TITLE: ALGICIDAL BACTERIA AND HAB TERMINATION

MILESTONE SHC 2.5.2: Transfer technology, tools, and knowledge related to harmful algal bloom events to assist communities, resource managers, and health officials.

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EXTERNAL COLLABORATORS: Florida Marine Research Institute, Mote Marine Laboratory, Monterey Bay Aquarium Research Institute, Scripps Institution of Oceanography

OBJECTIVES OF RESEARCH ACTIVITIES: To establish the presence of algicidal bacteria in U.S. coastal waters, to evaluate the potential role of such bacteria in promoting the decline or termination of harmful algal blooms, and to characterize the mechanistic basis of the algicidal activity.

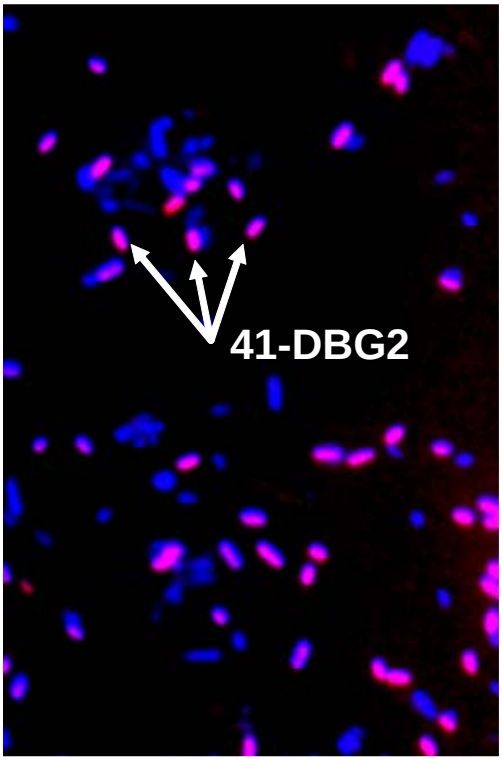
DESCRIPTION OF RESEARCH ACTIVITIES: Research on the interaction of algicidal bacteria and red tide algae has provided a new means to understand microbial processes influencing harmful algal bloom (HAB) population dynamics and promoting bloom termination. Moreover, interest in algicidal bacteria has been enhanced further by their potential use as part of HAB management strategies. The primary focus of the current project is to develop the appropriate tools that will allow detailed study of algicidal bacteria population dynamics and killing activity in both the laboratory and the field. Concurrent detection of these bacteria and the algicides they produce will aid in defining their possible role in regulating the growth of HAB species in natural systems. To this end, we have designed strain-specific oligonucleotide probes targeting unique regions of the small subunit (16S) rRNA. These probes have been applied using fluorescent *in-situ* hybridization (FISH) to visualize algicidal bacteria and follow changes in their abundance within mixed bacterial assemblages associated with cultures of *K brevis*. Additional probes for other bacterial groups, as well as the technique of denaturing gradient gel electrophoresis (DGGE), are being used to assess changes in the associated bacterial community during killing of *K. brevis* by algicidal bacteria. Our ultimate aim is to apply these approaches (FISH & DGGE) for the field-based detection and enumeration of algicidal bacteria and co-occurring microbes during the process of HAB development and decline.

In terms of the killing agent(s) produced by algicidal bacteria, these as yet unidentified compounds released into the medium are being investigated by chemical and physical manipulation of culture filtrates. In addition, we are attempting to elucidate the mechanism by which these algicides kill their target algal species. Finally, through the use of bioassay-guided fractionation followed by LC-MS and NMR analysis, algicidal agents will be identified and methods developed for their measurement in the field. Defining the role of algicidal bacteria in algal bloom termination will provide a basis for new generation management efforts necessary to control harmful algal blooms.

Graphic/Image/Figures

Selected Highlights. The following are several important findings from work conducted in 2002:

- It was determined that several algicidal bacteria isolated thus far exhibit a threshold concentration of ~10^6 cells ml^{-1}, below which they are unable to cause a net decline in levels of the target algal species, *K. brevis*.
- The apparent resistance of certain *K. brevis* clones to attack by algicidal bacteria was discovered to result from an inhibition of algicidal bacteria growth by antagonistic bacteria associated with these algal cultures. Remarkably, the susceptibility and resistance to attack by algicidal bacteria can be transferred between *K. brevis* clones by exchanging their respective ambient bacterial floras.
- The dissolved algicidal agent produced by one bacterium appears to be a heat-labile compound greater than 500 daltons, possibly proteinaceous.



Epifluorescence image of mixed bacterial assemblage stained with DAPI for total bacteria (blue) and a Cy-3 conjugated strain-specific probe for 41-DBG2 (pink), demonstrating the ability to distinguish algicidal bacteria from co-occurring bacteria. Photo by X. Mayali.

Publications/Reports:

Mayali, X. and Doucette, G.J. 2002. Microbial community interactions and population dynamics of an algicidal bacterium active against *Karenia brevis* (Dinophyceae). *Harmful Algae* 1:277-293.

Presentations:

Mayali, X. and G.J. Doucette, Algicidal bacteria: ecologically significant or artifacts of laboratory experiments? ASLO/Ocean Sciences Meeting, 2002. Honolulu, HI.

Mikulski, C.M., Doucette, G.J. Development and application of LSU rRNA probes for *Karenia brevis* in the Gulf of Mexico, USA. Molecular Probe Technology for the detection of Harmful Algae: a workshop on harmful algal bloom monitoring, 2002. Martin Ryan Institute (MRI), National University of Ireland, Galway.

Mikulski, C.M., G.J. Doucette, and S. Morton. Development and application of LSU rRNA probes for *Karenia brevis* in the Gulf of Mexico, USA. Xth International

Conference on Harmful Algae, 2002. St. Petersburg, FL