

Physiology and Molecular Ecology of *Synechococcus* WH8102

DOE Genomes To Life project
CARBON SEQUESTRATION IN *SYNECHOCOCCUS* SP.:
FROM MOLECULAR MACHINES TO HIERARCHICAL MODELING

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Objective: The object of this paper is to provide a summary of the literature on the physiology and molecular ecology of bacteria and in particular, *Synechococcus* WH8102 and the marine *Synechococcus/Prochlorococcus* group to aid in formulating use cases and requirement documents for our sub-project on “Data-driven hierarchical models. This document is not intended to be a comprehensive review. Excellent current detailed reviews are available on the physiology and molecular ecology of the *Synechococcus/Prochlorococcus* group (Scanlan and West 2002, Partensky 1999). This paper compliments our other papers on the “Current state of the data in the cyanobacterium *Synechococcus*,” “Population Dynamics, Marine Community Ecology, and Oceanography of Marine Microbes,” and “Simulation Techniques and Software Engineering Patterns.”

“Microbial ecology offers a logical framework for the analysis of feedbacks and interconnections between the physical, chemical and biological features of an environment. The extent to which the rules and/or theories of macroecology apply to microbial ecology is an intriguing open question. (Newman and Banfield 2002).”

Detailed understanding of microbial ecology will require

- Determining the constituents of biological communities
- Determining the ways in which the members interact
- The nature of system inputs (sun light, nutrients)
- Outputs (CO₂, solutes)
- Ways in which organisms respond to and change the environment.

In monitoring ecological networks we need not only to understand the interactions, but also to have a metric for monitoring the overall state of the system. The application of molecular techniques to the study of microbial ecology has created a new window on microbial population dynamics and community structure. The goals of the field of molecular ecology are to (Scanlan and West 2002);

1. Better understand the diversity of microbial populations.
2. Define how populations and even single cells are effected spatially and temporally by biological and physiological factors.
3. Identify niches occupied by specific populations.

The Diversity of Microbial Populations*Genetic Diversity*

Bacteriologists have not yet reached a consensus for defining the fundamental unit of biological diversity, the species. In general bacterial diversity is organized into discrete

phenotypic and genetic clusters, which are separated by large phenotypic and genetic gaps, and these clusters are recognized as “species”. Because diversity of bacteria and the past reliance on a few phenotypic characters, bacteria are often classified taxonomically with less importance placed on their evolutionary relationship than in eukaryotic systematics. Additionally, the different mechanisms of recombination and rates of recombination lead to a much broader sampling of whom DNA can be exchanged with. For instance in the cases of *Helicobacter pylori* and *Neisseria gonorrhoeae* recombination occurs at least one order of magnitude more frequently than mutation (Suerbaum et al. 1998, Feil and Spratt 2001). There is often considerable variation in genomic content within a named species (ex. Alm et al. 1999, Perna et al. 2001). Since we know that gene transfer can occur between species as well as between bacterial division, bacteria species are not irreversibly separated.

Bacterial diversity can be defined as the number of prokaryotic species and their relative abundance in a community, or as the amount and distribution of information in a community (Farquar et al 2000). Because a typical named bacterial species contains many ecotypes, a named bacterial species may be more like a genus than a species (Cohan 2002). Below are some of the flavors of species definition used for bacteria:

Phylogenetic definition: “monophyletic and genomically coherent cluster of individuals that show a high degree of overall similarity in many independent characteristics, and is diagnosable by a discriminative phenotypic property (Tyrell 1999).

Sequence definition: An assemblage of strains sharing 70% or more DNA homology (Berman-Frank 2001).

Ecological concept: The species consists of organisms occupying the same niche. Thus the species and niche concepts are linked.

Studies of genetic diversity in *Synechococcus* are just beginning. Relatively high genetic diversity exists within the marine *Synechococcus* group based on restriction fragment polymorphism analysis (Douglas and Carr 1988) and sequencing of the 16s rRNA gene and ITS region (Scanlan and West 2002). It appears that genetically defined *Synechococcus* clades that are physiologically distinct can exist at the same site (Rocap 2002). It is not clear whether this is the result of sympatric “speciation” or the result of recent mixing of previously stratified waters. (Scanlan and West 2002). Martin (2002) has just reviewed the statistical techniques used to measure bacterial diversity.

Recombination and Horizontal Gene Transfer

No studies have directly assessed mutation, recombination or horizontal gene transfer rates in the *Synechococcus/Prochlorococcus* group. It should be possible to make rough estimates of mutation rates and variation in mutation rates among clades using the existing sequence data. However, we have every reason to suspect that recombination and HGT may play as large or a larger role than mutation in the evolution of (Feil and Spratt 2001, Gogarten et al. 2002). Cyanobacteria appear to be particularly susceptible to HGT (Ochman et al. 2000) and *Synechococcus* and *Prochlorococcus* are not an exception (Hess et al. 2001). HGT between within groups of closely related bacteria will slow gene transfer similar gene conversion processes in eukaryotes. However, recombination and HGT across species-level and above barriers could promote environmental adaptation and the evolution of new traits (i.e. antibiotic resistance).

Phenotypic Diversity

Fundamental understanding of the molecular biology and biochemistry of bacteria has led to the development of molecular probes (Figures 1 and 2) that can be used as proxies for a physiological state or response. These molecular tools are being developed to address several major areas

- The extent of genetic diversity within and between bacterial populations and species.
- The relationship of this diversity to physiology and function.
- Estimating growth rates and losses to predators and viruses.
- The role that environmental factors (such as UV damage).

In many ways Figures 1 and 2 contain information we would like to build into the modeling process to understand how genotypic differences and various environmental parameters effect fitness and other related phenotypes. This would effectively enable us to generate hypotheses using laboratory data produced in the GTL programs that would be relevant and testable in natural population studies. Thus approaches for addressing the relationship between levels of transcripts and proteins and the physiological status of the organism are critical for interpreting ecological studies and in the design of probes. In turn, the application of additional probes will be pivotal in addressing what external parameters are controlling *Synechococcus* growth rates. Examples of applications of molecular probes to the marine cyanobacteria include the use of antibodies to detect phosphate stress in natural populations (Scanlan et al. 1997), *ntcA* gene expression levels as an indicator of nitrogen status (Lindell and Post 2001), and the use of RT-PCR to assess the role of the cell division initiator *ftsZ* in the synchronized cell division of *Prochlorococcus* populations (Holtzendorff 2002).

Limited work has been done on correlating genetic diversity with specific physiological adaptations and specific environmental niches in marine *Synechococcus*. Thus far the only the non-flagellar swimming phenotype shows congruence with phylogenetic trees. Other known physiological differences between marine *Synechococcus* strains include response to nitrogen depletion, preference for urea or nitrate as a nitrogen source, and cell cycle behavior. However these traits have not been systematically investigated in relation to across *Synechococcus* taxa. Ratios of phycourobilin (PUB to phycoerythrobilin (PEB) differ among strains. However, some strains are capable of chromatic adaptation (Palenik 2001). The light harvesting complexes represent one possible target for understanding the relationship between variation in genes between populations and ecological niche differentiation. For a summary diagram of physiological traits overlaid on a phylogenetic tree see Scanlan and West 2002.

Most marine *Synechococcus* strains that have been isolated are capable of utilizing nitrate as a N source for growth involving a permease. However mechanistically this process is not similar to most fresh water cyanobacteria that use an ABC transporter system (Scanlan and West 2002). This is one more example that there is variation in genome content for fundamental processes between the fresh water and marine cyanobacteria. The carbon sequestration process differs within the *Synechococcus/Prochlorococcus* group and between this group and the rest of cyanobacteria as a result of HGT and gene loss (Hess et al. 2001, Table 1). Further elucidation of nutrient physiological process through experimentation and genome sequence analysis is critical for assessing growth rates, since they are likely to represent control points for bacterial photosynthesis. Hopefully the forthcoming paper on WH8102 highlights relevant data that can be used in the modeling process.

Table 1 - Limited mechanisms for CO₂ uptake and HCO₃ transport in *Synechococcus* WH8102 and *Prochlorococcus*.

Process	Genes	Syn. sp. WH8102	Pro. marinus MED4	Pro. marinus MIT9313	Synechocystis sp. PCC6803	Nostoc sp. PC7002
Active CO ₂ uptake	ndhD3/ndhF3/ CupA inducible	-	-	-	x	x
Active CO ₂ uptake	ndhD4/ndhF4/ CupB constitutive	x	-	-	x	x
HCO ₃ transport	cmpABCD ABC transporter	-	-	-	x	x
HCO ₃ transport	sbtA1/nptJ Na ⁺ dependent	-	-	-	x	x
HCO ₃ transport	ictB	x	-	x	x	x
Carbonic anhydrase	icfA	x	-	-	x	x

Trophic Interactions Controlling Bacterial Diversity

The relative higher number of planktonic species relative to the limited amount of resources in seemingly non-structure aquatic environments led to the “Paradox of the plankton”. This question continues to interest theoreticians and experimentalists (Huisman and Weissing 1999, Czarán et al. 2002). From a biogeochemical perspective, bottom-up control suggests that high microbial diversity is needed for processing all the different types of substrate molecules (resources) produced in the systems. Top-down control mechanisms focus on the role of predation by protozoa or parasitism such as by host specific viruses. Thus bottom-up and top-down models are fundamentally different concepts for coupling diversity and biogeochemical cycles. In addition, simulations of resource competition models show that oscillations and chaotic fluctuations in species abundance can support the coexistence of many species on limited resources (Huisman and Weissing 1999)

The current dogma is that top-down control is more likely in aquatic and other less stratified environments and that bottom-up control probably more important in soils and sediments where there is considerable spatial heterogeneity. “Hence, the control the of diversity in such models becomes a hierarchical systems where the total amount of limiting resource determines the total amount of biomass that can be produced. Size-selective grazing determines how the biomass is distributed into functional groups (communities), and the host specificity of viruses determines how the functional groups are divided into species (Torsvik et al. 2002).” However, in marine cyanobacteria both predators and bacterial viruses have recently been shown to play an important role in trophic interactions (Christaki et al. 2002, Mann 2003, McDaniel et al. 2002). Grazing by nanoflagellates has a diel component with consumption peaking in the night (Christaki et al. 2002). The lytic phase of cyanobacterial phage induction has been shown to have a temporal (seasonal component) that tracks the rise of *Synechococcus* population numbers (Figure 3).

Recent work has addressed the question of bacterial diversity within populations of the same species. The differential production of bacterial toxins to ward off predators or to more

effectively compete against other bacteria (Czaran et al. 2002) is another mechanism that can promote diversity. These mechanisms can lead to a version of the “rock-paper-scissors” game played out in a spatial context (Czaran et al. 2002, Kerr et al. 2002). These papers reflect an increase appreciation of the role of the spatial scale of ecological processes and the role of non-hierarchical competitive processes (Kerr et al. 2002). Although, in laboratory studies of evolving *E. coli* populations transitive relationships were dominant (de Vissar and Lenski 2003), the production of toxins or mechanisms of viral resistance could lead to non-transitive relationships. The role of toxins in an aquatic environment is less clear due to rapid diffusion of the toxin. However, the water column of the open sea typically contains about 1,000,000 bacteria per milliliter as determined by direct counts and as discussed below there are several spectacular examples where bacteria can sense gradients in aquatic environments.

How are cells and populations affected spatially and temporally by biological and chemical factors?

In the discussion above on the “rock-paper-scissors” game simulations involving spatial aspects of the environment (a Petri dish) were important for the outcome (Kerr et al. 2002) “From the perspective of a bacterium 2 μm in length, the surface of a matchbook represents an area roughly the size of Rhode Island, or the Grand Duchy of Luxembourg. To the same bacterium, an Italian espresso compares in volume to Lake Baikal, the largest freshwater lake on Earth, while a hot air balloon takes on the proportions of Earth itself (Leveau and Lindow 2002)” This contrast in world view should make obvious the fact that we cannot afford to neglect the element of scale as we try to understand the behavior of bacteria and other microbes. There is a limited understanding of how microbes perceive their immediate environment, or changes to it, on a scale that is most relevant to them.

A large fraction of the planktonic bacteria (20 to 70%) are motile suggesting that adaptations for chemotaxis in a heterogeneous environment (Fenchel 2002) are important. Although, we may not expect concentration gradients to develop in the ocean, modeling shows that even in rough water, motile bacteria can still take advantage of point sources by using chemosensory behavior (Fenchel 2002). A dramatic example of bacterial taxis to a small point source is shown in the aggregation of bacteria around a lysed ciliate (Fenchel 2002). As mentioned above there is polymorphism for swimming within the marine *Synechococcus* group possibly reflecting selection for movement to take advantage of point source nutrients.

The use of molecular probes is likely to have a significant impact on understanding the role of temporal heterogeneity. A common assumption in aquatic environments is that seasonal changes in the climate may play a relatively minor role in oceanic populations because the temperature fluctuations are more moderate. However, a mixing of the water column may actually lead to an increase in diversity, since major and frequent disturbances, such as those seen in arable pasture soil decrease diversity (Torsvik et al. 2002).

Summary

We must recognize that to date, it has not been possible to take apart a natural system, analyze it and model it at the cellular level or the ecological level (Newman and Banfield 2002). Therefore we need to focus data that allows us to transverse the hierarchy. Data generated in the field of molecular ecology will be central to understanding how genotypic differences and various environmental parameters affect fitness and other related phenotypes. Because the field offers approaches for addressing the relationship between levels of transcripts and proteins and the physiological status of the organism, this would effectively enable us to build models using laboratory data produced in the GTL programs that would be relevant and testable in natural population studies.

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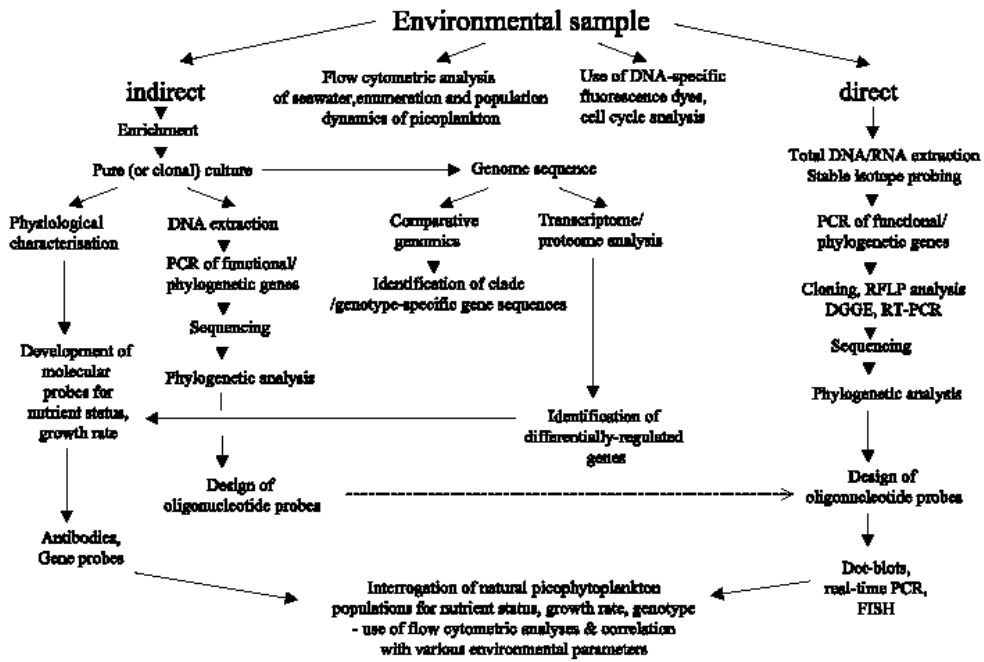


Fig. 1. Strategies for the development of molecular probes for use in interrogating natural picophytoplankton populations, a coupled molecular and ecological analysis of cultures.

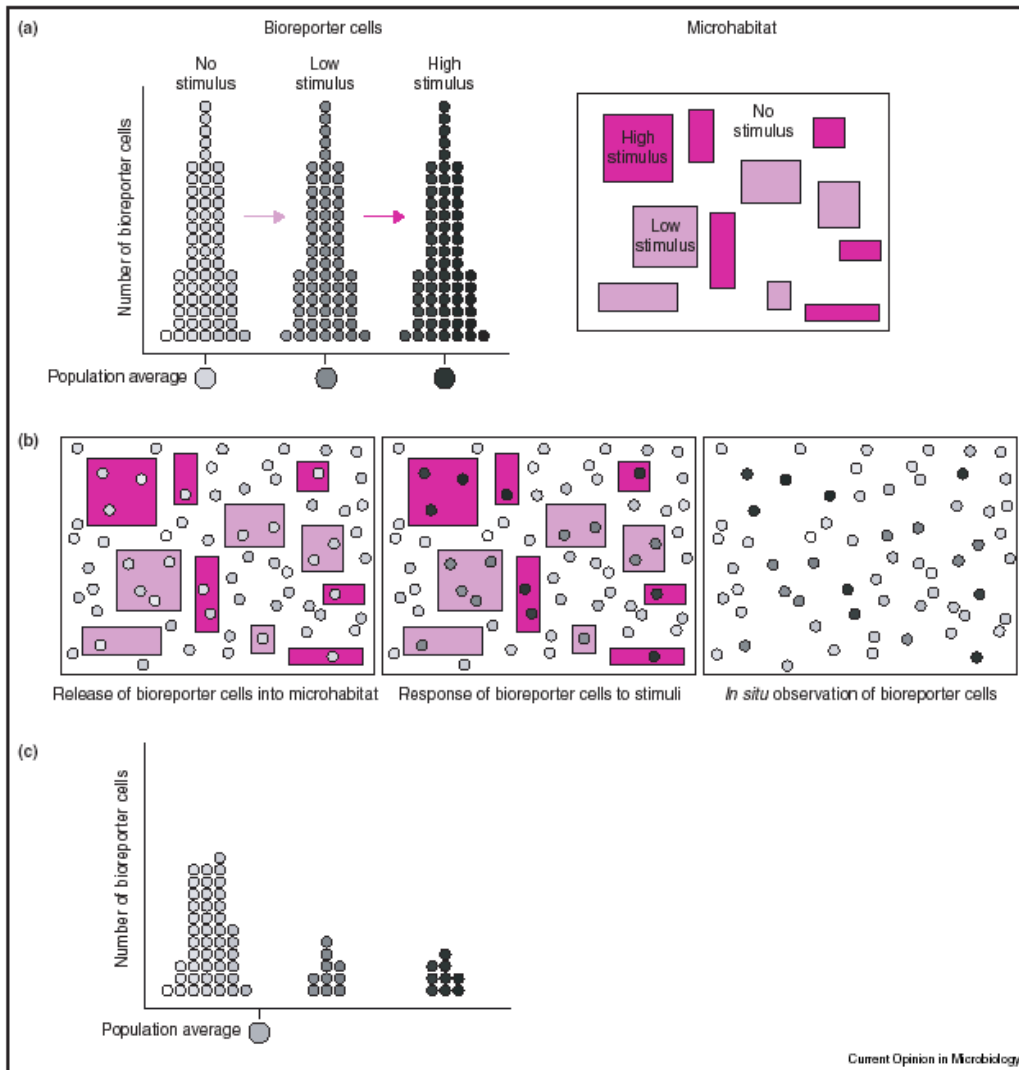


Figure 2 (from Leveau and Lindow 2002) “The utility of bioreporters for microbial exploration of a microhabitat. **(a)** We start with a population of bioreporter cells for which it has been established in culture that they exhibit elevated reporter gene activity in response to a known metabolical, physical, chemical or biological stimulus. For many bioreporters, reporter gene activity is proportional to the magnitude of the stimulus (no, low or high exposure, in this example). Bioreporter activity is expressed as the abundance of reporter protein averaged over the entire population, or on a cell-to-cell basis (for example, in a histogram). The bioreporter can be used to search a microhabitat for the stimulus to which it is responsive. As symbolized by the coloring, there may be variation within the habitat in terms of stimulus magnitude, but this variation is essentially unknown. **(b)** Following release of the bioreporter cells into the habitat, and after a defined period of time or at different time intervals, bioreporter cells can be examined for reporter protein directly in their natural surroundings, that is, if both the reporter protein and the habitat allow. Note that the coloring in the final panel has been removed to indicate that variation in stimulus exposure is not an observable feature, but instead the kind of information that we try to infer from the bioreporters’ behavior. **(c)** Alternatively, or in addition, cells may be recovered from the habitat and analyzed for reporter activity. Depending on the reporter gene that is being used, this activity is expressed as an average over all the bioreporters recovered from the habitat, or as a distribution of reporter activity among individual bioreporter cells...”

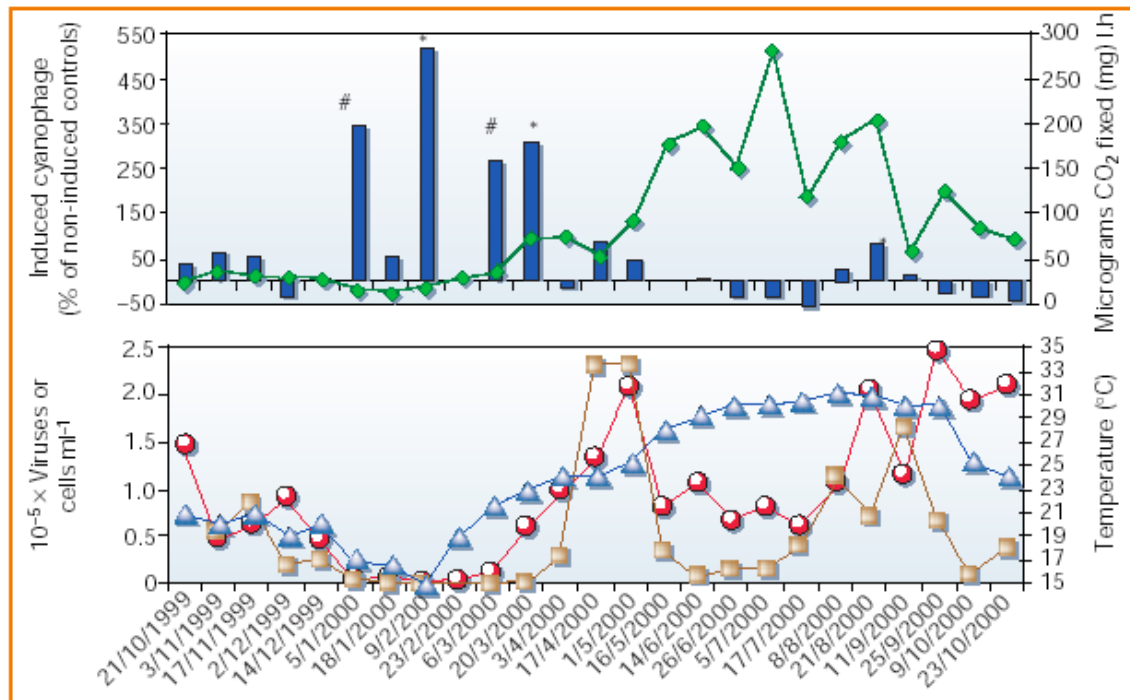


Figure 3 (From McDaniel et al. 2002) “Seasonal induction of prophage in natural *Synechococcus* populations. Top, variation in cyanophage induction (blue bars) compared with primary productivity of *Synechococcus* (green line) for the year ending in October 2000. l.h, litre hour. Bottom, corresponding values for *Synechococcus* abundance (red), cyanophage counts (brown) and temperature (blue) over the same period. Prophage- induction results are expressed as a percentage change in treatment compared with control (asterisks and hash symbols indicate statistical significance at the 95% and 90% confidence interval, respectively; the significance of each induction event was determined by comparison of treatment and control levels of cyanophage by paired t-test in three pseudoreplicates of each sample).”

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