**Environmentally Regulated Genes in M. aeruginosa, a Fresh Water**

Our long term goal is to understand how environmentally regulated genes are expressed in the toxin-producing cyanobacterium Microcystis aeruginosa UV027, a fresh water organism reported to be a health hazard to animals and humans. We are presently examining regulation of the synthesis of the cyclic heptapeptide toxin, microcystin-RR (MCYST-RR) in Microcystis aeruginosa UV027. This toxin is produced non-ribosomally. It inhibits PP1 and PP2A-type eukaryotic protein phosphatases resulting in impairment of signal transduction and cell-cycle regulation.

Using a lambda ZAP II M. aeruginosa UV027 genomic library and PCR, we cloned both
the peptide synthetase operon and the second operon, found upstream and transcribed in the opposite direction, which encodes a polyketide synthase and other genes for the synthesis of MCYST-RR. These putative operons are similar to those reported for MCYST-LR in M. aeruginosa K-139 and PCC 7806 except for one region in the peptide synthetase operon which encodes arginine in MCYST-RR rather than lysine.

We isolated the 726 bp promoters for the two operons and have ligated them to the reporter gene gfp replacing the mcyD gene and the reporter gene luxAB replacing mcyA. This construct has been cloned into the shuttle vector, pMaL-D7 (constructed by us from a plasmid isolated from M. aer.UV025), to enable us to examine regulation of toxin production by light. Suspected regulatory sequences in the promoter region in the plasmid have been mutated in order to examine their role in regulation.

We are also searching for regulatory genes, starting with phytochromes, found in other cyanobacteria, that might be involved in regulating toxin synthesis. A phytochrome gene from strain UV027 has been cloned, and its role, if any, in MCYST regulation will be analyzed.

Studies have been initiated with M. aeruginosa UTEX 2063 which does not synthesize MCYSTs. Our data indicates that it lacks the MCYST operons. This strain will be used for examining regulation by reconstituting genes into this natural “knock-out” strain.

Selected Publications:

- Miller, David, Arora, Shalini** and Raps, Shirley, 1998. Construction of a Shuttle Vector and Its Use in Gene Transfer in Microcystis. The VIth Cyanobacterial Workshop Exploiting the
Cyanobacterial Genome, Asilomar Conference Center, Pacific Grove, CA. B26 July 24-27.