A Review of Coral Bleaching Resistance Genes in *Symbiodinium*

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***Abstract*- Coral bleaching, or the loss of corals' symbiotic algae, is the result of environmental stressors such as excessive heat, light, acidity, and pathogenic microorganisms. This phenomenon causes coral death and may lead to irreversible environmental damage in the form of habitat loss for a quarter of all marine life. Recent studies suggest that thermal stress may impair algal processes such as photosystem I&II, protein phosphorylation, oxidation counseling and programmed cell death, which could all be factors driving algal, or *symbiodinium*, expulsion. Some genes identified in thermally stressed algae were differentially expressed compared to non-stressed algae. These genes include heat shock proteins and antioxidant enzymes such as superoxide dismutase, which indicates they may play a major role in the bleaching process. Recently, an increasing number of coral and algal symbiont genomes have been sequenced. This, along with advances in transformation techniques such as Crispr/Cas9 genome editing, will advance our understanding of coral bleaching and bring remediation closer to success than ever before.**

***Index Terms*-** Coral bleaching, Resistance genes, Symbiodinium, Synthetic Biology

I. INTRODUCTION

The most diverse marine habitats in the world, coral reefs, are suffering. Models have predicted that within this century, most coral reefs will be severely damaged or lost due to coral bleaching (loss of their algal symbionts) unless novel approaches to restore corals are established (Levin et al. 2017). Many stressors contribute to coral bleaching, including extreme temperatures, ocean acidification, high irradiance, heavy metals, and pathogenic microorganisms (Warner et al. 1999, Douglas 2003). *Symbiodinium*, the obligate symbiont of coral, produces nutrients via photosynthesis, which are translocated to the host and can provide up to 100% of coral's required energy in nutrient-deprived tropical waters (Davies et al. 2018). Correspondingly, coral provides algae with access to light, inorganic nutrients, and dissolved inorganic carbon all within a protected microenvironment (Davies et al. 2018). However, with global temperatures on the rise, this symbiotic relationship has been threatened (Douglas 2003). Coral bleaching affects not only the host-symbiont system, but also the reef fishes that rely on these structures (Pratchett et al. 2011). Pratchett et al. suggested that up to 75% of reef fishes heavily rely on coral reefs for food, shelter, and settlement. Loss of coral cover would eventually lead to loss of abundance and biodiversity in these reef environments, which would threaten human food security and lead to billions of US dollars in economic damage (Pratchett et al. 2011). Levin et al. and other researchers are exploring synthetic approaches to coral bleaching by performing genomic analyses and identifying genes that could be linked to bleaching events in the coral and algae (Levin et al. 2016). There are different strains in *symbiodinium*, and some of them are more resistant to bleaching. Differential expression between sensitive and resistant strains may indicate that the genes associated with thermal-sensitive strains can be up or down regulated in order to prevent coral bleaching. Then, these genes can be used to determine the source of bleaching resistance. Furthermore, new advances in biotechnology such as the CRISPR/CAS-9 system and biolistics show promise in genetic modification of coral's symbiotic algae. These new genetic manipulation techniques for coral reef management could not only promote sustainability, but could also benefit human health and food security. (Levin et al. 2017).

I. RESEARCH ELABORATIONS

A. Research design

Articles were compiled by several GSU iGEM members in order to structure a review of studies related to coral bleaching resistant genes within the genus Symbiodinium.

Database
https://docs.google.com/spreadsheets/d/e/2PACX-1vRzSPKnMx0KjTpqEuGQGvVPmyQiDpq_GOyPbsiGoZVWTyiNanwEn8BkjweupikgcPf237OPRYWlPt1c/pub?output=pdf

III. RESULTS AND FINDINGS

A. Causes and Effects of Coral Bleaching

When faced with thermal stress, *Symbiodinium's* responses can be classified into three distinct stages: 1) Downregulation of plastid transcription; 2) Photosystem damage causing functional failure of the chloroplast, and 3) Degradation of existing functional transcripts (Mcginley et al. 2012). High temperatures have been shown to damage the symbiont's photosystem II (PS II) (Warner et al. 1999). Damage to the symbiont's PS II will cause a decline in the D1 protein reaction center of that photosystem (Warner et al. 1999). The balance between the rate of light-induced damage to photosynthetic proteins and the rate of subsequent cellular repair, including reinsertion of the DI subunit, would be broken by high thermal stress (Weis 2008, Mcginley et al. 2012). This could then result in a loss of transfer of photosynthate to the coral host, thereby adversely affecting the host-symbiont relationship (Warner et al. 1999). Another potential consequence of PS II damage is the overproduction of reactive oxygen species (ROS) (Mydlarz et al. 2010). Reactive oxygen species such as superoxides and peroxides are produced when excessive heat reduces the consumption of ATP and NADPH by the enzyme rubisco, which, in turn, results in a buildup of excitation energy in the PS II (Weis 2008). ROS leakage can oxidatively damage both coral and algal membranes, proteins, and DNA, which is correlated to the expulsion of the algal symbiont from the host cells (Mydlarz et al. 2010). With the increase of ROS concentration and the destruction of photosynthetic function, the antioxidant system in the symbiote collapses and cannot detoxify ROS. The resulting positive feedback loop of accumulating ROS causes further damage to the photosynthetic cells and eventually the expulsion of the algae from the host (Weis 2008).

B. Target Genes

Although thermally tolerant strains of *Symbiodinium* already exist in nature, they tend to have slow growth rates compared to thermally sensitive ones (Karim et al. 2008). The optimal solution would be to have thermally tolerant strains of fast-growing algae. Coral bleaching could potentially be solved by taking advantage of endogenous genes in thermally tolerant *Symbiodinium* that are correlated to bleaching resistance by either up or down regulating them in thermally sensitive strains. Analysis of critical target genes during a stress period allows for the identification of bleaching resistance sources. Two transcriptional analyses (Levin et al., 2016 and Gierz, Forêt, & Leggat, 2017) of symbiodinium in response to heat stress involved genes related to antioxidant defense, photosynthesis, fatty acid desaturase, meiosis, and RNA binding. In Gierz, Forêt, & Leggat's study comparing the expression levels of thermally stressed symbiodinium clade F to the same clade at control temperatures, HSP70, HSF, UCH (ubiquitin carboxyl-terminal hydroxylase), chlorophyll A-B binding protein, VDE (violaxanthin de-epoxidase), fer4_17 (4FE-4S di cluster domain), ICL (isocitrate lyase family), MORN, SAE2 (DNA repair protein endonuclease), and FATC were upregulated. On the other hand, FHA, DNA photolyase, fer2 (2FE-2S di cluster domain), VDE, cty-B559, cyt-B5 (cytochrome b5-like heme/steroid binding domain), pkinase (protein kinase domain), rad51, TBPIP (tat binding protein), RRM_2 (RNA recognition motif) were all downregulated (Gierz et al. 2017). However, there did not seem to be any pattern with respect to downregulation versus upregulation of these various categories. Levin et al. compared differentially expressed genes (DEGs) between thermally sensitive and thermally tolerant clade C1 and revealed that Hsp70 and Hsp90 were upregulated in only the thermal tolerant strain on day 13 (Douglas 2003, Levin et al. 2016). Heat shock proteins (Hsps) are molecular chaperones that partake in protecting cellular functions related to protein folding. In that same transcriptomic analysis, DEGs responsible for metabolism, biosynthesis, oxidoreductase activity, and motile cilium were upregulated and appeared to give symbiodinium adaptive mechanisms against heat stress. Upregulated genes including Fe-Sod, Ccpr, Gpx, Txn, Cyp450 reveal the importance of ROS scavenging for thermal tolerance. Recent research by Krueger et al. into antioxidant genes Fe-SOD, Mn-SOD, APX and KatG suggests that the enzyme superoxide dismutase (SOD) acts as the first line of ROS defense in *Symbiodinium*, as it can catalyze the disproportionation of O_2- into H_2O_2 and O_2 to reduce the damage of superoxide to cells (Krueger et al. 2015, Polle 2001). Despite one source, Levin et al., stating that the lack of Fe-SOD is a major contributor of thermal tolerance in *Symbiodinium*, another study by Goyen et al. suggested that the Mn-SOD gene was more common than Fe-SOD among *Symbiodinium.* Fe-SOD had little to no expression in some *Symbiodinium* while both secretory and mitochondrial pathways in *Symbiodinium* contain Mn-SOD. In addition, Mn-SOD may be located in peroxisomes, which produce molecules that readily react to form ROS. Finally, Fe-SOD and Mn-SOD have very similar functions and structures. (Goyen et al. 2017)

IV. CONCLUSION

Based on the information gathered, the most relevant target genes for coral bleaching resistance are related to ROS scavenging and protein folding in response to heat stress. The genes that stood out the most were those that coded for heat shock protein, heat shock factor, and superoxide dismutase. Synthetic biologists can now take these genes and introduce them into the genomes of thermally sensitive strains of *Symbiodinium.* Hopefully, this will confer bleaching resistance and prevent the destruction of one of the most biodiverse ecosystems on the planet.

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