Neutralizing Red Tide Threats via Brevetoxin Nullification

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Reviewed on 11 May 2019; Accepted on 17 June 2019; Published on 28 October 2019

Red tide algal blooms are affecting the coastal ecosystems by emitting harmful Brevetoxins that eliminate large portions of fish and shellfish populations. The aerosolized form of Brevetoxin also causes respiratory issues in mammals, and general consumption results in Neurotoxic Shellfish Poisoning. Brevetoxin production is theorized to be tied to PKS (polyketide synthase) clusters. Disrupting the gene that codes for the production of the PKS could therefore stop the production of the Brevetoxins. Assuming the PKS cluster could be located, CRISPR-cas9 could be used to edit the gene or genes that disturb the ability of PKS clusters to function. This then could result in the disabling of Brevetoxin production. The PKS knockout gene could be delivered to red tide algae via an algae-infecting phage in lieu of a gene drive. This phage could possibly affect other organisms; therefore a specific phage must be used to ensure that only the red tide algae is infected and transformed. The impact of altering PKS clusters (other than the probable cessation of Brevetoxin production) has not yet been discerned in algae, and the unintended effects of the disruption of PKS cluster processes on healthy algae are unknown.

Keywords: Red tide, algae, brevetoxins, polyketide synthase, knockout gene

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Background

Harmful algal blooms (HABS) have become more pervasive as rising ocean temperatures due to climate change have stimulated more intensified and long-lasting algae growth. These circumstances lead to an increase in the toxicity of the tides inhabited by these HABs. Sometimes called "red tide" events, these HABS are easily identifiable by the way that the high concentrations of algae blooms discolor coast waters, making them appear reddish or pinkish (Brawley et al. 2017). Red tide blooms threaten both human lives and the balance of the ecosystem because of the high levels of toxins produced by the algae in the area. This ecosystemic instability results in decreased biodiversity in these coastal environments (Baden, et al., 2005).

Although the environmental triggers for Brevetoxins are still uncertain, the harmful effects are expressed through many oceanic organisms such as shellfish and fish. Massive fish die-offs have been reported in and around algal bloom sites. Humans can be affected by Brevetoxins through shellfish or seafood consumption. Brevetoxins disrupt standard neurological processes in organisms, causing death in oceanic organisms and poisoning in humans through binding selectively to site 5 on mammalian voltage-sensitive sodium channels within dendrites and neurons (Gold et al. 2013). The disruption of regular function within the sodium channels causes the respiratory and cardiac systems to fail due to uncontrolled sodium flow, which leads to spontaneous firing

(Condello et al. 2016). Brevetoxins also appear to impact calcium homeostasis and encourage the release of histamine, which causes bronchoconstriction. These issues combined are medically defined as neurotoxic shellfish poisoning (Condello et al. 2016).

The consumption of infected seafood, especially bivalve shellfish, is the root cause of neurotoxic shellfish poisoning in humans (Erdner et al. 2019). Brevetoxins accumulate within both the viscera and lean muscle tissue of organisms, and is both heat and acid stable. It is, therefore, not easily destroyed during the cooking process, allowing human individuals to consume large doses of Brevetoxins (Condello et al. 2016). Humans can also inhale aerosolized Brevetoxins and develop asthma-like symptoms.

The mechanism of synthesis for Brevetoxins within algae is largely unknown, yet it is clear that Brevetoxins are not proteins, and cannot be coded for by a single gene. Organisms of the genus Karenia, such as Karenia brevis, to which many toxin-producing algae species belong, have genomes almost thirty times longer than a human genome, making it difficult to identify gene clusters containing PKS sequences (Thompson 2012). This obstacle would then interfere with traditional approaches to disrupt the Brevetoxin production, making the method to solving this issue even more difficult to find.

Supposedly, Brevetoxins are produced as secondary metabolites by polyketide synthases (PKS)(Condello et al. 2016). The relationship between the enzymes produced by polyketide genes and their relations and interactions to the Brevetoxins produced as a result remains unknown. Further research is required to ascertain the locus of the polyketide synthase coding gene and the linkage of the gene to Brevetoxin production.

Systems Level

Eliminating the neurotoxic effects of red tide algae has no obvious environmental repercussions. The removal of Brevetoxin from the oceanic environment would eliminate the threat of neurotoxic shellfish poisoning and prevent red tide-induced shellfish die-offs.

However, there is uncertainty as to what preventing Brevetoxin production would have on the algae itself and the surrounding environment, which stems from limited basic knowledge of the systems and functions of Brevetoxin itself (Gold et al. 2013; Meyer et al. 2013). Toxic secretions of algal blooms have not been linked to any direct cause, although rising ocean temperatures leading to explosive algae growth is a likely cause (Florida Fish and Wildlife Conservation 2019). Therefore, manipulating the gene within red-tide algae that controls Brevetoxin secretion would be a solution with many unpredictable outcomes in regards to the algae itself.

Device Level

Brevetoxins are not proteins and cannot themselves be genetically manipulated to regulate their production. An engineered gene that shuts down PKS synthesis could be introduced to red tide algae via an algae-infecting phage as a gene drive facsimile.

A virus family that has bacteriophages, called siphoviridae, has been found to be virulent within red tide algal blooms (Thompson 2012). Although its ability to infect the algae blooms is prevalent, it might require a lot of genetic engineering to do so without eliminating the algae. Since the siphoviridae family contains a variety of genera, a candidate for transporting the knockout gene may be within the family, but the extent of this genera's virulence is unknown and requires further research. Another viable phage could include the strain HcDNAV, a double-stranded virus that infects Dinophyceae algae (Takano er al. 2018).

However, little is known about the activities of these phages. To effectively transmit the gene, the viral infection rate must be higher than the rate at which the algae die. Again, the problem with these virus strains is that studies would need to be completed to determine their effectiveness and capacity to take in the PKS knockout gene. Furthermore, the location and nature of PKS genes remains largely unresearched. More research would be required to determine the specific Biobricks necessary for development of a knockout gene and viral infiltration of the algae.

Parts Level

Due to the fact that very little is known about Brevetoxins and in-depth research about red-tide algae species and their viral infectors is lacking, a concrete parts level to this proposed method cannot yet be promoted.

PKS synthases appears to function similar to operons. However, this is only a speculation, and studies to determine their systems and interactions with viral strains would need to be completed. The exact location and specific functions of the PKS gene clusters within the K. brevis genome remains unidentified. The systems through which these clusters function are also generally unresearched (Thompson 2012).

More information is therefore necessary when determining viable viral strains to use as phages, as well as that

virus' interaction with the surrounding oceanic ecosystem and its ability to be genetically manipulated using CRISPR cas9 technology.

Safety

The issues with implementing our proposed PKS-knockout gene include possible environmental repercussions and other unanticipated side effects within the human population.

Tests on red tide algae to insert the PKS knockout gene would need to be completed in the sterile conditions of a laboratory. However, inducing red tide events within laboratory conditions has proven extraordinarily difficult, as the environmental and internal factors that trigger these events are still subject to speculation.

Since the scientific field of microbiology has limited research on the specific genetic pathways in the algae, the risk posed to the organism and environment of genetic manipulation using a knockout gene is not completely understood. The algal reactions to the inserted phage and knockout gene could possibly trigger other genetic reactions and chemical emission like that of the Brevetoxin. Another possibility is that the gene being targeted for knockout could also control other processes vital for the survival of the organism and the attempted inactivation would result in death (Chen et al. 2006).

If the knockout process does not result in the interruption of Brevetoxin secretion, the environment would still be affected by the red tide toxins and would thus be damaged by species loss. Altering genetic pathways without understanding the extent and specificity of their structure and triggers can cause potential problems for both the environment and organism. This means that the experiment would only be safe to enact if the algae's genes were thoroughly mapped and located, and their functions understood.

Discussions

Since this was a theoretical paper, no physical experimentation was completed to test our postulations. However, the implementation of this proposed system has the potential to be beneficial to human and environmental systems. Massive oceanic wildlife die-offs could be prevented, and the threat of neurotoxic shellfish poisoning could be lessened significantly.

The results of this experiment, whether successful or not, would either prove or deny the link between PKS synthases and Brevetoxin production, therefore propelling future research. If the system of the experiment was to function perfectly, but no change in the red tide concentration was observed, it could be concluded that PKS synthases do not control Brevetoxin production. If the inverse were to occur, the experiment would prove that PKS synthases are instrumental in Brevetoxin production. With the then obtained data, the possibility of decreasing the harm caused in the ecosystem by these emitted Brevetoxins would be feasible.

As research on red tide algal blooms, red tide species, and the Brevetoxins that cause neurotoxic shellfish poisoning is significantly limited, more specific research into these different elements is necessary. In order to comprehend why these algal blooms occur, studies that test for potential environmental triggers that cause red tide algal blooms and discern what the function of Brevetoxin is (defense, competition control, etc.).

The effects of eliminating red tide algae species would also have to be more intensely studied. Since red tide species often transition from a harmless state to producing Brevetoxin, eliminating the species could have a ripple effect within the ecosystem, as algae are key producers of oxygen and sources of food for zooplankton and crustaceans.

Furthermore, the study of viruses that infect the algae species would need to be expanded upon. Finding a phage that is perfectly suited to the conditions of the experiment is necessary to insert the proposed PKS knockout gene. To that end, more research on genome and systems of algae would have to be done in order to construct an actual gene knockout pathway.

Our original idea was to use Brevetoxin's natural antagonist, Brevenal, as a way to demote the toxic effects in the fish and shellfish. In this idea, we would use CRISPR-cas9 to increase the production of brevenal in algae. However, we found this to be an invasive method since the Brevenal also binds to the site five in the voltage-gated sodium channels but does not significantly affect the organisms as we know so far. It is also easier to approach the toxin production directly, rather than after it is produced and can already harm the organisms in the environment.

As a completely different experiment, disrupting the quorum-sensing abilities of the algae is another potential approach to eliminating Brevetoxin production that could be considered. However, it is uncertain whether red tide events are algal reactions to increased population densities. An experiment conducted that disrupted and algae's quorum-sensing abilities, even if no change was observed, would confirm that Brevetoxin is a byproduct of competition-based systems of defense.

Acknowledgements

We would like to thank our teacher, Anna Minutella, for guidance in this project's contents and providing the necessary class time and resources. Our mentor, Khaled Said, also deserves recognition for helping in the initiation of the project, by mentioning that the Brevetoxins are not proteins, and cannot be synthesized by one gene, which has directed the projected design of the paper completely.

This project was accomplished through participation in the BioBuilderClub, an after-school program organized by BioBuilder Educational Foundation. BioBuilderClub engages high school teams around the world to combine engineering approaches and scientific know-how to design/build/test their own project ideas using synthetic biology.

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