Motility in the marine environment: an adaptive response to patchy nutrient distributions?

Dana E. Hunt
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Abstract:

While the ocean is generally assumed to be well-mixed, from the vantage point of bacteria the ocean may in fact be a mosaic of evolving microenvironments. The ability of bacteria to sense and respond to these nutrient gradients may influence bacterial productivity and confer selective advantage to motile organisms. The phycosphere surrounding photosynthesizing phytoplankton is thought to contain enhanced levels of carbon and nitrogen containing substrates that can be used by heterotrophic bacteria for growth. This study establishes that cyanobacterial form strain-specific associations with heterotrophs over many years of co-culture, suggesting interactions that may be mirrored in the natural environment. Media in which cyanobacteria had been cultured resulted in positive taxis in six of the seven environmental isolates tested, suggesting that even the potentially low levels of nutrients released by cyanobacteria can induce taxis. Additionally, seawater isolates display a tactic response to a variety of monomers that make up cyanobacterial exopolysaccharide and the amino acid leucine but not serine or glutamine. The strongest tactic response was evident in response to glucose, with severe retardation of spreading on soft agar plates. Further study of the chemotactic response to glucose suggests that marine bacteria, reflecting their oligotrophic environment, may be able to sense glucose concentrations down to 10^{-12} M, several orders of magnitude lower than that detectable by *E. coli*. The fact that large numbers of isolatable marine bacteria are motile and chemotactic suggests that clustering around nutrient point sources (including phytoplankton) play a large role in bacterial ecology and productivity

Introduction:

What is the significance of motility in the marine environment? A large fraction of marine isolates have been found to be motile, but direct observation of seawater suggests a small fraction of total cell populations are motile. Flagellar motility is energetically expensive for organisms in low nutrient environments requiring greater than 10% of available resources, thus it seems logical that bacterial should derive a specific energetic benefit from this process. Theoretically, motility could confer two main advantages enhancing flux by reducing the thickness of the diffusive boundary layer or moving organisms towards sources of higher nutrient levels (Purcell 1977). Modeling has suggested that the most likely explanation is that this motility is used to respond to transient chemoeffectors.

Photosynthetic phytoplankton are thought to release significant amounts of nutrients into the marine environment through exudation, representing a potentially significant nutrient point source. Bell and Mitchell (1972) first suggested the importance of the "phycosphere", the region surrounding a photosynthesizing alga analogous to the rhizosphere of plants containing high levels of photoassimilated carbon. The compounds released by algae are thought to consist largely of polysaccharides (up to 80%) and may also contain other labile compounds including amino acids and vitamins (Myklestad 1995). Interactions between phytoplankton-heterotrophs may be highly specific with algal products serving as both bacterial attractants and repellents (Bell et al. 1972); and specific bacterial groups co-culturing with algae (Schäfer et al. 2002). Modeling suggests that up to 20% of the chemotactic bacteria could be clustered around phytoplankton cells (Bowen et al. 1993); and chemotactic tracking of motile algal cells has been observed ex-situ (Barbara et al. 2003). Yet, a mesocosm experiment yielded no evidence of bacterial clustering around algae (Müller-Niklas et al. 1996). Thus while no direct evidence has established the importance of bacterial clustering in response to cyanobacterial exudates evidence suggests this would be an adaptive technique in oligotrophic aquatic ecosystems.

The components released by algae have been observed to vary between species and exudation is thought to increase during nitrogen and phosphorous limitation (Myklestad 1995). Previous study of bacterial isolates revealed that bacteria co-occurring with *Microcystis aeruginosa* were better more likely to exhibit positive taxis towards *Microcystis* cells than isolates from a site not colonized by these cyanobacteria (Casamatta et al. 2000), suggesting that specific associations form between heterotrophic and photosynthetic bacteria. The association between an *Anabaena* sp. and its heterocyst epibiont has been previously studied in the course (Anderson, 2003), although chemotaxis toward *Anabaena* cells was not observed.

The compounds released by algal cells have been extensively characterized; there has been to my knowledge no comparable study of the production of labile compounds by marine cyanobacteria, although the composition of exopolysaccharide for many organisms is known. A large fraction of marine bacterial isolates are motile, the only logical explanation for this energetically expensive activity is that bacteria derive a substantial benefit from being able to respond to spatially variable nutrient conditions. This study will attempt to quantify the importance of this chemotactic response and perhaps better inform our models of productivity i marine ecosystems.

Materials and Methods:

Cyanobacteria:

Cyanobacterial cultures were obtained from John Waterbury (Woods Hole Oceanographic Institution) : axenic *Crocosphaera* WH8501 and non-axenic *Crocosphaera* strains E12 and 1051. No attempt has been made to purify the non-axenic strains from their co-existing heterotrophic organisms although they have been in culture for many years. Cyanobacteria were grown in a Sargasso-seawater based medium containing no combined nitrogen so that cells would grow diazotrophically with iron as the limiting growth factor. Cyanobacterial cultures were incubated on a 14 hour light 10 hour dark cycle at 28°C.

Isolates were obtained from non-axenic cultures using direct plating on a glucose minimal media (per liter: Na_2HPO_4 4.8g; KH_2PO_4 4.4g; NH_4Cl 1g; $MgSO_4$* $7H_2O$ 0.5g; NaCl 20g; post autoclaving 2g of filter-sterilized glucose, 1ml of trace metals mix and 1 ml of vitamins were added (Microbial Diversity 2004 Handbook)). Individual colonies appeared after one day on plates from strain E12 and after two days for strain 1051. Isolates were named after the culture from which they were obtained i.e. E12-1, E12-2, etc. Strains were streaked several times for isolation, the 16S gene was then PCR amplified and partially sequenced in order to obtain an identification of the organism. PCR was done using primers 8F and 1492R with 519R used as the sequencing primer.

Seawater isolates:

Heterotrophic bacterial isolates were also obtained from the coastal ocean at Woods Hole, MA from the end of the MBL pier at high tide using two different methods. The first set of isolates was obtained by direct plating of seawater on SWC media (per liter: 750mls seawater; 250mls DI water; 5g tryptone; 3g yeast extract; 3ml glycerol). The second set of isolates attempted a selection for bacteria that are motile in the environment and potentially chemotactic towards the exudate produced by *Crocosphaera*. Culture media from strain WH8501 was placed in a sterile capillary tube and incubated in contact with seawater. After 30minutes- the contents of the capillary tube were plated onto a SWC plate and colonies were picked and isolated after several days of growth. While isolates obtained using this method are not necessarily chemotactic towards exudates, they represent bacteria that are theoretically motile in the marine environment. Strains were streaked several times for isolation and the 16S gene was amplified and partially sequenced in order to identify isolates (as previously).

Chemotactic assays:

Swarm plates:

Crude chemotactic assays were conducted using swarm plate assays for the motile isolates. Swarm plates were made with 0.3% sloppy agar using a basal seawater medium (per liter 750mls of seawater, 250mls DI, 0.25g NH_4Cl, 0.2g KH_2PO_4, 1ml 1000x trace metals mix, 1ml vitamins mix) supplemented with 100µM of the compounds of chemotactic interest: D glucose, galactose, leucine, serine, rhamnose, D – arabinose, D-glucouronic acid, D+ cellobiose (United States Chemical Corp), L-glutamine, or _- L Arabinose (all chemicals from Sigma except as noted). Swarm plates were inoculated by directly injecting 1µl of a culture grown exponentially in SWC or glucose minimal media directly into the soft agar. The diameter of the ring of motile cells was measured after 24-48 hours of growth and compared to cells inoculated in non-supplemented plates. The plates were also scored to observe if cells growth was enhanced on the substrate compared to the basal seawater plates.

Chemotaxis assay:

Motile cells were grown to late log phase in SWC media with shaking at 30C. A cell aliquot was pelleted at 5000 rpm for 5 minutes. The cell pellet was washed in motility medium (10mM potassium phosphate, 100um Na EDTA supplemented with 25g/L NaCl for marine organisms, pH 7) three times with subsequent pelleting for 5 minutes at 2,500rpm. Cell cultures were visibly checked for motility using 40x phase contrast, then diluted in motility medium 100-1000x as appropriate for chemotaxis assays. The chemoeffector of interest or a blank of motility medium was placed in a sterile glass capillary tube and incubated in the cell culture solution for 30 minutes. The contents of the capillary tube was either directly plated or first diluted in sterile motility buffer and plated on SWC media. Colonies were enumerated after 24 hours of incubation at 30°C.

Results and Discussion:

Heterotrophic isolates obtained from non-axenic cyanobacterial cultures:

A set of isolates from each of the non-axenic *Crocosphaera* strains was obtained on glucose minimal media. Partial 16S sequences were obtained for a number of these organisms. Interestingly, the nine isolates from strain E12 were most similar via BLAST to *Pseudoalteromonas* sp. Md 213, with a minimum of 99% identity. Of the isolates analyzed from strain 1051 nine of the ten were most closely related to *Halomonas* sp. using BLAST, with the remaining isolate most closely related to a *Pseudomonas* sp. The specificity of these associations with the different cyanobacterial cultures suggests that selective pressure is exerted on the heterotrophic bacteria co-existing over many years of culturing perhaps by the culture media and the cyanobacterial products. In order to confirm that these bacterial "contaminants" of the cyanobacterial cultures are making use of cyanobacterial products one could attempt to culture them using spent cyanobacterial culture medium. Considering that the cyanobacteria were grown without a combined nitrogen source, unless these organisms also fix nitrogen it appears likely that they are using cyanobacteria as at least a nitrogen source.

Seawater isolates:

Isolates were obtained from coastal seawater off the MBL pier using both direct plating and plating of the contents of capillaries containing spent cyanobacterial medium. Table 1 lists the closest BLAST hit to identify the obtained isolates. Cultures obtained from the capillary tubes represent organisms that are both fast growing on complex medium and theoretically actively motile in the marine environment. Although all of the observed cultures were motile grown in complex medium, it appears that the vibrios are motile in the ocean judging from their over-representation in the "capillary supernatant" isolates. The species obtained as isolates are the common microbial weeds generally obtained using culture-based methods involving rich media and short incubation times: *Vibrio*, *Pseudoalteromonas*, and *Alteromonas* sp.

Table 1: Characterization of direct seawater isolates including information about the closest BLAST hit and percent identity of the unedited sequence read with that identification. Exponential phase cultures were also scored for motility and cell morphology.

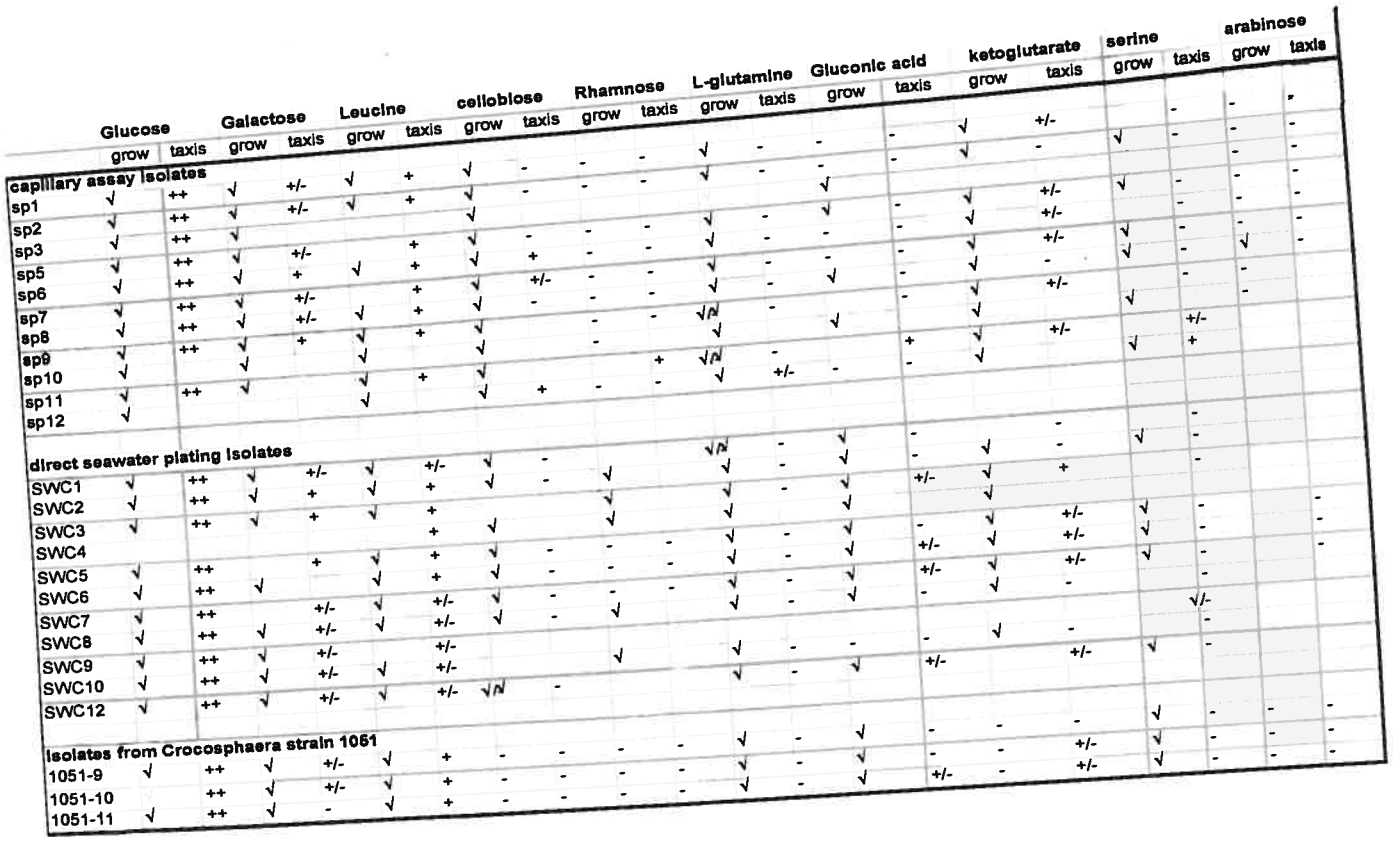
	Closest BLAST hit	Percent identity	Motile	Morphology/ comments
Capillary supernatant isolates				
SWC1	Vibrio sp. MD 208	98%	yes	short rods
SWC2	Vibrio sp. MD 208	98%	yes	short rods
SWC3	Photobacterium damselae subsp. Piscicida	99%	slow	short rods
SWC4	V.nereis (ATCC 25917T)	99%	yes	smaller, short rods
SWC5	Vibrio pomeroi	98%	yes	long chains of rods-snake-like swimming
SWC6	Pseudoalteromonas haloplanktis subsp. tetraodonis	99%	maybe?	longer rods
SWC7	Vibrio splendidus strain 636	100%		
SWC8	Vibrio splendidus	99%	darting	short rods and long filaments
SWC9	Vibrio splendidus	98%	fast	tiny, darting motility
SWC10	V.natriegens (ATCC 14048T)	97%	fast	tiny, run-reverse
SWC11	Vibrio alginolyticus strain NRL-SS41	98%	slow	small rods
Direct Seawater Plating Isolates				
Sp1	Pseudoalteromonas citrea	99%	yes	rods
Sp2	Vibrio tasmanius	100%	yes	short rods
Sp3	Pseudoalteromonas sp. SKA20	98%	fast	short rods
Sp4	Vibrio splendidus strain 636	100%		
Sp5	Pseudoalteromonas sp. TE1-1 16S	99%	slow	rods
Sp7	Pseudoalteromonas sp. SM9913	99%	wiggly	rods
Sp8	Pseudoalteromonas sp. SKA32	99%	yes	rods
Sp9	Pseudoalteromonas sp. ANG.ro2	99%		
Sp10	Vibrio tasmaniensis	100%	slow	rods
Sp11	Alteromonas sp	99%		
Sp12	Pseudoalteromonas sp. TE1-1	99%	yes	rods

Swarm Plate Assay:

The swarm plate assay was used as a rapid method of assessing the chemotactic response of organisms to various potential growth substrates compared to growth on seawater –based medium supplemented with minerals and vitamins. Potential chemoeffectors were chosen based on their presence as monomers making up the exopolysaccharide of many cyanobacteria (glucose, galactose, rhamnose, arabinose, glucuronic acid) (Nicolaus et al. 1999; Giroldo et al. 2003). Chemotaxis was also tested for several amino acids (serine,

glutamine, and leucine). Chemotaxis swarm plates supplemented with 100μM of the component of interest were scored based on the diameter of the spreading circle of bacteria through the medium compared to the blank. A chemotactic response was observed when spreading of the bacteria was significantly decreased in the presence of a chemoattractant. For some substrates- i.e. glucose there was almost no migration through the agar plates for any of the isolates, suggesting that glucose is a strong chemoeffector. For other substrates, notably serine and glutamate the spreading of the cultures appeared to be enhanced with a diameter up to 175% of the control value, suggesting that there may be an interesting response to these substrates; however, leucine, the other amino acid tested, appeared to generate a positive taxis response in many isolates. It may be possible that this represents a negative taxis as it is been noted that natural bacterial populations are negatively tactic towards serine {Barbara, 2003 #173}. This observation was further investigated using a capillary assay with no observed negative or positive taxis in response to serine for two separate isolates over a range of 10^{-3} to 10^{-10} M serine. Thus perhaps the presence of serine served to activate motility in these organisms as it did not appear to be a good substrate for growth, although this hypothesis warrants further testing. Other compounds such as cellobiose that in general made good growth substrates did not appear to induce a chemotactic response, which is interesting as cellobiose is a dimer of glucose. Galactose which is chemically identical to glucose but has a different arrangement of ring substituents appeared to induce only weak chemotactic responses. Several of these observations warrant further testing using a more sophisticated method such as the capillary assay.

Table 2: Swarm Plate assay results: after 24-48 hours the plates were scored based on the extent of growth on various added substrates (100μM) compared to growth on the control plate (seawater and agar). The check mark reflects definite growth, a check minus was difficult to discern and a minus reflected no observable growth. The chemotaxis was scored based on the diameter of the motile bacteria- a culture less than half the diameter of the control was scored as +; +/- reflects a diameter between half and 75% of the diameter of the control; while a – reflected a growth diameter greater than 75% of the control.



	Glucose		Galactose		Leucine		cellobiose		Rhamnose		L-glutamine		Gluconic acid		ketoglutarate		serine		arabinose	
	grow	taxis	grow	taxis	grow	taxis	grow	taxis	grow	taxis	grow	taxis	grow	taxis	grow	taxis	grow	taxis	grow	taxis
capillary assay isolates																				
sp1	√	++	√	+/-	√	+	√	-	-	-	√	-	-	-	√	+/-		-	-	-
sp2	√	++	√	+/-	√	+	√	-	-	-	√	-	√	-	√	-	√	-	-	-
sp3	√	++	√				√				√	-	√	-	√	+/-	√	-	-	-
sp5	√	++	√	+/-		+	√	-	-	-	√	-	-	-	√	+/-		-	-	-
sp6	√	++	√	+	√	+	√	+	-	-	√	-	-	-	√	-	√	-	-	-
sp7	√	++	√	+/-		+	√	+/-	-	-	√	-	√	-	√	+/-	√	-	√	-
sp8	√	++	√	+/-	√	+	√	-	-	-	√/√	-		-	√	-		-	-	-
sp9	√	++	√	+	√	+	√		-	-	√	-	√	-	√	+/-	√	-	-	-
sp10	√		√		√		√			+	√/√	-		-	√			+/-		
sp11	√	++	√		√	+	√	+	-	-	√	+/-	-	-	√		√	+		
sp12	√				√		√													
direct seawater plating isolates																				
SWC1	√	++	√	+/-	√	+/-	√	-	√		√	-	√	-	-	-	√	-		
SWC2	√	++	√	+	√	+	√	-	√		√	-	√	-	-	√		-		
SWC3	√	++	√	+	√	+			√		√	-	√	+/-	√	+				
SWC4						+	√		√		√	-	√	-	√					
SWC5	√	++		+	√	+	√	-	-	-	√	-	√	+/-	√	+/-	√	-		-
SWC6	√	++	√		√	+	√	-	-	-	√	-	√	+/-	√	+/-	√	-		-
SWC7	√	++		+/-	√	+/-	√	-	-	-	√	-	√	+/-	√	+/-	√	-		-
SWC8	√	++	√	+/-	√	+/-	√	-	√		√	-	√	-	√	-		-		
SWC9	√	++	√	+/-		+/-												√/-		
SWC10	√	++	√	+/-	√	+/-			√		√	-	-	-	√	-		-		
SWC12	√	++	√	+/-	√	+/-	√/√	-			√	-	√	+/-		+/-	√	-		
Isolates from Crocosphaera strain 1051																				
1051-9	√	++	√	+/-	√	+	-	-	-	-	√	-	√	-	-	-	√	-	-	-
1051-10		++	√	+/-	√	+	-	-	-	-	√	-	√	-	-	+/-	√	-	-	-
1051-11	√	++	√	-	√	+	-	-	-	-	√	-	√	+/-	-	+/-	√	-	-	-

Chemotaxis capillary assay:

In order to test taxis of isolates toward various individual components of cyanobacterial exopolysaccharide in a semi-quantitative method, the glass capillary assay was employed. A range of the isolates were tested for chemotaxis towards supernatant obtained from a growing iron-limited culture of *Crocosphaera sp.* WH8501. The results of these tests are given in table 3. The ratio of colonies obtained from plating of the capillary contents of spent medium versus uninnoculated medium varied from 1 to 9.24 suggesting that for some organisms this exudate is not a chemoattractant while other organisms displayed a strong tactic response to component(s) of medium in which cyanobacteria had grown. While replicate experiments did not yield exactly the same ratio value there was good agreement between the numbers. Additional replicates would have to be performed for rigorous statistical analysis. Additionally a control should be included to check for the potential chemo attractive properties of lysed cells as there was no way of measuring the extent of cyanobacterial lysis in the cultures

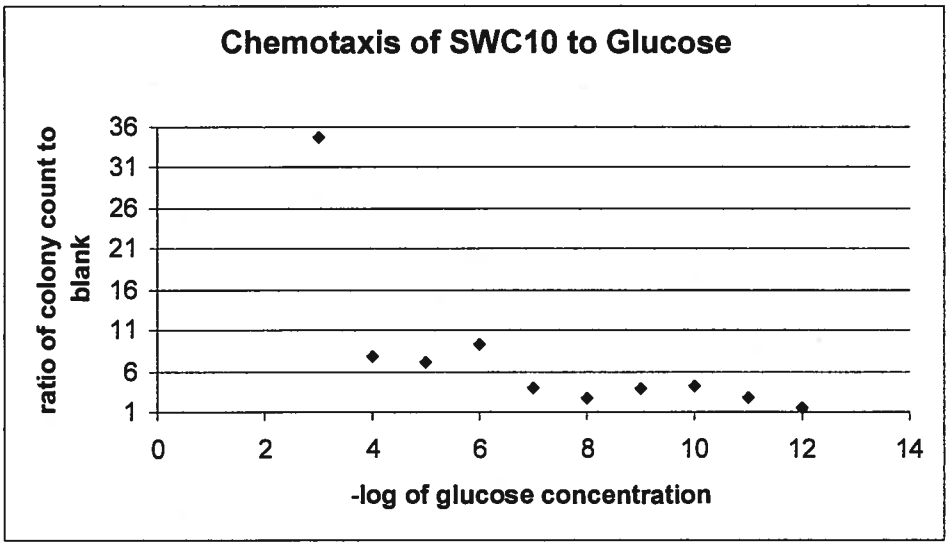
Table 3. Ratio of colony forming units obtained in cyanobacterial "spent" medium versus fresh medium for various environmental isolates

Organism ID	Ratio of colonies from spent versus fresh medium
SWC10	4.46

SWC10(rep)	4.94
sp1	1.24
sp1(rep)	2.32
6-E12	3.45
sp5	1.04
SWC8	2.29
SWC12	6.38
SWC4	9.24

Additionally, since glucose appears to be such a strong chemoattractant for all of the cells studied, the capillary assay was performed with glucose concentrations ranging from 10^{-2} to 10^{-12} M. The ratio of colony forming units for isolate SWC10 for this range of glucose concentrations compared to the control is plotted in Figure 1. The colonies for the 10^{-2} M glucose concentration were too dense to enumerate, but appeared to be more abundant than those on the10^{-3} M glucose plate, suggesting that cells may be able to recognize very high levels of glucose. The other interesting result is the low concentrations at which chemotaxis appears to be able to sense a glucose gradient. The receptors of *E. coli* cannot detect glucose below ~5* 10^{-9}M, while this vibrio isolate appears to exhibit taxis to glucose down to 10^{-12} M levels. More sensitive receptors may be a distinct advantage in the marine environment where nutrient levels are significantly lower than the gut environment experienced by *E. coli*.

Figure 1. Plot of glucose concentrations versus the chemotactic response level



Conclusions:

This project suggests that motile cells in the marine environment may be highly sensitive to low concentration nutrient point sources, such as those produced by phytoplankton leaking photoassimilated carbon and other nutrients. Interactions between phytoplankton and heterotrophs may be highly specific as suggested by previous research {Schäfer, 2002 #129} and

the observation in this study that the culturable heterotrophic contaminants of non-axenic cultures of *Crocosphaera* were highly strain-specific after years in co-culture. Since the medium in which these organisms are cultured contains no added combined nitrogen or carbon sources- it seems likely that these heterotrophs are making a living off of the cyanobacteria, whether through consumption of extracellular products or through feeding off of lysed cells. Additionally there may be important cross-talk between phytoplankton and heterotrophs and it would be interesting to test if cyanobacterial growth is enhanced by the presence of heterotrophs, especially if certain essential nutrients (P, etc.) were provided in the form of complex organic compounds. Another interesting observation that bears further investigation is that culturable seawater isolates are apparently highly sensitive to low concentrations of glucose and perhaps other compounds, although the largest chemotactic response was observed at high concentrations. This result is consistent with the observed multiphastic uptake kinetics in natural assemblages for glucose and amino acids suggests that bacteria are adapted to an environment with order-of –magnitude variations in nutrient levels (Azam & Hodson 1981, Fuhrman & Ferguson 1986, Ayo et al. 2001). While energetically expensive, motility must confer important selective advantages by enabling bacteria to make use of nutrient point sources such as those provided by marine cyanobacteria by exudation or cell lysis.

Cited Works

Anderson, C. (2003) "Isolation, growth and investigation of the bacterial epibiont of the heterocyst of an *Anabaena* sp." Microbial Diversity Summer Course 2003.

Barbara, G. M. and J. G. Mitchell (2003). "Bacterial tracking of motile algae." FEMS Microbiology Ecology **44**(1): 79-87.

Bell, W. and R. Mitchell (1972). "Chemotactic and growth responses of marine bacteria to algal extracellular products." The Biological Bulletin **143**(2): 265-277.

Bowen, J. D., K. D. Stolzenbach, et al. (1993). "Simulating bacterial clustering around phytoplankton cells in a turbulent ocean." Limnology and Oceanography **38**(1): 36-51.

Casamatta, D. A. and C. E. Wickstrom (2000). "Sensitivity of two disjunct bacterioplankton communities to exudates from the cyanobacterium Microcystis aeruginosa Kutzing." Microbial Ecology **40**(1): 64-73.

Giroldo, D., A. A. H. Vieira, et al. (2003). "Relative increase of deoxy sugars during microbial degradation of an extracellular polysaccharide released by a tropical freshwater Thalassiosira sp (Bacillariophyceae)." Journal of Phycology **39**(6): 1109-1115.

Müller-Niklas, G., M. Agis, et al. (1996). "Microscale distribution of bacterioplankton in relation to phytoplankton: Results from 100-nl samples." Limnology and Oceanography **41**(7): 1577-1582.

Myklestad, S. M. (1995). "Release of extracellular products by phytoplankton with special emphasis on polysaccharides." Science of the Total Environment **165**(1-3): 155-164.

Nicolaus, B., A. Panico, et al. (1999). "Chemical composition and production of exopolysaccharides from representative members of heterocystous and non-heterocystous cyanobacteria." Phytochemistry **52**(4): 639-647.

Purcell, E. M. (1977). "Life at low Reynolds-number." American Journal of Physics **45**(1): 3-11.

Schäfer, H., B. Abbas, et al. (2002). "Genetic diversity of 'satellite' bacteria present in cultures of marine diatoms." FEMS Microbiology Ecology **42**(1): 25-35.