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1st choice: Monitoring and Mitigation
2nd choice: Toxins, Biosynthesis, and Detection Methods

Life Without Chargaff's Rules: Thymine Dioxygenase as a Proposed Enzyme for the Synthesis of 5-Hydroxymethyl Uracil in Dinoflagellates

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The genomes of core dinoflagellates contain a fifth nucleotide base, 5-hydroxymethyl uracil (HOMeU). This fifth nucleotide complementary base pairs with adenine, replacing about forty percent of thymine in the genome. While the function of HOMeU is unknown, it is hypothesized that it either plays some role in the regulation of gene expression or is a vestigial structure from an ancestral genome. Thus, enzymes involved in the production of HOMeU are good candidates for harmful algal bloom (HAB) mitigation. We find that incorporation of HOMeU occurs during the polymerization of DNA rather than as a post-synthetic modification because i) HOMeU exists within the deoxyribose and ribose nucleotide pools and ii) the DNA polymerase is not able to distinguish thymine from HOMeU. It is likely that thymine is oxidized and hydrolyzed to form a HOMeU:adenine pair. We identified a hypothetical thymine dioxygenase (TD) transcript in *Amphidinium carterae* that is found in all dinoflagellates. The predicted activity of the enzyme is to produce 5-hydroxymethyl uracil, succinate, and CO_2 from thymine, 2-oxogluterate, and oxygen. *In vitro* studies with recombinant produced TD examined the substrate specificity and kinetics of this presumptive conserved protein. DMOG (Dimethyloxalylglycine), an antagonist of α-ketoglutarate cofactor, was examined as an inhibitor of the activity. Polyclonal antibodies then examined whether this enzyme is produced only in the core dinoflagellates that express HOMeU and not in other species of the broader dinoflagellata phyla. Our findings ultimately introduce new mechanisms for HAB mitigation should TD activity inhibition or protein knockdown prove lethal for dinoflagellates.