

## Chemoreception in a marine cryptophyte: Behavioral plasticity in response to amino acids and nitrate

**Abstract**—The behavioral responses of *Chroomonas* sp. to ammonium, nitrate, and 19 individual amino acids were examined by computer-assisted video motion analysis. Cells were cultured with either glycine or nitrate as the sole nitrogen source. Relative to seawater, rates at which glycine-grown cells stopped and turned were significantly reduced in response to  $10^{-6}$  and  $10^{-7}$  M glutamate, methionine, alanine, and aspartate. This response was akin to the tumbling behavior (chemotaxis) displayed by flagellated bacteria. When cultured in nitrate, *Chroomonas* sp. did not react to amino acids and ammonium but did significantly reduce stopping and turning in response to  $10^{-6}$  M nitrate. These results are the first to demonstrate chemoreception in any cryptophyte species. Because *Chroomonas* sp. commonly lives in habitats where light and inorganic nutrients are limiting, behavioral mechanisms that maximize use of both amino acids and nitrate would seem particularly adaptive.

The production of dissolved organic matter (DOM) in select microenvironments locally elevates concentrations of or-

ganic and inorganic substrates relative to surrounding habitats (Azam and Ammerman 1984; Alldredge and Cohen 1987). Numerous studies have shown that concentration gradients affect the motility of marine microorganisms (Malcróna-Friberg et al. 1990; Mitchell et al. 1995). Bacteria often swim in a straight line (smooth run) and then stop and turn (tumble) before swimming again. The chemosensory responses of microorganisms to concentration gradients can trigger changes in stop-and-turn frequency that interrupt swimming more or less often and thus alter the time-averaged swimming paths. The net result of these changes in swimming behavior is the migration of cells either towards or away from regions of elevated concentrations, a process called chemotaxis (Armitage 1992; Manson 1992). Positive chemotaxis, or chemical attraction, occurs when the stop-and-turn frequency of a cell decreases in response to contact with a region of relatively high concentration, irrespective of a change in swimming speed (Berg and Brown 1972; Adler 1975).

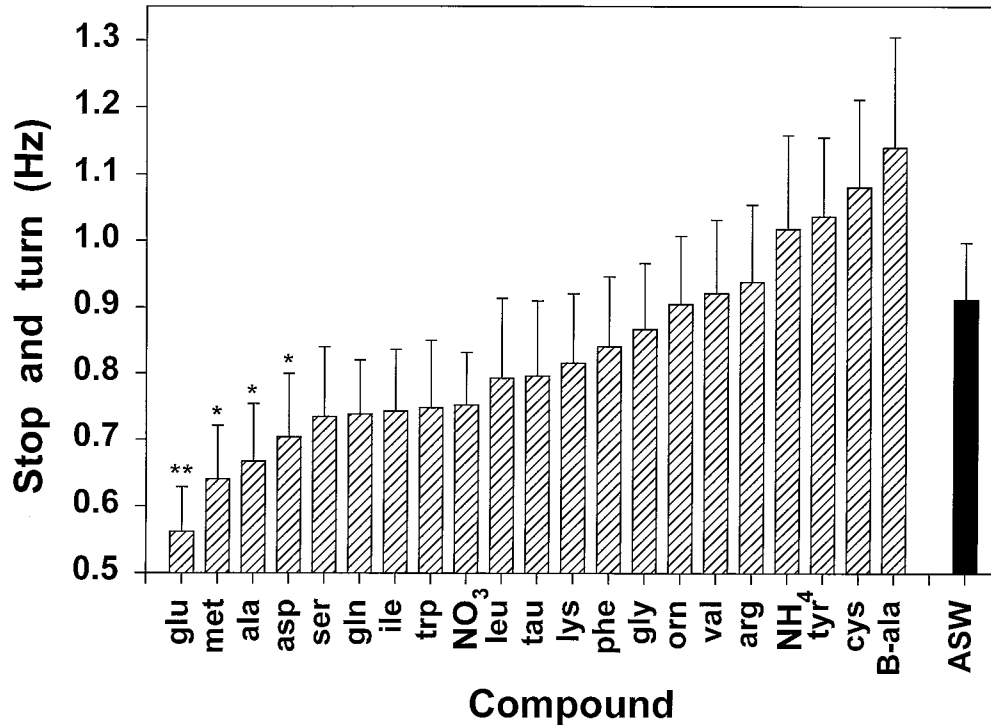


Fig. 1. Mean ( $\pm$ SEM [standard error of the mean]) stop-and-turn rates of glycine-grown cells in response to artificial seawater (ASW), ammonium ( $\text{NH}_4$ ), nitrate ( $\text{NO}_3$ ), and amino acids. Abbreviations for amino acids are standard three-letter codes. The Y-axis units are Hertz (Hz), the number of stops and turns per second. A minimum of 40 paths was analyzed for ASW and each chemical; compounds were presented at  $10^{-6}$  M. Relative to ASW, significant changes in stop-and-turn rates were determined by a one-way ANOVA followed by pairwise comparisons using Bonferroni's correction: \*  $P < 0.05$ ; \*\*  $P < 0.01$ .

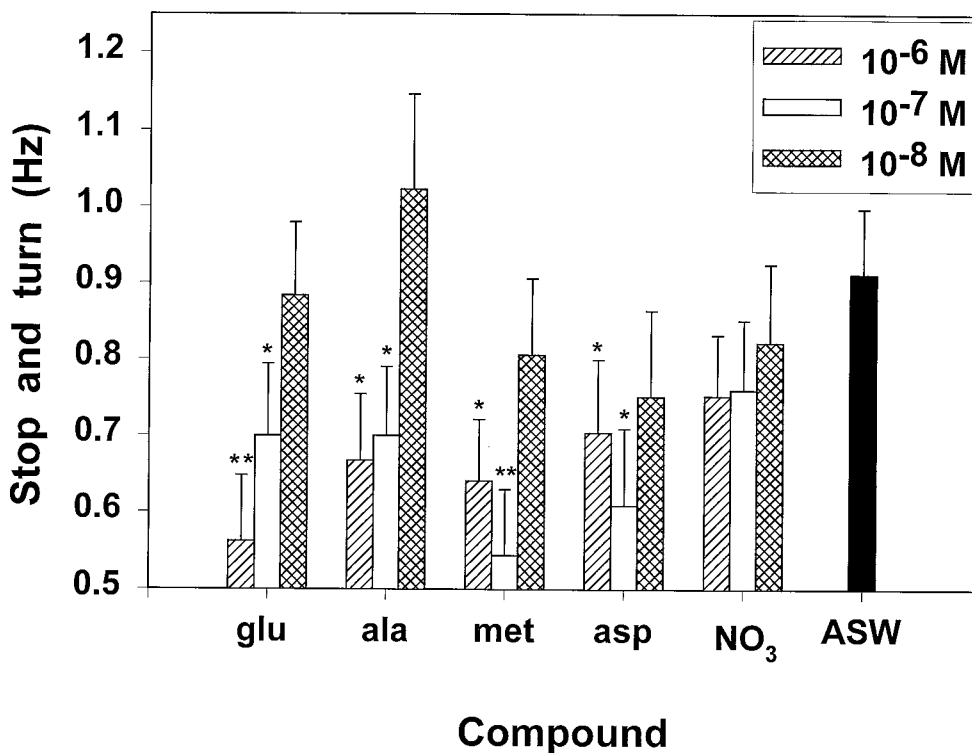


Fig. 2. Mean ( $\pm$ SEM) stop-and-turn rates of glycine-grown cells in response to artificial seawater (ASW) and to  $10^{-6}$ ,  $10^{-7}$ , and  $10^{-8}$  M glutamate (glu), alanine (ala), methionine (met), aspartate (asp), and nitrate ( $\text{NO}_3$ ). A minimum of 20 paths was analyzed for ASW and each chemical solution (compound and dose). Relative to ASW, significant changes in stop-and-turn rates were determined by a two-way ANOVA followed by pairwise comparisons using Bonferroni's correction: \*  $P < 0.05$ ; \*\*  $P < 0.01$ .

Chemotaxis can enhance the rates at which microbes exploit nutrient patches, but the chemosensory mechanisms that control patterns of motility have been investigated for only a few phytoplankton species. These species include green algae (*Chlamydomonas reinhardtii*, *Dunaliella tertiolecta*), cyanobacteria (*Synechococcus* spp.), symbiotic dinoflagellates (*Symbiodinium microadriaticum*), and benthic diatoms (*Amphora feaeformis*, *A. normanis*) (e.g., Levandowsky and Hauser 1978; Sjoblad and Frederikse 1981; Cooksey and Cooksey 1988; Willey and Waterbury 1989). Studies on the perception of chemical stimuli by microorganisms are lacking for small chromophytic flagellates, such as chrysophytes or cryptophytes, and free-living phototrophic dinoflagellates. Because these flagellates are numerically dominant components of many phytoplankton communities (Lewitus et al. 1998 and references therein), processes that relate to their chemotaxis and nutrient uptake are especially important.

We investigated the chemosensory mediated behavior of a marine cryptophyte, *Chroomonas* sp. HP9101. This 3- $\mu\text{m}$ -diameter cell was isolated by one of us (A.J.L.) from the Choptank River, a subestuary (salinity 7–12 ppt) of Chesapeake Bay. Cultures were prepared in artificial seawater medium (ASW; 17 ppt/pH 8) by dissolving Forty Fathoms salt mixture (Zimmer-Faust et al. 1996) in Nanopure-grade deionized water (18 m $\Omega$ ) and filtering to 0.45  $\mu\text{m}$ . Guillard's (1975) f/2 trace metal and vitamin solutions were employed.

Cells were grown for 1 week prior to use at 26°C and a 12:12 light:dark cycle with the addition of either  $6 \times 10^{-8}$  M glycine or  $6 \times 10^{-7}$  M nitrate (determined by high-pressure liquid chromatography) as the sole nitrogen source.

Trials were performed to establish the behavioral responses of *Chroomonas* sp. to chemical stimuli. In each trial, a 25- $\mu\text{l}$  aliquot of log-phase culture ( $10^5$  cells  $\text{ml}^{-1}$ ) was added to a Petroff-Hauser chamber, then a test or ASW control solution (5  $\mu\text{l}$ ) was introduced to the cell suspension by capillary action from one side of the cover slip. Cells were examined under phase contrast microscopy ( $\times 100$  objective and  $\times 10$  photopiece, Olympus model IX70), and images were video recorded for 20 s by a NEC TI-23A CCD camera, stored on tape using a SONY videocassette recorder (model SLV-686HF), and displayed on a Panasonic (model TR-930B) monitor. The recordings were initiated  $< 10$  s after stimulus (or control) introductions because cells behave under these conditions as if placed in a chemical concentration gradient (Armitage 1992; Manson 1992; Zimmer-Faust et al. 1996). All solutions were prepared with Nanopure-grade DI water and Forty Fathoms marine salts at the same salinity and temperature as the culture medium. Ammonium, nitrate, and L-amino acids (alanine, arginine, aspartate,  $\beta$ -alanine, cysteine, glutamate, glutamine, glycine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, serine, taurine, tryptophan, tyrosine, and valine) were each individually tested at  $10^{-6}$  M on glycine-grown cells. In additional trials, the

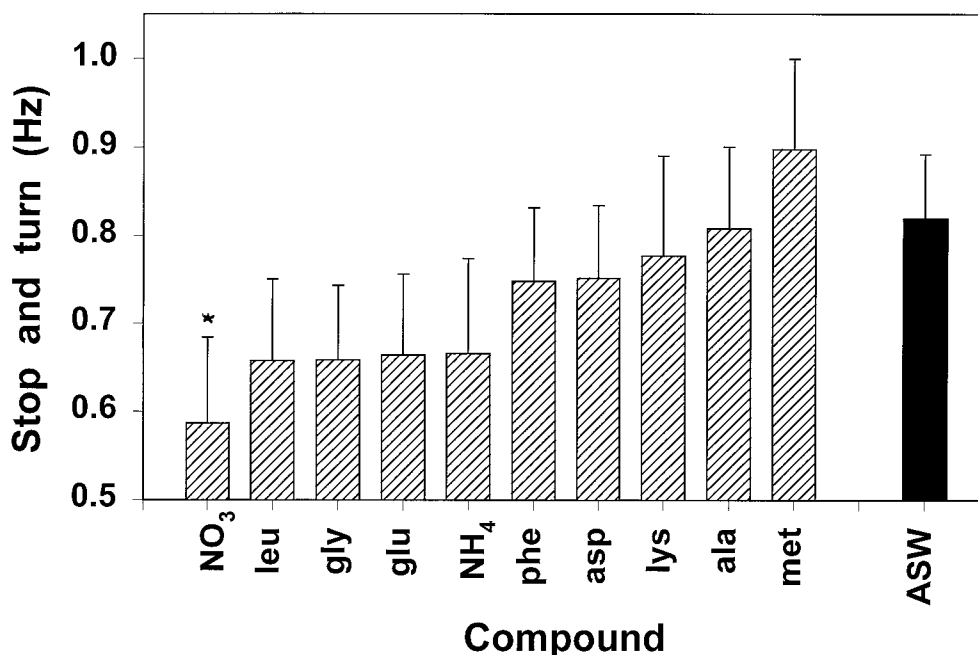


Fig. 3. Mean ( $\pm$ SEM) stop-and-turn rates of nitrate-grown cells in response to artificial seawater (ASW), ammonium (NH<sub>4</sub>), nitrate (NO<sub>3</sub>), and amino acids. Abbreviations for amino acids are standard three-letter codes. A minimum of 40 paths was analyzed for ASW and each chemical; compounds were presented at 10<sup>-6</sup> M. Relative to ASW, significant changes in stop-and-turn rates were determined by a one-way ANOVA followed by pairwise comparisons using Bonferroni's correction: \*  $P < 0.05$ . No significant effect of 10<sup>-7</sup> or 10<sup>-8</sup> M nitrate on stop-and-turn rate was found in additional trials (data not shown).

four most stimulatory amino acids (glutamate, alanine, methionine, and aspartate) and nitrate were presented to glycine-grown cells at 10<sup>-6</sup>, 10<sup>-7</sup>, and 10<sup>-8</sup> M. Finally, a limited set of compounds (alanine, aspartate, glutamate, glycine, leucine, lysine, methionine, phenylalanine, and ammonium) was assayed at 10<sup>-6</sup> M and nitrate was tested at 10<sup>-6</sup>, 10<sup>-7</sup>, and 10<sup>-8</sup> M on nitrate-grown cells.

The swimming paths of individual flagellates, smooth runs alternating with stops and turns, were constructed from digitized video images at 30 frames s<sup>-1</sup>, for a total of 600 frames per trial (for more details on methods, see Zimmerfaust et al. 1996). A minimum of five trials was performed for each chemical solution and ASW. A computer-assisted video motion analyzer (CAVMA; Motion Analysis Corp. Model 320 and custom programs written in ExpertVision) was interfaced with a Sun Microsystems SPARC 2 workstation to measure swimming speeds and stop-and-turn rates. No significant effect of any chemical on swimming speed was found.

The stop-and-turn behavior of cells depended on the nutrient environment. There was a significant negative correlation between cell stop-and-turn rates in response to amino acids, ammonium, and nitrate, and the type of culture medium (Pearson's product-moment correlation:  $r = -0.649$ ,  $df = 18$ ,  $P < 0.05$ ). For glycine-grown cells, stop-and-turn rates were significantly reduced in response to 10<sup>-6</sup> and 10<sup>-7</sup> M glutamate, methionine, alanine, and aspartate (Figs. 1, 2), and nitrate-grown cells reacted only to 10<sup>-6</sup> M nitrate (Fig. 3).

These changes in stop-and-turn rates were akin to the reduction of tumbling displayed in positive chemotaxis by bacteria (Armitage 1992; Manson 1992). Even without considering the elevated levels of organic substrates occurring near microaggregates or the dilution of test chemicals during introduction in experiments, amino acid concentrations that elicited behavioral responses by *Chroomonas* sp. were similar to those occurring in natural seawater (Coffin 1989; Antia et al. 1991; Decho et al. 1998). Three sets of physicochemical descriptors for each amino acid were extracted by principal components analysis of a multiproperty matrix, corresponding to side chain hydrophilicity, bulk, and electronic properties (Hellberg et al. 1987; Browne et al. 1998). No significant correlation was identified between any descriptor and stop-and-turn rate (Pearson's product moment correlation:  $P > 0.20$ , all comparisons), and thus there is not yet a clear structure-function relationship between amino acids and their role(s) as environmental signal molecules.

A working hypothesis to explain the chemosensory mediated behavior of *Chroomonas* sp. is that when nitrate concentrations are sufficient for photosynthesis and cell division, *Chroomonas* sp. ignores amino acids that potentially reduce rates of protein synthesis by inhibiting nitrate reductase activity (Antia et al. 1991). But when photosynthesis is limited by either low light or low nitrate concentration, cells become positively chemotactic in response to amino acids. That is, amino acids supply both the carbon and nitrogen needed for protein synthesis. In the turbulent mixed layer of the ocean, positive chemotaxis (through a reduction in the

rate of stopping and turning) may increase the exposure of cells to patchy nutrients (Bowen et al. 1993). If amino acids and nitrate are indeed taken up and employed in carbon or nitrogen metabolism, prolonged exposure to these substrates could lead to higher rates of cell division and population growth. *Chroomonas* sp. commonly inhabits waters where light and inorganic nutrients are limiting (Lewitus and Kana 1994). Mechanisms for maximizing use of both amino acids and nitrate through chemotaxis thus seem particularly critical for this species.

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