**Ecological Distinctness of Bacterial Ecotypes Predicted from Sequence Data Analysis and Evolutionary Simulation: Evidence from Distribution, Gene Expression, and Population Dynamics.**

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Molecular studies of community composition and structure conducted on cyanobacterial mats inhabiting the effluent channel of Mushroom Spring, Yellowstone National Park, have previously shown evidence that native *Synechococcus* populations group into ecologically distinct species-like units, or ecotypes. However, concern over the low resolving power of the genetic markers used in these studies (16S rRNA and 16S-23S rRNA internal transcribed spacer region) has directed us to examine more rapidly evolving loci, such as those encoding proteins, for detection of ecotypes. We are currently studying *psaA* which encodes a core protein in Photosystem I. We PCR amplified, cloned, and sequenced *psaA* variants from Mushroom Spring 60°C, 63°C, and 65°C mat samples, or subsections through the upper photic layer obtained using a cryotome, and performed an evolutionary simulation analysis (Ecotype Simulation) that predicts and demarcates putative ecotype populations by assuming the existing ecotype diversity was generated through periodic selection, ecotype formation, and genetic drift. Phylogenetic clades that were demarcated as putative ecotypes each contained a dominant allelic variant, as well as rarer singleton and doubleton variants; each variant was marked with a single nucleotide polymorphism unique to that putative ecotype. Denaturing gradient gel electrophoresis (DGGE) analyses of PCR-amplified mat *psaA* genes revealed discrete bands with sequences corresponding to dominant allelic variants of different putative ecotypes. These bands were distributed uniquely in samples collected along flow and vertical gradients, where temperature and light are known to vary. DGGE and cloning analyses also suggest that putative ecotypes increased or decreased in abundance in response to environmental perturbation (e.g. 90% light reduction). Reverse transcriptase PCR was used to show different temporal patterns of expression through the dark-to-light transition period. Dominant DGGE bands corresponded to the dominant allelic variants of putative ecotypes indicating ecotype-specific gene expression patterns. Results suggest that putative ecotypes predicted from Ecotype Simulation are ecologically distinct. We are currently exploring 454 pyrosequencing

methods to provide more robust analyses of all variants within *psaA* defined putative ecotypes, their distribution along gradients, responses to environmental change, and gene expression patterns.