Zooxanthellae resistance to coral bleaching using heat shock proteins

Jeffery P. Conners, Eva I. McKone, Nadia Shah, Abhay Yajurvedi

BioBuilderClub, Andover High School, Andover, MA, USA

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Zooxanthellae are algae that give coral their renowned color and for mutualistic relationships they are essential to maintain coral health. Zooxanthellae photosynthesizes to provide coral with sugars and oxygen, and in turn, coral serves as a habitat for zooxanthellae. However, when the ocean temperature increases by one degree celsius than average for over a month, the zooxanthellae are ejected from the coral, ultimately leaving the coral vulnerable to deterioration (Bleaching Background). This is commonly known as coral bleaching. The effects of coral bleaching negatively impact the ecosystems of many organisms, and since corals are immobile, they cannot escape it. Due to the rise in ocean temperatures, coral bleaching has become more prominent; as a result, we propose a solution to make zooxanthellae more tolerant to warmer waters. We plan to create a transgenic microalgae—with our chassis a zooxanthellae genus known as *Symbiodinium sp.*—which will provide a heat-stable variant alongside the existing zooxanthellae. The process will be initiated with a temperature-induced promoter, and therefore our construct will take effect once the ocean temperature is higher than average for a period of four weeks. Our proposed construct will express more heat shock proteins (HSPs) to control the ejection of zooxanthellae. When exposed to thermal stress, such as an increase in temperature, HSPs have the potential to regulate the sensitivity of cells. In our case, the construct should specifically express HSP70. When the transgenic algae produces these heat shock proteins, they will be contained inside the cell to prevent overexpression and maintain control over the system. With our project, we hope to address the critical issue of coral bleaching and ultimately preserve ecosystems across the ocean.

Keywords: Heat shock proteins, HSP70, Zooxanthellae, Coral bleaching, Microalgae, Thermal stress

Mentors: Alberto Donayre, Lindsey L'Ecuyer, Khalid Shah

Direct correspondence to llecuyer@k12.andoverma.us

Watch a video introduction by the authors at https://youtu.be/8z0tZrEjZjI

Background

Coral bleaching is the process by which algae is expelled from coral due to stressors such as heat and occurs when ocean temperatures are higher than average for a period of four weeks (Bleaching Background). These critical algal structures that reside in coral are known as zooxanthellae, or dinoflagellates, and have a mutualistic relationship with coral. While the coral itself provides a habitat for the zooxanthellae, the zooxanthellae in exchange nourishes the coral with sugars and oxygen through photosynthesis (NOAA, 2021). At the molecular level, photons enter through the thylakoid membrane during photosynthesis in coral by dinoflagellates. Once absorbed by the photosystem, sugar and oxygen byproducts are created. However, when ocean temperatures increase, the thylakoid membrane and photosystems are overwhelmed by this excess heat, inhibiting photosynthesis (Palumbi, 2014). During an excess amount of photons and heat, the energy is converted into reactive oxygen molecules, harming both the zooxanthellae and coral (Palumbi, 2014). As a result, the dinoflagellates are ejected from the coral polyps (Figure 1). Without zooxanthellae, coral loses its pigment and source of nutrients, and is reduced to what is known as a “coral skeleton” (GBRMPA, 2017). This relationship is paramount for the maintenance of coral reefs around the ocean and the life of organisms that depend on reefs.

Heat shock proteins (HSPs) are a type of protein that act when cells are exposed to various stressors that differ from their normal conditions. Some of these include water deprivation, infection, inflammation, exposure to toxins, and thermal stress (Santoro). Of these stressors, change in temperature (thermal stress) is a common one; it has the potential to kickstart apoptosis. The role of HSP’s is to prevent programmed cell death and provide the cell with thermal stability, ultimately maintaining homeostasis . Another role of HSPs is to simply keep cells in order by “chaperoning” other proteins. Despite the fact that heat shock proteins are present in all cells, they can be upregulated when in the presence of stressors (Scientific American, 2008). Here, we use HSP70 to control the sensitivity of the zooxanthellae cells to thermal stress.

Systems Level

Theoretically, our construct would prevent the effects of coral bleaching by acting as a supplement to the existing zooxanthellae. In a coral-reef system, dinoflagellates reside in the tentacle cells of coral. As coral bleaching primarily targets the photosystems within the chloroplasts, the HSP70 in our chassis would hopefully increase its resilience to high temperatures, ultimately preserving the mutualistic relationship between the coral and zooxanthellae.

Device Level

In choosing a host for our design, we considered the idea of a mutualistic relationship in which multiple organisms work together to ultimately benefit a system (New England Complex Systems Institute). Temperature plays a key role in the construct, and so, with the idea of creating a heat-stable variant to work synonymously with the zooxanthellae that make up the coral, we found that *Symbiodinium sp*. would be the most efficient chassis. *Symbiodinium sp*. are a microalgae that are also a type of symbiotic zooxanthellae and a genus of dinoflagellate. They are well known for their mutualistic relationships with coral reefs (Moberg & Folke, 1999), and can be considered indispensable to the prevention of coral bleaching. A heat-stable symbiotic relationship would require the *Symbiodinium sp*. to provide oxygen and photosynthetic products to the coral; in turn, it would receive hosts from the coral and a permanent habitat (Kumar, 2021) (Figure 2).

Symbiodinium sp. can be collected by isolating it from coral tissue. A hypothetical lab procedure to isolate and grow our chassis would involve centrifuging coral tissue material with sea water to isolate the *Symbiodinium sp*. Following this, the algae would be treated with a saltwater nutrient solution such as F/2 (UTEX) and set the experimental temperature and light intensities to conditions that mimic the ocean (Lasker) in order to grow. This would permit us to synthetically engineer the algae and implement our construct including the HSP70 gene.

Parts Level

Since our construct is based on a change in ocean temperature, we intend to use a temperature induced promoter, Part BBa_K2559007, to initiate it. This part regulates the expression of the HSP70 gene using the temperature of the system. Once the ocean temperature is higher than average for a period of four weeks or longer, our construct will take effect. Next, we intend to use the HSP70 gene to regulate the sensitivity of zooxanthellae to temperature. HSP70 is a type of heat shock protein, which is a group of proteins produced when cells are exposed to different stresses, a prominent one being thermal stress. When the temperature of a system increases, HSPs have the potential to regulate the sensitivity of cells and prevent them from reacting to the temperature change. The HSP70 gene will be expressed in the *Symbiodinium sp*., and its stability will provide a heat-stable variant alongside the existing zooxanthellae. We will conclude our construct with a commonly used terminator, Part BBa_J52016. (Figure 3)

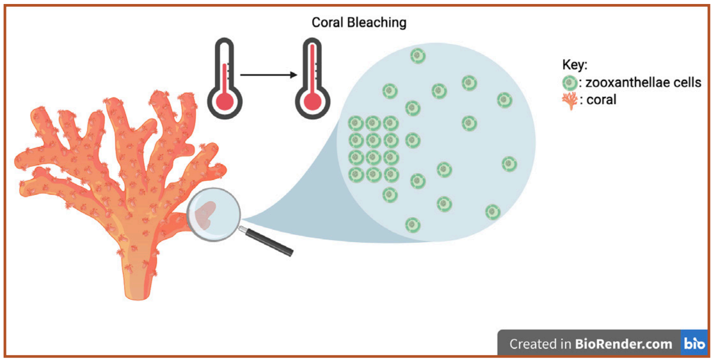

Figure 1. *Coral bleaching on a cellular level.*

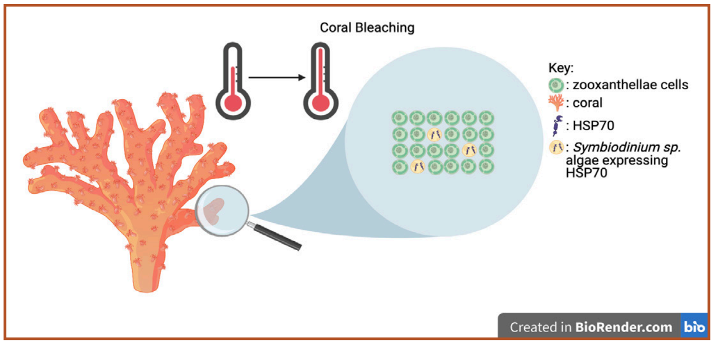

Figure 2. *The prevention of coral bleaching with the implementation of our construct.*

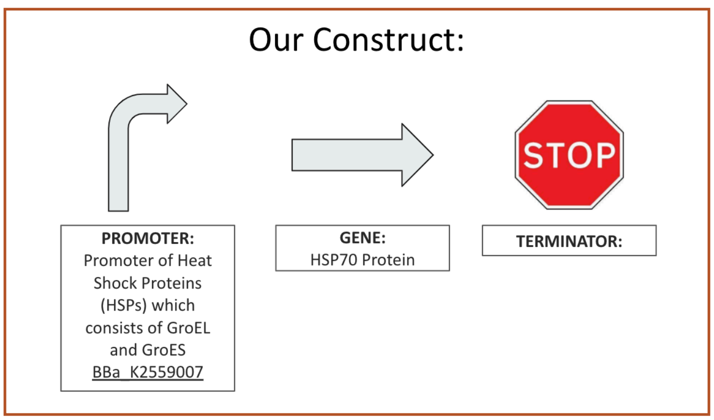

Figure 3. *The design construct; features a temperature-induced promoter, the HSP70 gene, and a terminator to conclude.*

Safety

In order to safely create our construct, we decided to use *Symbiodinium sp*. as our chassis. In nature, this form of dinoflagellate already has a symbiotic relationship with coral. Thus, by using it as a vessel for our genetic material, we would not be introducing new organisms into the aquatic ecosystem. Additionally, we chose to use a temperature induced promoter. When the temperature of the surrounding water returns to its average, the promoter will cease to initiate biological activity. This essentially would prevent an overproduction of HSP70. There have been few studies in regards to the consequences of excess HSP70 and thus, the effects are unpredictable. Should HSP70 have a negative impact, we would modify the construct.

In order to test our construct, we would begin in a contained environment. After having tested our system thoroughly, we would try to increase the testing space and see how the modified zooxanthellae interacts with a larger area. If our chassis is able to maintain the health of the coral under high temperatures while sustaining its own conditions without damaging environmental conditions, we would release it into reefs.

Discussion

Over the past few decades, the effects of climate change have been rapidly increasing, and in particular, these effects have severely impacted the world's coral reefs. Presently, more than fifty percent of the Earth's coral reefs have been damaged; (Camp et al. 2018) our project could potentially protect and preserve the remaining coral reefs from the coral bleaching process.

Working with biological organisms comes with a variety of things to be taken into consideration. With our project, for instance, there is little known about the over-expression of HSP70 in photosynthetic organisms and thus, the interaction between the modified zooxanthellae and its environment would be uncertain. It would be beneficial to test the effects of HSP70 on other organisms and the environment before proceeding. One such example would be testing the dinoflagellates in contaminated water to ensure that there would be no negative effects when exposed to impurities. Another limitation to our project is that we can only test our construct in a lab setting; this may not yield as accurate results compared to testing in the ocean. However, if we eventually decided to test and release our system into a live ecosystem, we would need to take into account how biologically ethical it would be. Hopefully, our construct will prove to be an effective way of preserving coral reefs and that we may be able to test it in a biologically safe way.

Next Steps

Looking to the future, to test our construct, we would like to perform an experiment in which we take two samples of zooxanthellae that are native to a body of water, such as the Great Barrier Reef. We would then like to place the zooxanthellae into two bins of water which are greater than the temperature that they would experience in the water. To ensure our construct works, one of the groups of zooxanthellae would have the construct applied, and the other would not. After a month, we would compare the proportion of coral that underwent coral bleaching in each group respectively. After reviewing the results, we would test for statistical significance using a Two Proportion Z-interval; using this test would allow us to determine the probability of seeing the results we obtained (Figure 4). We could then use this probability to decide if our construct did prevent bleaching. Extrapolating our design, we would eventually like to use a similar construct on other plants affected by climate change. As time goes on, it will be pertinent for the scientific community to develop more solutions to aid species directly affected by global warming.

In the course of researching for our project, we identified many potential extensions for another group to pursue, one of which is applying our construct or a similar one to plants on land that will be affected by climate change. We believe that the potential application of a similar construct to species on land will be beneficial. Another way for a group to further the research presented is to conduct an experiment similar to the one outlined in the Next Steps section.

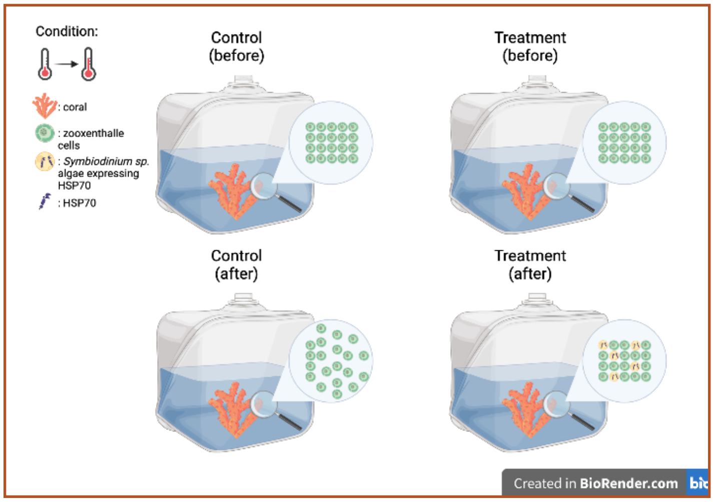

Figure 4. *Two Proportion Z Interval Experiment*

Author Contributions

Each member played a key role in the creation of this project. All authors not only worked on the general research for the project but also worked on specific sections too. Author, N.S., centered her focus on the function and role of HSP70 and was a key contributor to the parts level, device level, and background. N.S. also crafted the diagrams. Author, E.M. specialized in coral and zooxanthellae biology and developed the idea of creating resilient dinoflagellates. E.M. mainly worked on the background, systems level, and safety. J.C. worked primarily on the development of potential experiments that could be used to test the construct. J.C. presented the idea of incorporating statistics into the project to better determine results. In addition, J.C worked in part with N.S. to think about extensions that future groups could do to further the project. A.Y. played a crucial role in the editing and citations process. A.Y. also focused on the abstract, background and safety.

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References

Camp, E. F., Schoepf, V., Mumby, P. J., Hardtke, L. A., Rodolfo-Metalpa, R., Smith, D. J., & Suggett, D. J. (2018). The future of coral reefs subject to rapid climate change: lessons from natural extreme environments. Frontiers in Marine Science, 5, 4. https://doi.org/10.3389/fmars.2018.00004

GBRMPA (2017) Fact sheet: coral bleaching. The Great Barrier Reef Marine Park Authority. Available at https://www.gbrmpa.gov.au/ https://www.gbrmpa.gov.au/__data/assets/pdf_file/0007/252385/GBRMPACoralBleaching_FactSheet_Updated5May2017.pdf .

Hirooka S;Hirose Y;Kanesaki Y;Higuchi S;Fujiwara T;Onuma R;Era A;Ohbayashi R;Uzuka A;Nozaki H;Yoshikawa H;Miyagishima SY; (n.d.). Acidophilic green algal genome provides insights into adaptation to an acidic environment. Proceedings of the National Academy of Sciences of the United States of America. https://pubmed.ncbi.nlm.nih.gov/28893987/.

Kobayashi, Y., Harada, N., Nishimura, Y., Saito, T., Nakamura, M., Fujiwara, T., … Misumi, O. (2014, September 29). Algae sense exact temperatures: small heat shock proteins are expressed at the survival threshold temperature in Cyanidioschyzon merolae and Chlamydomonas reinhardtii. Genome biology and evolution. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4224343/.

MG;, S. (n.d.). Heat shock factors and the control of the stress response. Biochemical pharmacology. https://pubmed.ncbi.nlm.nih.gov/10605935/.

Moberg, F., & Folke, C. (1999). Ecological goods and services of coral reef ecosystems.

Ecological Economics, 29(2), 215–233. https://doi.org/10.1016/s0921-8009(99)00009-9

NOAA. (2021) Zooxanthellae, what's that? National Oceanic and Atmospheric Administration (NOAA), US Department of Commerce; Zooxanthellae: Corals Tutorial. Accessed June 3, 2021. https://oceanservice.noaa.gov/education/tutorial_corals/coral02_zooxanthellae.html

Obornik, M., & Lukes, J. (2013). Symbiodinium - an overview | ScienceDirect Topics.

Sciencedirect.com. https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/symbiodinium

Palumbi, S. (2014). Coral bleaching: A breakdown of symbiosis. Coral Bleaching Animation– HHMI Biointeractive Video. YouTube, uploaded by Biointeractive, November 2014. https://www.youtube.com/watch?v=_ZfGIKiSwwQ&t=5s

Scientific American. (2008). Roles of Heat Shock Proteins. Scientific American. https://www.scientificamerican.com/article/roles-of-heat-shock-proteins/.