

Reef Relief

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Abstract

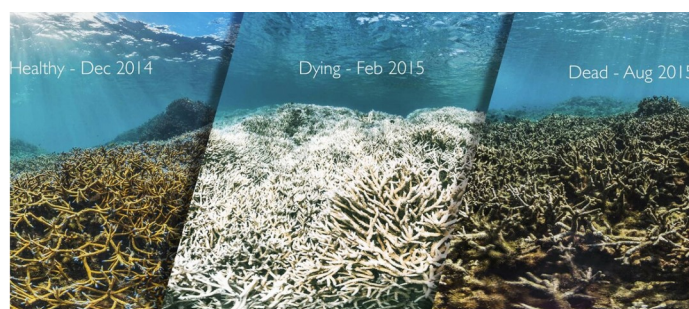
Bleaching & death of coral is mostly due to global warming (heat and light stress) and consequent eviction of symbiotic zooxanthellae from their polyps. Creation of a genetic circuit with NO sensor and proline biosynthetic genes to quench excess reactive oxygen species produced by zooxanthellae under such conditions.

Introduction

Corals provide the photosynthetic zooxanthellae with shelter, carbon dioxide and various nitrogen compounds and zooxanthellae, a dinoflagellate mainly of the genus *Symbiodinium* living inside the polyps provide the corals with glucose, glycerol, amino acids and helps in forming the calcium carbonate skeleton, creating coral reefs.

Combined heat and light stress lead to the formation of reactive oxygen species (ROS) like singlet oxygen (1O_2), superoxide anions and hydroxyl ions in the zooxanthellae. This causes bleaching and irritates the coral. The corals thus evict the dinoflagellates as a defence mechanism but they cannot survive without them and soon die.

My aim is to create a genetic circuit that quenches the excess ROS so that bleaching and irritation can be prevented.



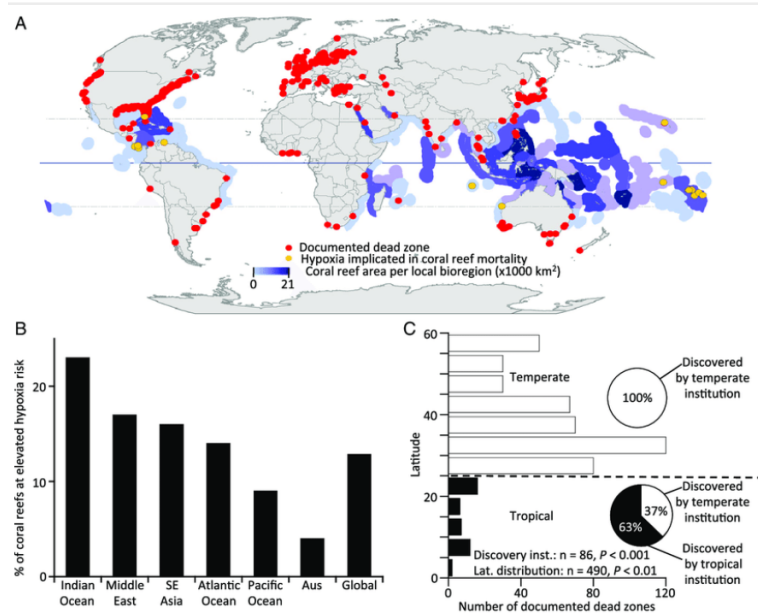
The same reef in American Samoa before, during, and after a coral bleaching event. Credit: XL Catlin Seaview Survey.

Background

Despite covering <1% oceanic area, Coral reefs support about 25% marine species like clownfish, manta rays, reef sharks, turtles and many more – acting as sites of shelter, reproduction, feeding and nursery areas.

Corals also protect the coastlines from flooding and erosion during storms. They are filter feeders and keep the shore waters clean. Corals and microorganisms associated with it form basis for advanced food and medicine technology. Healthy coral reefs provide jobs for local community, boost tourism and fishing industry, generating as much as \$375 billion annually around the globe.

However, in recent years, coral reefs have been damaged permanently by rising sea temperatures. Up to 90% of coral cover is lost in Maldives, Sri Lanka, Kenya, Tanzania, and Seychelles, >75% coral reefs are threatened in Atlantic Ocean and mass bleaching and death of corals has been observed in South Indian, South Pacific Oceans and even the Great Barrier Reef.



Credit: Researchgate.net

Marine ecosystems have collapsed largely. Worldwide economy can crumble.

Existing methods

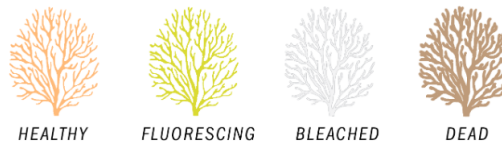
Several genes that promote thermal stress tolerance in zooxanthellae like Fe-sod, Mn-sod, Pxr & hsp70 have been identified and CRISPR Cas9 could be used for genome editing. However, dinoflagellates exhibit evolutionary divergence from other eukaryotic organisms & unusual biological features like a large genome and permanently condensed liquid-crystalline chromosomes have hindered research progress. Even if it is possible, Symbiodinium genus is highly diverse and several coral species associate with specific zooxanthellae only. Uptake of stress-tolerant but non-native Symbiodinium types could adversely affect coral growth and fertility. This means we would have to genetically modify many clades/species. I want to propose a model that increases stress tolerance with the help of chemical sensors and quencher proteins and is not species-specific.

Proposed solution

Intracellular ROS cause photooxidative damage to the photosynthetic apparatus (mainly PSII), accessory pigments, proteins, lipids of the zooxanthellae. The vivid colours of the corals are due to the pigments present in zooxanthellae. So, this causes bleaching of the corals. Excess ROS also escapes into the extracellular medium. If it exceeds the host antioxidant capacity, it starts irritating the coral which makes them evict the microorganisms.

So, should we target the extracellular ROS? On further research, I found out that ROS in extracellular medium like superoxide provides pathogen resistance to the coral host.

Moving on to intracellular ROS, Nitric Oxide (NO) has been detected before the accumulation of ROS/ before observing the fluorescing stage. It acts as a signalling molecule.



Credit: Time.com

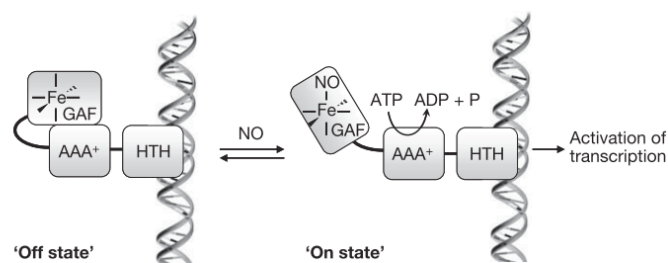
NO also reacts with superoxide to produce peroxynitrite which induces photooxidative damage. Should we stop the production of NO then? NO however is necessary for gene transcription, translation, other useful signalling pathways and metabolic processes. Thus, I aim to quench excess intracellular ROS.

Genetic Circuit

- It has been experimentally proved in isolated thylakoid membranes that the amino acid L-proline is a potent ROS (superoxide, peroxide, hydroxide, singlet oxygen) scavenger and pH buffer.
- Genes required for biosynthesis of L-proline – PRODH, P5CDH, P5CS1, P5CS2, P5CR are isolated. Ligase is used to join them and create the gene of interest.
- NorR (NO responsive transcription factor) is isolated from bacteria *E. coli* to be used as promoter.
- Amino-terminal GAF_{NorR} domain will act as the inhibitor.
- A terminator will be present at the end of the gene.

The NO Biosensor and its working

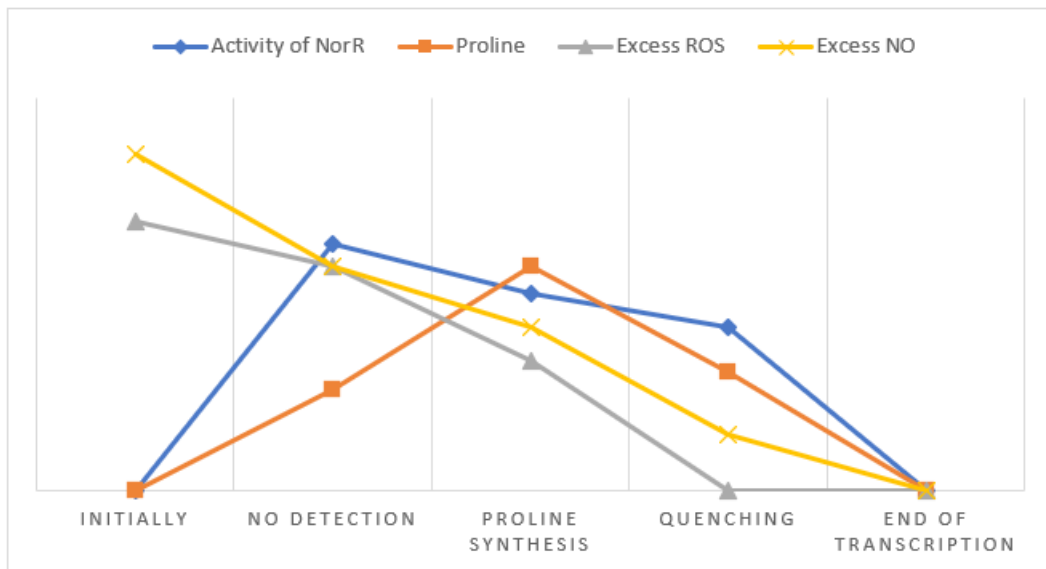
- If NO is present then GAF_{NorR}, the regulatory domain of NorR, containing a mononuclear non-haem iron centre reversibly binds NO. Binding of NO stimulates ATPase activity of the central AAA⁺ domain of NorR enabling the activation of transcription by RNA polymerase. Proline synthesis starts.
- If GAF_{NorR} is unable to detect NO, then it inhibits the activity of AAA⁺, NorR remains inactive and transcription does not occur. Proline synthesis stops.



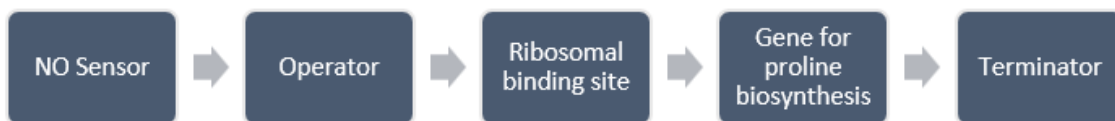
The NO Sensor Credit: NATURE Vol 437

This provides dual benefits: successful quenching of excess intracellular ROS by the proline synthesized and prevention of proline accumulation that can harm the cell's own metabolism.

The amount of NO \propto activity of NorR \propto amount of proline that can be produced \propto 1/amount of ROS. Again, during quenching, the proline & ROS conc. reduces.

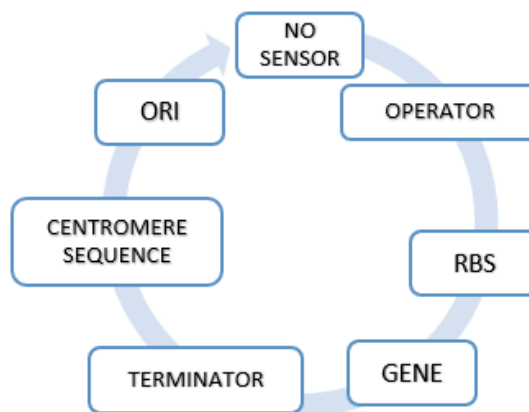


A probable concentration graph



Genetic Circuit

This can be incorporated into a compatible vector to ensure that the genes work in the eukaryote. The vector must also have a centromere sequence to allow the correct partition of plasmid in dividing dinoflagellates – so the future generations are also armed with this. An origin of replication (ORI) point must also be present for proper replication of plasmid.



Plasmid Design

The vector can be introduced into the zooxanthellae via gene gun method. It will bombard the cells with DNA coated metal (preferably gold) particles using helium as propellant. The presence of stiff cellulose plates on the surface of dinoflagellates discards the possibility of severe damage to cells. The gene gun also successfully penetrates cells deep within a tissue – useful in this case.

Acknowledgment

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