use. The practice of these steps prevents cross contamination of bacteria either from previous work or storage. Before, during, and after an experiment, we use aseptic technique to prevent growth of unwanted bacteria that could lead to inconclusive results as well as guarantee the safety of team members and students. Working in the lab requires us to wear personal protective equipment, such as lab coats, goggles, and appropriate footwear. We also have additional safety protocols that we practice because of the limited waste disposal methods in our lab. By practicing sterile techniques and wearing personal protective equipment, we keep our team, our school community, as well as our experiment, as safe as possible.

Due to the nature of iGEM and our project, real-world safety implementation is of huge importance. In a few years time, our team plans to reach the end goal of introducing our project into industrial and commercial sectors to have positive effects outside of the lab, without the potential for harm. When we think about the application of our project, there are many variables that we must consider. Addressing all of these variables will require an extensive amount of planning, research, and years of development.

At the end of our last iGEM season, we began the basic planning for the industrial implementation of our project. The first thing that we took into account when designing the biological systems was to take precautions to ensure that the organism would not grow uncontrollably. We used a lab strain of *Escherichia coli* K12, a non-pathogenic form of the bacteria used in many labs with a modified genome (Chart et al. 2000). Though it grows successfully in the lab, the bacteria has difficulty surviving outside of laboratory conditions. We designed our construct with an isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible promoter to activate the expression for the keratinase enzyme. With the use of this molecular biology reagent, IPTG, it allows us to control the expression of the protein in low quantities so that we can confirm that the intended protein is expressed. As a safeguard, we can chemically denature the enzyme or terminate the cells if we so desire. Our team plans to add a "kill switch" to our final construct before we introduce it into industry. This is a safety mechanism, usually triggered by a chemical stimulus, that would prevent the bacteria from growing out of control. They work by degrading the bacteria's DNA so that it is unable to continue to produce the keratinase enzyme as well as break down the cell completely to kill the microorganism. It could also be used to control the expression of our keratinase enzyme. This would be a final measure to ensure that our bacteria would not be able to survive if it were to escape our designed containment system.

Aside from the design of our biological construct, we are also looking into engineering physical containment systems and controls. We have researched systems that are currently in use industrially. Our team is focusing on the poultry industry and the municipal wastewater treatment facilities. One system that we became knowledgeable about that can help with the easy and safe implementation of our construct is the bioreactor. A bioreactor is an apparatus in which a biological reaction or process is carried out, especially on an industrial scale. They are used in many wastewater treatment facilities. Utilizing a bioreactor would allow for safe and easy integration of our system into an already existing system. Bioreactors are made for different purposes; not every bioreactor is alike, and our design is no exception.

Our intent is to use one of these systems as they are commonly used, allowing for easy integration into industrial sectors, and our bacteria would thrive as a result of maintained optimal conditions.This system would allow the bacteria to grow efficiently and degrade the hair or feathers in a controlled fashion. This stable environment will prevent uncontrollable rates of growth and mortality of the bacteria, something that poses potential hazards in the industrial application of our construct. Since a bioreactor is a semi-sealed containment system, it will ensure that the protein byproduct of keratin degradation will not leak into the environment, resulting in negative effects, as well as prevent the enzyme from causing harm downstream. Finally, certain kinds of bioreactors could completely remove the bacteria from the water before reintroducing into the environment and, eventually, the public.

Working in a high school lab means that we have had to transform a simple lab prep room into a fully functional microbiology lab, which presented numerous safety concerns. With a considerably low budget, the majority of our equipment is either second hand or DIY. In previous years, we had developed a functional synthetic biology lab with a feasible budget for high school students. Creating this budget included plenty of hands-on experimentation to enable us to build our own DIY equipment. We learned the mechanics behind our equipment and how to customize them so that the appliances functioned the way that we desired, while simultaneously meeting safety requirements in our lab.

In order to upgrade the lab, we created equipment through DIY projects. Our DIY projects included an incubator shaker table and a centrifuge. The incubator shaker table was assembled using a rubbermaid container lined with tin foil, with a heating lamp and a baking pan attached to a record player. We tested our shaker table's security by using non-flammable materials and testing that it is not flammable as a result of a rapid increase in temperature. Our centrifuge was manufactured from a paint can, a dremel tool, and a 3D printed drill bit used to hold the centrifuge tubes. We have made certain that this is safe by ensuring that it is only operated while team members, as well as our faculty advisors, are present. This is monitored by placing the paint can lid on the centrifuge while it is on and operating it in the fume hood with the glass down.

Ethics

Questions regarding the ethics of synthetic biology are issues that have been present since synthetic biology was an emerging field. These questions and concerns have stemmed from various aspects such as safety, misuse, religious compatibility, genetically modified organisms (GMOs) and the potential of bioterrorism. Even at a high school level, these are the kinds of things we need to contemplate when carrying out a project at an international level. Some people fear a "hacker subculture." It is the attitude of "showing off" and hurting others that encourages the misuse of synthetic biology. If standardized parts are made easily accessible to high school students, the concern is how people with this "hacker" mentality would be prevented from creating their own lab and engineering dangerous viruses or biological weapons to be used against humanity.

One of the most prominent criticisms of synthetic biology is the use of our chassis, *E. coli*. *E. coli* is a bacteria found in the environment, in food, and in the intestines of people and animals. Most strains are harmless, particularly the strain K 12, which we use in our project. However, some strains can promote illness, such as pneumonia. As a result, using this bacteria may strike fear in individuals who are not aware that the strain we are utilizing in our project is lab strain. Some individuals are also wary about high school students pursuing synthetic biology, especially in a low-budget high school science lab. As previously stated, we as a high school team prioritize safety and practice protocols to ensure success and safety. By allowing high school students to be introduced to this field of science, many opportunities are presented that would not be available through the typical science curriculum. The majority of our team is taking a synthetic biology course for credit that has been approved by the Alberta Board of Education. This serves as background for the students who wish to pursue synthetic biology or any other field of science in post-secondary, and allows them to get credits for their scientific endeavours.

Through iGEM, we have addressed questions regarding how ethically safe our construct is, how we will implement our construct in an ethical manner, whether it is safe to work in a lab as high school students, and whether our scientific development contradicts our faith. By addressing the questions presented to us, we have gained knowledge about ethics that we would have not even considered through typical high school experience alone. Perhaps the most significant concern that we address is the faith aspect of what we do as an iGEM team.

Faith

Albert Einstein once said that "Science without religion is lame; religion without science is blind." Science and religion compliment each other and prove to have a symbiotic relationship. Religion explains values, what matters, and the unknown; but science can help explain what is known and what things are. Religion can provide a foundation of morals for science (Catechism of the Catholic Church 1994). As a Catholic school, faith is a chief matter that we must consider while constructing and pursuing our project. There is one inquiry that we must constantly ask ourselves when experimenting with our project, "are we playing God?" and "are we going too far in manipulating living beings for our own purposes?" The Catholic church has expressed their perspective on genetic engineering, stating that the engineering of bacteria must not be sinful, but ethical. Most importantly, scientists must not be creating new life or destroying life unnecessarily—in other words, playing the "role of God" (Heavey 2013). Many seem to believe that the Catholic church vehemently opposes scientific research, especially in a field such as synthetic biology. However, this is a common misconception that stems from the beliefs of other, more fundamentalist sects of Christianity. The Catholic church recognizes that science and religion are two different things and aims to answer two different questions. But as stated previously, religion and science often work together to answer coinciding questions. One recent example of effective cooperation between the church and the scientific community is Pope Francis' stance on climate change. The Pope believes that it is a moral obligation for us to take care of our planet and we will have to adhere the warnings of climate scientists in order to do this. Religion continues to be a constant challenge for our iGEM team. Overall, we feel that what we do as an iGEM team meets the conditions of Catholicism, and we will continue to keep these aspects in mind as we progress further with our project.

Education

Our project and experiences that we have had are mostly centred around the theme of pursuing education. As stated earlier, many members of our team are doing synthetic biology as a course for high school credit. Through our pursuit of those credits, as well as participating in the iGEM competition, we have learned so much. All of the learning that we have done and knowledge we have obtained is primarily through experience. In a typical classroom setting, there is a teacher who teaches the students lessons. They then evaluate the students on their knowledge of the lessons that have been taught. This is not the case with iGEM. Alternatively, our learning is all hands on and our teachers are learning with us, on an equal platform. Due to the lack of knowledge we had as we introduced iGEM in our high school, we have reached out to many professionals within the iGEM community. As a result, we have had the privilege of working with various mentors and professionals in the field of synthetic biology in both our own lab and university labs. Unlike traditional learning, we are not only evaluated on our ability to summarize and regurgitate information, we are evaluated on our ability to explain with precision and deep understanding, displayed through presentations of our project, poster sessions, and even through the construction of our website. We have attended jamborees that required an oral and visual presentation as well as a poster presentation. Judges circulate and inquire about the project. In situations such as these, students must be able to explain their project, information spanning from the science to the ethics. Students become able to explain their endeavors to people who do not know any aspect of the project and have to apply their knowledge when they are asked a question by a judge, or even another participant. Because the competition is filled with professionals in the field, students have to understand their project, as everyone at iGEM understands the principles of synthetic biology. This encourages team members to really comprehend and think critically about their project, all while applying the knowledge that they have gained.

Participating in synthetic biology as a high school team is a very multi-faceted research experience. We as students have pursued knowledge in many different ways such as reading other people's research to use as a basis for our own projects, writing out our own lab and research results to display to the public, creating our own lab protocols, knowing how to work efficiently in our lab, and collaborating with other teams. Effective researching is also a skill that we are expected to acquire because troubleshooting our failures and modifying our learning methods is incredibly important. Knowing how to read research papers is an ability that is of great significance, as we have to know how to publish our own results and design our own protocols based on previous work. Writing out our research is a vital skill, especially in the iGEM competition, because we are required to incorporate our work into a wiki page and a poster. We also have to be knowledgeable in presenting our information in a potent way, through infographics, presentations, and general interactions with people at jamborees. We must take into account the many different perspectives that people have of synthetic biology and the bioethics surrounding this field of science.

One of the major aspects of the iGEM competition is collaboration between teams. This is an important aspect of iGEM, as it helps to build the international community of iGEM members working together to create a collaborative environment that is about scientific development rather than competition. This collaboration component allows for students to get help from other teams, learn from other teams, and focus on scientific development. So far we have made three major collaborations concerning our project. The first two connections we made were with two undergraduate iGEM teams who had previously tried to tackle this project but later moved to pursue different projects. The first undergraduate team that we collaborated with was the University of Chicago iGEM team, who worked on the development of a project aimed at breaking down feathers in 2013. (University of Chicago iGEM team wiki, 2013). The second undergraduate team we collaborated with was the University of Sheffield 2014 iGEM team, who worked on a construct that was intended to break down hair as a side project to their main grease degradation project. (University of Sheffield iGEM wiki, 2014). We asked these teams a variety of questions regarding why their attempts to express the keratinases in *E. coli* failed, and they gave us troubleshooting ideas and general iGEM advice. Our third collaboration was made with another local high school team from Consort, Alberta. We collaborated by sending each other manuscripts outlining our project and some of our future planning. We gave each other feedback and questions to address. It is very valuable to have other people's input on a project like this as a way to incorporate different perspectives that people may have, opinions they hold of the project, the field of research, or the way we present our findings. We have had to acknowledge these stances and beliefs, and by doing so we have gained perspective from many different viewpoints. Through the collaboration aspect of iGEM, we have come to know and learn from others, developing an international community focused on the development of science. All of these experiences have contributed to the continuation of synthetic biology being a well rounded field of study.

Conclusion

When looking back on the growth of our iGEM program since its beginnings within our school, we have taken notice of the experiences and knowledge we have gained, which is far beyond our imagination. From the after-school meetings to the late nights working on our protocols, we have expanded our comprehension past the scientific aspect of synthetic biology into the ethics and faith behind hands-on education. Through the work in our low-budget high school lab, we have learned to adapt our protocols and create our own equipment when necessary. Through the sharing of our lab space with our neighbouring school, students, staff, and teachers, we have learned to take appropriate precautions in order to ensure safety and cleanliness in our lab. We have also taken into consideration the many ways that we can implement our project in a safe manner. The more we learn about synthetic biology, the more familiar we become with the problems it poses. Among these concerns is whether or not high school students should pursue this field of science, and can do so safely. Additional concerns address the kind of biological materials we are actually working with, and how the accessibility of this field could harm people through acts of bioterrorism.

We, as students, are studying synthetic biology in the pursuit of education and knowledge. Despite the common misconception that science and religion must exist in conflict, we have come to the understanding that they can, in fact, coexist and complement each other beautifully. Not only has iGEM provided us the opportunity to experiment in a lab, it has also allowed us to indulge in self-explorative learning that goes beyond the teaching that any textbook and memorization-based education could provide.

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