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SHORT COMMUNICATION

Diverse populations of lake water bacteria exhibit chemotaxis towards inorganic nutrients

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Chemotaxis allows microorganisms to rapidly respond to different environmental stimuli; however, understanding of this process is limited by conventional assays, which typically focus on the response of single axenic cultures to given compounds. In this study, we used a modified capillary assay coupled with flow cytometry and 16S rRNA gene amplicon pyrosequencing to enumerate and identify populations within a lake water microbial community that exhibited chemotaxis towards ammonium, nitrate and phosphate. All compounds elicited chemotactic responses from populations within the lake water, with members of *Sphingobacteriales* exhibiting the strongest responses to nitrate and phosphate, and representatives of the *Variovorax*, *Actinobacteria ACK-M1* and *Methylophilaceae* exhibiting the strongest responses to ammonium. Our results suggest that chemotaxis towards inorganic substrates may influence the rates of biogeochemical processes.
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In aquatic environments, organic and inorganic nutrients are distributed heterogeneously, with microscale hotspots found throughout the water column (Azam, 1998; Blackburn *et al*., 1998). Microbial cells are generally unable to uptake nutrients that are more than a few cell diameters away; therefore, motility is a key determinant of the size of the microhabitats within which different populations perceive and exploit nutrient sources (Stocker, 2012). Non-motile cells can explore ~80 nl water per day, which when compared with ca. 1 ml per day for motile cells is very small (Stocker, 2012). Furthermore, as motility is typically associated with the ability of cells to sense and direct movement along chemical gradients (chemotaxis), many motile populations are not only able to explore large volumes of water, but are also able to move directly towards perceived resource hotspots (Fenchel, 2002; Stocker *et al*., 2008; Stocker and Seymour, 2012).

Aquatic bacterial chemotaxis occurs in response to a wide range of organic substrates, including amino acids (Barbara and Mitchell, 2003), sugars (Malmcrona-Friberg *et al*., 1990) and organic sulphur compounds (Miller *et al*., 2004). In contrast, chemotaxis towards inorganic substrates is poorly characterised despite bacterial growth in aquatic ecosystems often being limited by dissolved inorganic nutrients (Church, 2008), and the requirement for some inorganic chemicals in anaerobic respiration (Thauer *et al*., 1977). In addition, as chemotaxis studies have focussed on single populations of cultivated bacteria, the generality of this trait is unknown. To date, chemotaxis towards inorganic substrates has been observed towards: (1) ammonium and nitrate by the marine cyanobacterium, *Synechococcus* (Willey and Waterbury, 1989); (2) ammonium by the purple non-sulphur photosynthetic bacterium *Rhodobacter sphaeroides* (Ingham and Armitage, 1987; Poole and Armitage, 1989); (3) nitrate and nitrite by *Shewanella putrefaciens* (Nealson *et al*., 1995) and several strains of denitrifying bacteria (Kennedy and Lawless, 1985; Lee *et al*., 2002) and (4) phosphate by a heterotrophic marine *Thalassospira* sp. (Hütz *et al*., 2011).

In this study, we used a syringe-based assay (Supplementary Figure S1), inspired by the traditional capillary assay (Adler and Dahl, 1967), coupled with flow cytometry and 16S rRNA gene amplicon pyrosequencing to enumerate and identify populations within a lake water microbial community that exhibited chemotaxis towards ammonium, nitrate and phosphate. Chemotaxis assays were performed by submerging 1-ml syringes containing 80 µl of 0.1 M chemoattractant (nitrate/ammonium/phosphate) into

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lake water for 30 min (Supplementary information). Each chemoattractant was prepared in 0.2 μm-filtered lake water to ensure that the chemical characteristics of the background solution in the syringe was similar to that in the lake. Filtered lake water control assays with no added chemoattractant were performed in parallel and facilitated the measurement of stochastic movement of cells into our assays (Supplementary Figure S2). All assays were replicated six times, providing three samples for cell counting and three samples that were pooled for 16S rRNA gene amplicon sequencing. This novel approach facilitated culture-independent characterisation of chemotactic populations within a lake water community. To ensure that counts in the assays reflected chemotaxis rather than growth stimulation, we enumerated cells in 0.2 μm-filtered lake water with and without added nutrients. The controls were incubated for 30 min, but the tips of the syringes were not submerged in lake water (Supplementary Figure S3).

Cell counts in the nutrient-spiked chemotaxis assays were approximately six times higher than in the control assays ($P<0.001$, generalized linear model; Supplementary Figures S2 and S3), but did not differ from one another. Cell counts in nutrient stimulation assays, however, did not differ from those associated with the filtered lake water control (Supplementary Figure S3). These results indicate that nitrate, ammonium and phosphate elicited strong chemotactic responses from at least one population within the lake water community and that slight differences in the diffusion coefficients of the attractants (ca. 0.17, 0.18 and $0.05 \times 10^4\,cm^2\,s^{-1}$ for NO_3^-, NH_4^+ and PO_4^{3-}, respectively) did not lead to differences in the strength of response.

Three members of the *Sphingobacteriales* exhibited the strongest chemotactic response to nitrate and phosphate (Figures 1 and 2). Phylogenetic analysis (Supplementary information) revealed that these *Sphingobacteriales* populations belonged to the family *Chitophagaceae.* Within this family, members of the genera *Niastella* and *Chitinophaga* are known to exhibit gliding motility (Lim *et al.*, 2009; Weon *et al.*, 2009; Del Rio *et al.*, 2010), and some *Chitinophaga* species are known to reduce nitrate as a terminal electron acceptor (Lim *et al.*, 2009). Interestingly, the only other *Sphingobacteriales* population observed in the lake water at >1% relative abundance, a *Pedobacter*-like organism, did not exhibit chemotaxis towards nitrate (Figure 1). This is consistent with observations that *Pedobacter* isolates do not reduce nitrate (Steyn *et al.*, 1998). Therefore, the positive chemotactic response towards nitrate observed for the *Sphingobacteriales* populations may reflect their ability to detect and exploit nitrate as an alternative electron acceptor. Chemoattraction may also reflect the ability for cells to move towards nitrate in response to inorganic nutrient deficiency; however, there are examples, where bacteria do not metabolise attractants.

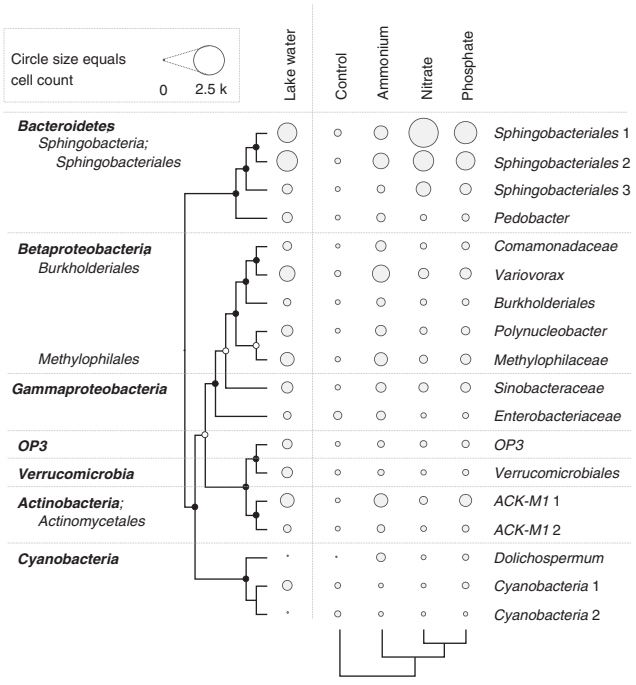

Figure 1 Bacterial operational taxonomic units (OTUs) present at >1% abundance in any sample. The size of each circle represents the relative abundance of each OTU normalised by the total cell count within each sample. The OTUs are organised by phylogenetic relatedness based on a maximum likelihood bootstrapped tree (1000 iterations) of full-length sequences that were identified as the nearest BLAST matches for each OTU. Bootstrap values are represented as follows: 75–100% (closed circles), 50–74% (open circles), <50% (no circle). The compositional similarity of each community is represented at the base of the heatmap by a complete-linkage cluster analysis tree of OTU abundances.

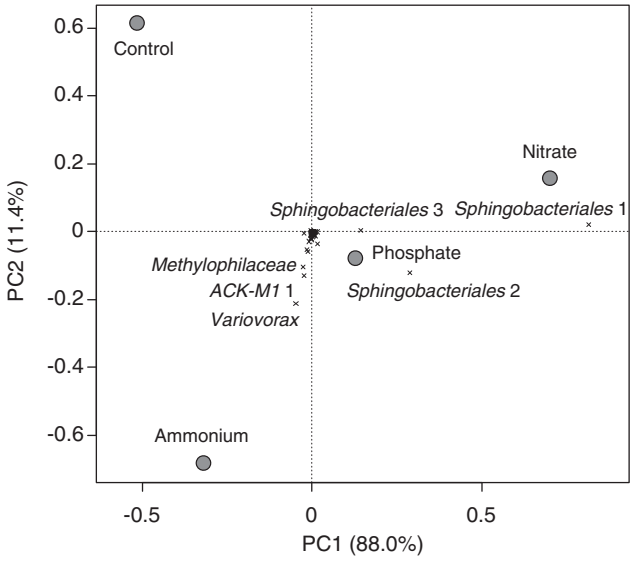

Figure 2 PCA ordination highlighting differences in operational taxonomic units (OTU) abundance between the chemotaxis assays. OTU abundances were calculated by normalising the relative abundances of each OTU by the total cell count within each sample. The OTUs are represented as crosses, with the taxonomic affiliation of the most discriminating OTUs highlighted in text.

For example, *Escherichia coli* is attracted to, but does not metabolise methyl-aspartate (Adler, 1969).

Our results indicate that chemotaxis towards inorganic phosphate is exhibited by a range of bacteria, in particular, the *Sphingobacteriales*, which also showed high levels of chemotaxis towards nitrate (Figures 1 and 2). Previously, chemoattraction to phosphate has only been demonstrated in a free-living planktonic bacterium belonging to the genus *Thalassopira* (Hütz *et al*., 2011), which was isolated from the oligotrophic eastern Mediterranean Sea. Interestingly, no differences in the swimming speed of the *Thalassopira* species was observed when exposed to organic carbon or inorganic phosphate (Hütz *et al*., 2011), suggesting that its motility was not differentially regulated by the type of nutrient supplied. This observation is consistent with our data, which showed that the populations responding to nitrate were similar to those that respond to phosphate (Figures 1 and 2). This may be indicative of cross-regulation of chemotaxis machinery by nitrate and phosphate sensory systems or environmental co-dependency on these compounds. Our results substantially expand the known diversity of bacteria that exhibit chemotaxis towards phosphate.

Ammonium elicited the strongest response among the tested inorganic nutrients for the majority of dominant populations, although none of these responses were as strong as those observed for nitrate and phosphate by the *Sphingobacteriales* (Figure 1). Organisms closely related to *Variovorax*, *Actinobacteria ACK-M1,* also known as the acI group (Warnecke *et al*., 2004), and a member of *Methylophilaceae* exhibited the strongest responses to ammonium (Figures 1 and 2). *Variovorax paradoxus* has been previously reported to swarm in response to ammonium (Jamieson *et al*., 2009), and ammonium is known to influence the composition of *Actinobacteria ACK-M1* assemblages in lakes (Newton *et al*., 2007); however, we are not aware of studies demonstrating that representatives of the *Methylophilaceae* and *Actinobacteria ACK-M1* group exhibit chemotaxis towards ammonium. This lack of information highlights that our approach has the potential to greatly improve knowledge of the microbial lineages that exhibit chemotaxis towards a wide range of compounds in natural environments. Our assay should also facilitate functional characterisation of assemblages of chemotactic populations when coupled with metagenomics and metatranscriptomics. This approach would help to reveal the range and prevalence of mechanisms of movement and environmental sensing used by chemotactic populations in natural environments. Gliding motility, for example, is used by *Myxococcus xanthus* during chemotaxis to phosphatidylethanolamine (Kearns and Shimkets, 1998), but it is unknown whether this type of motility is common among chemotactic microorganisms. Similarly, much of what is known about how microbial chemoreceptors respond to changes in concentrations of chemical attractants or repellents is based on *E. coli* and *Salmonella typhimurium* (Falke and Hazelbauer, 2001). Current evidence indicates, however, that chemosensory systems that mediate microbial chemotaxis differ considerably between species (Montrone *et al*., 1998; Boin *et al*., 2004; Szurmant and Ordal, 2004; Meier *et al*., 2007). The extent of these knowledge gaps highlights that our understanding of chemotaxis in natural environments is far from complete.

Our study indicates that a wide range of lake water bacterial populations have the capacity to move towards inorganic nutrients. This observation was previously overlooked due to culture-based analyses. Chemotaxis towards inorganic nutrients is a behaviour that likely enables them to exploit microscale gradients of limiting resources in the environment. It has previously been suggested that bacterial chemotactic exploitation of microscale patches of organic compounds in marine habitats can influence carbon cycling processes and influence microbial community interactions (Azam, 1998; Blackburn *et al*., 1998; Fenchel, 2002). Our observations indicate that strong, but phylogenetically variable, chemotactic responses towards inorganic compounds may similarly influence nutrient cycling processes and microbial competitive interactions within aquatic ecosystems.

Conflict of Interest

The authors declare no conflict of interest.

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