Cyanobacterial Populations in San Francisco Bay

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Introduction and Objectives

Anthropogenic inputs of metals to coastal environments have the potential to alter ecosystem productivity beginning with direct effects on phytoplankton. Metal inputs can also have the more subtle effect of changing phytoplankton species composition. Such a change then propagates through the ecosystem as grazers on particular phytoplankton groups are reduced or favored. A comprehensive review of these issues with specific reference to San Francisco Bay has recently been prepared (Tetra Tech, 1999).

Marine cyanobacteria, in general, are thought to be particularly sensitive to copper toxicity based on laboratory studies (Brand *et al*., 1986). In field studies high copper levels in small coastal bays have been correlated with the reduction in cyanobacteria of the genus *Synechococcus* (Moffett *et al*., 1997). In San Francisco Bay, cyanobacteria have been regarded as being "not commonly found" based on a review by Cloern (Cloern 1996) although this review was summarizing the phytoplankton populations of the spring bloom. Some data have suggested that cyanobacteria are present in San Francisco Bay (Murrell and Hollibaugh, 1998), however we have little quantitative information on cyanobacterial abundance and its spatial and temporal variations. This information would be particularly important if cyanobacteria were regarded as indicator species for metal-impacted environments.

Cyanobacteria use proteins called phycobiliproteins to harvest light for photosynthesis. All cyanobacteria use the biliproteins phycocyanin and allophycocyanin for light harvesting. Some cyanobacteria also contain the biliprotein phycoerythrin. Cyanobacterial isolates without this protein typically appear green, but cyanobacterial isolates with this protein are red to brown colored. When examined with blue light excitation on an epifluorescence microscope or flow cytometer, cyanobacteria with phycoerythrin will be detected because phycoerythrin absorbs this blue light and fluoresces. Cyanobacteria without phycoerythrin are not easily detectable under these conditions.

A flow cytometer uses a laser for fluorescence excitation and hydrodynamic focussing of a sample to rapidly examine the fluorescence properties of individual cells. Analyzing cell counts with a flow cytometer can be much faster than using a microscope. The instrument has been used extensively to analyze *Synechococcus* and other cyanobacterial populations in marine environments (Olson *et al*., 1990) . It has been used less often in analyzing coastal or estuarine systems. Large particles more common in natural coastal samples can clog the sampling system for example. We wanted to utilize the flow cytometer to see if we could detect cyanobacteria in San Francisco Bay and, if they were found, to analyze their spatial and temporal variation in the bay. Rapid analysis of cyanobacteria in samples might make their use as an indicator species more attractive for water quality monitoring.

Preliminary Results

We have examined the concentration of phycoerythrin-containing cyanobacteria in the San Francisco Bay ecosystem using flow cytometry analysis of samples from the February, April, and July Regional Monitoring Program cruises. Samples were fixed with glutaraldehyde and frozen for analysis back in the laboratory. Samples were thawed and filtered through a 100 µm screen to avoid large particles. A bead standard was added to all samples. The cells counts obtained by the flow cytometer were corrected to account for counting efficiency of the known bead standard.

In February and April 1999 the levels of phycoerythrin-containing cyanobacteria in the South Bay were at or near the detection level of the instrument while levels in the North Bay were easily detectable at around 1,000 to 6, 000 cells/ml. In July 1999, however, cell concentrations in the South Bay were up to 50,000 cells/ml, levels similar to those seen in Southern California coastal waters, while in the North Bay cell levels were similar to those seen in April.

A sample from the South Bay was not fixed with glutaraldehyde and, after shipment to the laboratory, subaliquots were added to sterile seawater enriched with nutrients. Some of these subaliquots were filtered through 1.2 µm filters to enrich for cyanobacteria. After enrichment under white light conditions, the samples were examined and plated on agar plates of the same media. Colonies of cyanobacteria were isolated and re-grown in the original media.

Enrichments from samples from the South Bay showed the presence of at least three different cyanobacterial types--two likely related to *Synechococcus* and one resembling *Synechocystis* in that in forms small rafts of cells. For the former, one *Synechococcus* type isolate is green (likely lacking phycoethryin) while one type is red (contains phycoerythrin).Thus although the South Bay shows high copper and other metal levels it seems to support the growth of a diverse cyanobacterial population. The biochemical adaptations of these cyanobacteria to the metal levels in their environment remain unknown.

Future Directions

Cyanobacteria are present in San Francisco Bay and interestingly in the South Bay where metal levels are relatively high. Their presence could be explained by:

1) Copper levels are not toxic because of the presence of other metals such as manganese that ameliorate the copper toxicity.
2) The cyanobacterial species found in the South Bay are less sensitive to metals than the species studied by Brand (Brand *et al*., 1986). If they are less sensitive, what adaptations do they possess that are absent from the strains studied by Brand? Are these adaptations characteristic of particular cyanobacterial “species”? If so, can one define cyanobacterial species that might be indicators for metal impacted environments?

These questions can possibly be answered using the isolates we have brought into culture by studying their sensitivity to copper at different copper/manganese ratios for example. We can also begin to compare what proteins they express at high copper levels compared to strains studied by Brand.

The flow cytometer approach using a 488 nm laser only readily analyzes cyanobacteria with phycoerythrin, but cyanobacteria without phycoerythrin were found in our enrichments. In the future we would also like to compare an epifluorescence microscope approach for counting cyanobacteria with the flow cytometer. In this way would understand what percentage of cyanobacteria are of the phycoerythrin- containing type and what percentage have pigments similar to the phycoerythrin lacking (green-colored) *Synechococcus* and *Synechocystis* type cultures.

References

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