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Chemotactic Responses of Marine *Vibrio* sp. Strain S14 (CCUG 15956) to Low-Molecular-Weight Substances under Starvation and Recovery Conditions

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The chemotactic responses by starved cells of marine *Vibrio* sp. strain S14 differed from those elicited by cells that were not nutrient limited. The rate of chemotaxis at different concentrations of several attractants varied for starved and growing cells. *Vibrio* sp. strain S14 showed positive chemotaxis to leucine, valine, arginine, and glucose at the onset of energy and nutrient deprivation. A continued, though decreased, positive response was demonstrated for leucine, arginine, and glucose at 10 h of starvation. Cells starved for 3 h displayed a stronger response to glucose than those starved for shorter or longer times. However, cells starved for 5 and 10 h responded more strongly to a lower concentration of glucose than did cells starved for 0 and 3 h. Starvation for 24 h elicited no measurable chemotaxis to leucine, arginine, or glucose. The motility decreased by over 95% in the cell population after 24 h of starvation, which resulted in a low sensitivity in the chemotaxis assay. A switch in the response to valine was observed by 3 h of starvation. The addition of nutrients to 22-h-starved cells elicited a temporary positive chemotactic response to leucine by 2 and 4 h of nutrient recovery, while cells at 1 and 6 h of recovery showed no response. At 2 h of recovery, the greatest response was recorded to 10^{-4} M leucine, whereas at 4 h it was to 10^{-2} M leucine. Ten to fifty percent of the 22-h-starved cell population regained their motility after 4 h of nutrient-aided recovery. It is possible that two types of chemosensory systems exist in marine bacteria. Starved and growing cells responded to different concentrations of the attractant, and growing cells displayed a saturated chemotactic system with leucine as the attractant, unlike the response during starvation.

The accumulation of macromolecules and nutrient ions at organic and inorganic surfaces in marine waters results in sites of substrate elevation in an otherwise nutrient-deficient environment. Aggregates and macroscopic particles constitute enriched microenvironments for both autotrophic and heterotrophic processes (4, 6, 32). Motile bacteria may respond to the nutrient concentration gradient at such surfaces by positive chemotaxis. Jackson (17) suggests that chemotaxis enhances bacterial interactions with aggregates in marine waters, allowing bacteria to stay for significant times at the surface and allowing for attachment and colonization. As such, chemotaxis may serve the bacteria primarily as a food-finding device (15).

Studies of the chemotaxis of marine bacteria have been performed (7–9). However, chemotaxis by energy- and nutrient-limited marine bacteria has been investigated to only a limited extent (13, 34). Such studies suggest that the ability of the nutrient-depleted bacterial cell to respond to substrate addition may influence the survival capacity of the cell. It has also been demonstrated that marine bacteria exhibit functional uptake systems during prolonged starvation (11, 13, 19, 23, 28).

In order to obtain further information on the survival behavior of energy- and nutrient-limited copiotrophic (30) bacteria, we conducted a series of experiments to study the chemotactic responses by marine *Vibrio* sp. strain S14 under starvation and substrate-aided-recovery conditions.

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MATERIALS AND METHODS

Organism, cultivation, and starvation conditions. Marine *Vibrio* sp. strain S14 (CCUG 15956) was used. It was first isolated by Humphrey et al. (16) and was further described and characterized by Mårdén et al. (23) and Albertson et al. (3). Motile cells each possess a single, polar flagellum. S14 cells were preserved in 15% glycerol plus VNSS (see below) and stored at −70°C. A new stock culture was established from an ampoule for each new set of experiments. The bacteria were grown in V medium (VNSS) (35) for marine bacteria which contained the following: peptone, 1.0 g; yeast extract, 0.5 g; glucose, 0.5 g; starch (soluble), 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; Na_2HPO_4, 0.01 g; and nine-salt solution (NSS), 1,000 ml. The NSS consisted of the following: NaCl, 17.6 g; Na_2SO_4, 1.47 g; $NaHCO_3$, 0.008 g; KCl, 0.25 g; KBr, 0.04 g; $MgCl_2 \cdot 6H_2O$, 1.87 g; $CaCl_2 \cdot 2H_2O$, 0.41 g; $SrCl_2 \cdot 6H_2O$, 0.01 g; H_3BO_3, 0.01 g; and double-distilled water, 1,000 ml (22). Agar was added at a concentration of 15 g liter^{-1} to give VNSSA plates.

One hundred-milliliter cultures were grown in 300-ml Erlenmeyer flasks at 25°C in a rotary incubator (125 rpm, 25°C). Bacteria were harvested and washed twice in NSS when cultures reached an optical density at 610 nm of approximately 0.12 (10^7 bacteria ml^{-1}). Care was taken to keep the flagella intact by means of low-speed centrifugation (3,000 × *g*), and washing and resuspending procedures were performed gently. Bacteria were diluted in starvation medium (NSS) to a density of 10^7 cells ml^{-1}. A 15-ml aliquant was transferred to each of four 20-ml sterile glass beakers. Bacteria were starved at 25°C statically. At different time

intervals (0, 3, 5, and 10 h) chemotaxis assays were performed.

Chemotaxis assay. All glassware was washed thoroughly, rinsed, and left in a 180°C oven for at least 5 h. The chemotaxis assay is based on the Adler assay (1) with the following alterations. A 20-ml glass beaker was used instead of the flat chamber on a glass slide; four capillaries (Drummond, 1 μl) were used for each individual experiment instead of one capillary. Elastic clay was used to stick the capillaries to the edge of the beaker. The chemotactic solutions were used at concentrations of 10^{-1}, 10^{-2}, and 10^{-4} M. NSS served as the control.

Each assay was run for 30 min. At completion of the assay time, each set of four capillaries (per concentration) were broken aseptically and the contents were mixed with 5 ml of NSS. Dilution series, in steps of 10×, were performed. One hundred-microliter aliquots from appropriate dilutions were spread on VNSSA plates and incubated for 24 h at 25°C, and the colonies were counted. The minimum numbers of colonies counted were 20 for each agar plate.

Experiments for each chemotactic stimulant were repeated five times. The means from the five data sets are shown in Fig. 2 and 4. Plate counts were made twice, in duplicate, and for each a mean value was derived. The chemotactic response was expressed in terms of relative response, defined as the number of cells that accumulated in the capillary containing the stimulant divided by the number of cells that accumulated in the capillary without the stimulant. By expressing the data in terms of a relative response, it was possible to correct for differences in motility and reductive division (24) of cell populations. Values >1 gave the fold increase in cells responding to the attractant.

Chemotactic stimuli. Stock solutions were made of L-leucine (1.31 g in 100 ml of NSS), L-valine (1.17 g in 100 ml of NSS), L-arginine (1.74 g in 100 ml of NSS), and glucose (1.8 g in 100 ml of NSS). All chemicals were of fine grade (Sigma) to obtain maximum purity for the chemotaxis assay. Chemotactic solutions were adjusted to equal the pH of the control (NSS, pH 7.6) and subsequently filter sterilized (Millipore, 0.22-μm pore size). The chemotactic solutions were made fresh for each set of experiments.

Motility test. Bacterial motility was checked microscopically at various starvation times (0, 1, 3, 5, 10, and 24 h). A 2-ml aliquant was removed, and to this 0.125 μl of glutaraldehyde (GA) (2%) was added. This was the basis for the total cell counts. Parallel sampling, without the addition of GA, provided the basis for counting of the nonmotile bacteria. The idea was to always be able to count nonmotile cells and then to derive the numbers of motile cells by the equation referred to below. Cell counts were performed with a Helber counting chamber. The number of motile cells as a percentage of the total population was derived from the following equation: percent motile cells = {[number of cells (+GA) − number of nonmotile cells (−GA)]/no of cells (+GA)} × 100. The experiments were repeated three times, and the mean value of three runs are depicted (see Fig. 1).

Recovery medium and experimental conditions. Cells starving at a density of 10^7 ml^{-1} in NSS for 22 h were used in recovery experiments. Glucose, $(NH_4)_2SO_4$, and K_2HPO_4 were added to the cell suspension at final concentrations of 2.0, 1.1, and 0.27 g $liter^{-1}$, respectively. The addition of the nutrients marked time zero for the recovery study. At 1, 2, 4, and 6 h, the chemotaxis assay was performed with leucine as the chemoattractant at concentrations of 10^{-1}, 10^{-2}, and 10^{-4} M. NSS without leucine was the control. Cell motility was monitored microscopically, and samples for transmission electron microscopy (TEM) preparations were taken at various intervals.

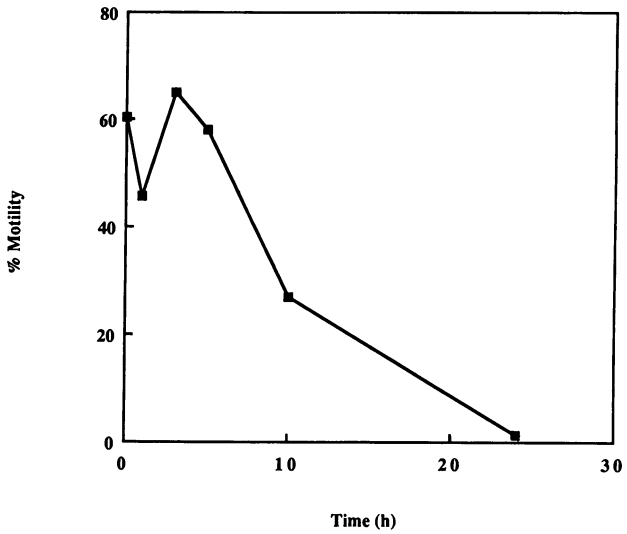

FIG. 1. Decrease in percentage of motile cells in a population of *Vibrio* sp. strain S14 during starvation.

Control experiments. The process of cell recovery measured as motility after starvation times of 10, 24, 48, and 168 h was monitored by video recording. At various time intervals a 50-μl aliquot of culture was placed on a glass slide which was immediately observed under a microscope attached to a television screen and video recorder. The observation time was from 30 to 60 s, and the test was performed twice for each sample.

Flagellum staining and TEM preparations. At different time intervals samples were prepared for flagellum staining. Fifty microliters of cell suspension was placed in a petri dish. A Formvar-coated copper grid (200 μm) was placed on top of the drop and was left there for 30 min. Subsequently, the grid was dipped carefully for 5 s into buffer (NSS) and then into a staining solution containing 1% tungstate–2% ammonium acetate–3 mM $MgCl_2 \cdot 6H_2O$ diluted in buffer, into buffer again, and finally into distilled H_2O. All solutions used were pH adjusted (pH = 7) and filtered (Millipore, 0.22-μm pore size) before use. Grids were examined in a Philips EM 301 electron microscope.

RESULTS

***Vibrio* sp. strain S14 cells undergoing starvation.** The number of motile *Vibrio* sp. strain S14 cells decreased to 1 to 2% by 24 h of starvation (Fig. 1). TEM photographs showed a decrease in the number of flagellated S14 cells with increasing time of starvation (not shown). TEM photographs of S14 cells at the onset of starvation demonstrated the presence of a single, polar flagellum. The length of the flagella ranged from about 10 to 15 μm. After 24 h of starvation, few flagellated cells were observed by TEM.

In the absence of exogenous energy and nutrient sources, *Vibrio* sp. strain S14 cells showed positive chemotaxis to leucine, valine, arginine, and glucose at the onset of starvation. The chemotactic response by S14 cells at the onset of starvation for the highest attractant concentration used ranged from a 100-fold response for valine to a 15-fold response for glucose (Fig. 2).

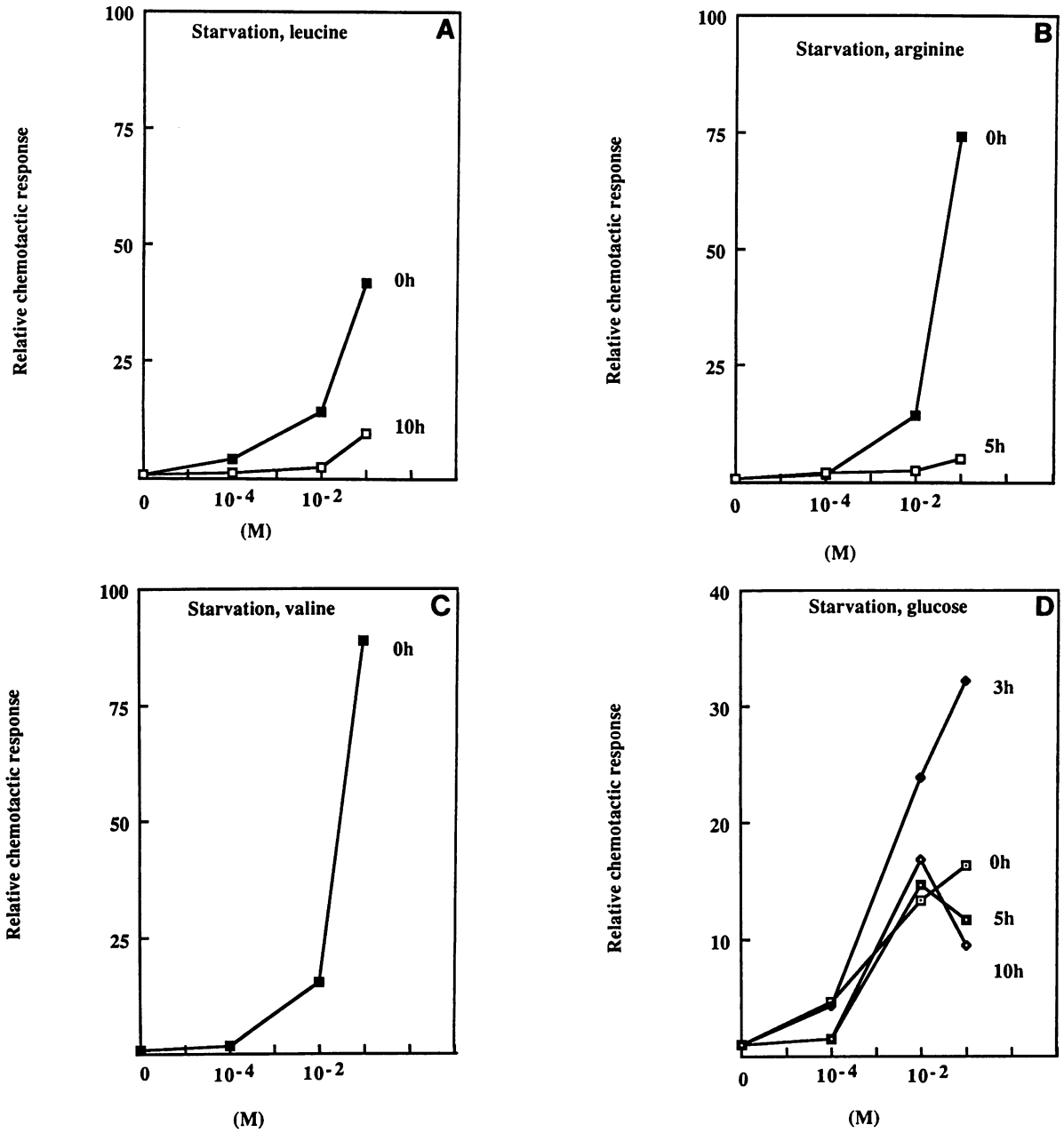

FIG. 2. Chemotactic responses of *Vibrio* sp. strain S14 cells to test chemostimuli at various starvation times.

Positive chemotaxis to leucine, arginine, and glucose remained throughout 10 h of starvation, although for leucine and arginine, the responses decreased with increasing time of starvation. *Vibrio* sp. strain S14 cells starved for 3, 5, and 10 h were no longer attracted to valine.

Chemotaxis to glucose was more complex (Fig. 2D). At 0 and 3 h of starvation the cells showed the strongest attraction to the highest concentration (10^{-1} M), while 5- and 10-h-starved cells were most attracted to glucose at a concentration of 10^{-2} M. Unlike the results with the amino acids, to which cells showed the greatest responses at the onset of starvation (0 h), with glucose, cells responded most strongly at 3 h.

Samples for chemotaxis assays were initially also taken at a sampling time of 24 h. However, at this time, the numbers of motile cells were generally too low to obtain sufficient counts by this method and, as a consequence, this sampling time was later excluded.

It should be noted that the results represent the fold increase in numbers of cells that were attracted to the chemostimulant in the capillary compared with the number of cells that moved into the capillary by chance. In this assay system it was only the motile cells that were capable of responding chemotactically, and thus only such cells were assayed. The difference in motility at different times of starvation is therefore taken into account.

Recovery from starvation of *Vibrio* sp. strain S14 cells. After 22 h of starvation, an increase in the number of motile *Vibrio* sp. strain S14 cells was not observed prior to 1 h after the addition of nutrients (Fig. 3). By 1.5 h, 5 to 10% of the

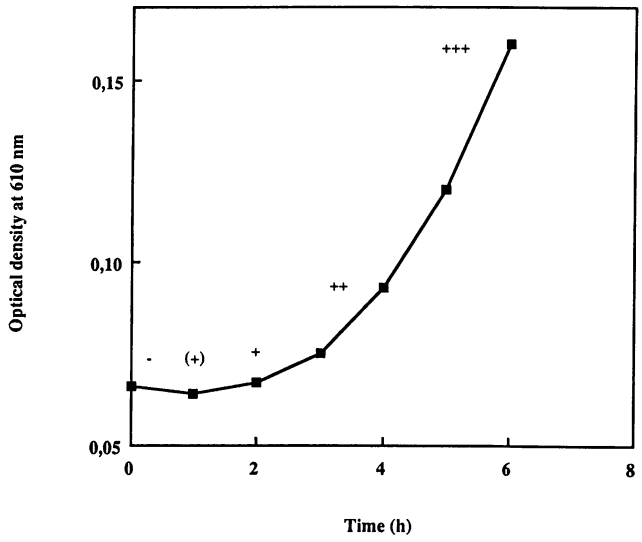

FIG. 3. Increase in optical density of *Vibrio* sp. strain S14 cells during nutrient-aided recovery from starvation. Motile cells: − none observed; (+), few observed; +, 5 to 10% observed; ++, 10 to 50% observed; +++, ≥50% observed.

S14 cell population was motile. At 4 h of recovery, 10 to 50% of the cells were motile, and by the completion of the observation period, after 6 h, 50 to 100% of the cells had regained motility.

While more than 10% of *Vibrio* sp. strain S14 cells that had been starved for 10, 24, or 48 h regained their motility within 120 min of recovery, there were no observable motile cells after 5 h of recovery of 168-h-starved S14 cells. The viability remains 100% after at least 2 weeks of starvation of S14 cells (3).

During recovery from starvation, *Vibrio* sp. strain S14 cells showed a positive chemotactic response to leucine at 2 and 4 h but not at 1 or 6 h after nutrient addition (Fig. 4). Moreover, after 2 h of recovery, cells responded most strongly to leucine at a concentration of 10^{-4} M, whereas after 4 h, 10^{-2} M leucine elicited the strongest response.

DISCUSSION

Motile cells have the possibility of encountering nutrient gradients that surround aggregates in marine waters (4) and gaining access to favorable nutrient sites. This random movement, in combination with a chemotactic response involving direct swimming toward higher nutrient concentrations, is suggested to be an efficient means for substrate scavenging by marine bacteria (5, 17).

Maintained motility is reported also for bacteria exposed to nutrient limitation (31, 34). Although the majority of cells lost motility, a fraction (1 to 2%) of the total population of *Vibrio* sp. strain S14 cells was still motile after 24 h, and motile cells (about 5%) were observed even after 70 h of starvation. The almost total loss of motility corresponded to a loss of flagella, as shown by TEM photographs of 24-h-starved cells. The decrease in motility corresponded to the utilization of energy storage polymer (poly-β-hydroxybutyrate) of S14 cells during starvation, as found by Mårdén et al. (24). It is possible that the loss of easily utilizable endogenous energy sources corresponds to an energy level below the threshold to drive the flagellum motor. Recently Eisenbach (12) reported that when *Escherichia coli* had no

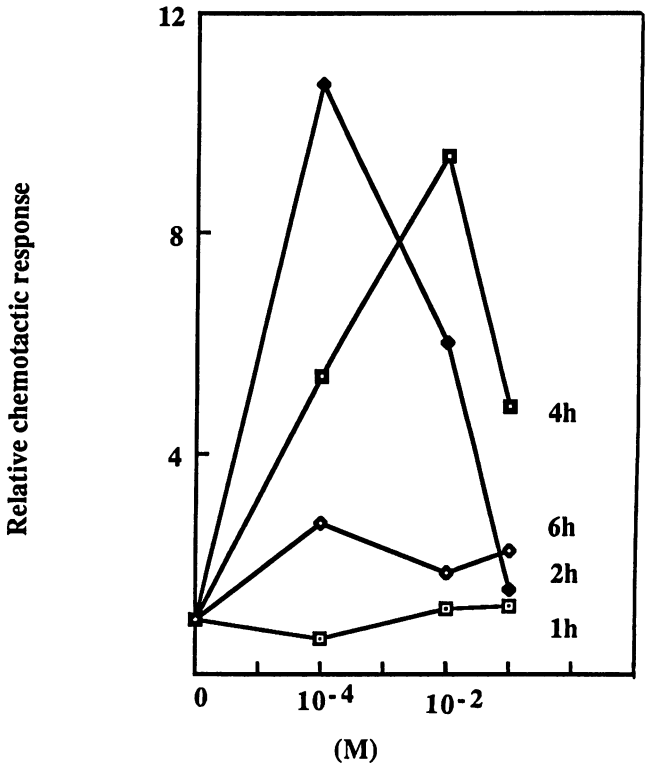

FIG. 4. Chemotactic response of *Vibrio* sp. strain S14 cells to leucine at various times during nutrient-aided recovery.

external supply of energy, a second mechanism for driving the motor, other than the proton motive force, is induced. This may serve as a backup mechanism that enables the bacteria to swim away from the energy-poor environment. Similarly, such a secondary mechanism may account for the motility observed after 70 h of starvation for approximately 5% of the S14 cell population.

The chemostimuli included in this study were chosen for the following reasons. *Vibrio* sp. strain S14 has shown different uptake systems for leucine under starvation-induced conditions than under nonlimiting conditions (23, 28). It may be of interest to observe whether a coupling exists between the leucine uptake systems and the chemosensory response for leucine. Also, leucine is reported to be a chemorepellent for *E. coli*. This is an interesting observation as leucine is one of the essential amino acids. Is the negative chemotactic response shown for *E. coli* toward leucine a common phenomenon for procaryotes, and is it altered during energy and nutrient limitation? Arginine has been employed as a chemoattractant in previous work that evaluated the chemotactic responses by marine bacteria under nutrient deprivation (13). Valine was chosen as an additional amino acid since it is known that for *E. coli* it is also a repellent. Glucose is a common carbohydrate in marine waters, and *Vibrio* sp. strain S14 induces, or derepresses, a high- affinity, protein synthesis-dependent uptake system for glucose subsequent to 70 h of starvation (3a).

The results showed that *Vibrio* sp. strain S14 responded differently to three amino acids and to glucose during the first 10 h of starvation. Although positively attracted to leucine, arginine, and valine at the beginning of starvation, after 3 h the cells were attracted to leucine and arginine but not to valine. It would be interesting to include experiments

to determine whether valine becomes a repellent to starving S14 cells. Valine is known as a repellent for *E. coli*, which uses the same class of receptor as for leucine. At 0 h of starvation *Vibrio* sp. strain S14 cells responded most strongly to the highest concentration of attractant used, i.e., 10^{-1} M. From the graphs shown in Fig. 2A to C it is clear that saturation (26) of the chemosensory system did not occur at any concentration of these attractants in the initial phase of starvation. These results can be compared with results obtained for *E. coli* cells from a log-phase culture: the majority of the compounds tested elicited a peak value in the chemotactic response (25). In contrast to the chemotactic response of *E. coli*, the results of our study showed that leucine was an attractant for *Vibrio* sp. strain S14 cells.

The response of *Vibrio* sp. strain S14 cells to glucose was different and more complex than that to the amino acids (Fig. 2D). At 0 and 3 h the chemosensory system remained unsaturated, even at 10^{-1} M glucose. However, after this time, saturation occurred at 10^{-2} M. This saturation effect is similar to the peak values for *E. coli* responses to sugars (2). The peak value of a chemotactic response denotes the attractant concentration at which an increase in concentration does not elicit any greater chemotactic response by the cell (26).

The chemosensory system of bacteria may be closely linked to the uptake and transport systems (10). It has been shown that under nonlimiting nutrient conditions bacterial cells use low-affinity uptake systems but that under limiting nutrient conditions there is a switch to high-affinity ones (11, 13, 19). For *Vibrio* sp. strain S14, Mårdén et al. (23) have shown that a low-affinity uptake system for leucine is used by cells during growth and during the initial phase of starvation. However, at 24 h of starvation both the low- and, with increasing V_{max}, a high-affinity system can be detected. This change in uptake capacity for leucine is concomitant with a loss in the chemotactic response to leucine by *Vibrio* sp. strain S14 cells.

Constant, or even enhanced, chemotactic responses by various microorganisms under nutrient-limited conditions have been reported (33, 34). For *Vibrio* sp. strain S14, the majority of cells were nonmotile after 24 h, so it is not known if the chemosensory system was still functional. However, the results indicate that the system may be shut down after 24 h of starvation, since the response to arginine and leucine became weaker over 10 h of starvation.

The dual uptake system may allow bacteria to conserve energy by being nonmotile when nutrient concentrations are low but to use a search strategy in the vicinity of higher solute concentrations, enabling them to locate or remain in the vicinity of a nutrient source. Similarly, the high-affinity uptake system for glucose of *Vibrio* sp. strain S14 cells is not induced or derepressed prior to 70 h of starvation (3a).

The lipid composition in the cell and outer membranes of *Vibrio* sp. strain S14 changes during starvation (21). The overall change leads to a total decrease of the lipid content after 24 h of starvation. The greatest changes were observed at starvation times of 3 and 5 h. This is parallel to the enhanced chemotactic response to glucose by *Vibrio* sp. strain S14 after 3 h of starvation. Changes in the lipid composition of the cell membrane toward shorter and temporarily unsaturated fatty acids may influence the chemotactic behavior at that starvation time. That the lipid composition as well as the lipid content in the cell membrane may influence activities such as uptake and chemosensory systems has been suggested by several investigators (20, 27, 29).

An increase in the numbers of motile, and therefore flagellated, cells occurred upon addition of nutrients to the 22-h-starved *Vibrio* sp. strain S14 cells. The first increase in the number of motile cells was observed after 1 h, and by 1.5 h of recovery approximately 5 to 10% of the cell population was motile.

The rate of synthesis of flagellin and its incorporation into the flagellum is reported to be 0.2 $\mu m\ min^{-1}$ for *E. coli* (14). A full-size *E. coli* flagellum is estimated to be 10 to 20 μm in length (14). If a similar rate of synthesis can be applied to *Vibrio* sp. strain S14, it would take approximately 1 to 1.5 h to synthesize a full-length flagellum. This time corresponds to that found for S14 cells to regain motility during recovery from starvation. Resynthesis of the flagellum is likely to be the case on the basis of results from TEM photographs of 24-h-starved cells. By this time, few or no flagellated cells were seen. There was no evidence of shearing of the flagellum as there were no free flagella in the surrounding area.

Vibrio sp. strain S14 cells starved for 48 h and subsequently given nutrients responded by an increase in motility after approximately 2 h of recovery. However, there was no increase in the number of motile cells observed within 5 h of recovery in a 168-h-starved cell culture. This shows that prolonged starvation includes a prolonged lag period between addition of nutrients and phenotypic expression. The observation time (5 h) may have been too short, as cells in colonies, recovered on rich medium plates after 8 weeks in a starvation medium, have been found to be motile (K. Malmcrona-Friberg and A. Goodman, unpublished observations).

When *E. coli* grows in the presence of glucose in minimal salts medium, flagellar formation is transcriptionally repressed (18). Flagellum synthesis is derepressed with increasing levels of cyclic AMP. A different result was found for *Vibrio* sp. strain S14 cells when they were subjected to energy and nutrient recovery. The addition of glucose in the recovery medium did not inhibit increased motility by *Vibrio* sp. strain S14. Glucose may, however, inhibit a chemotactic response with time of recovery in nutrient media, as by 6 h of recovery there appeared to be little chemotactic response to leucine.

Vibrio sp. strain S14 cells starved for 22 h exhibited a chemotactic response to leucine at 10^{-4} M after 2 h of recovery. The delay in chemotactic response may have been caused by a total shutdown of the chemotactic system under prolonged starvation and the time required for reformation of the system when energy became available. The appearance of the chemotactic response paralleled the restoration of motility. Therefore, it may be the lack of motility which regulates the chemosensory system.

The chemotactic response by *Vibrio* sp. strain S14 to a concentration of 10^{-4} M leucine after 2 h of recovery indicated the presence of a sensitive sensory system which was not induced or derepressed during starvation or log-phase growth. As the S14 cells continued to recover in the presence of nutrients, utilization of a chemotactic response may have become less necessary. This could be seen after 4 h of recovery, at which time the sensitivity of the response had decreased. No measurable response was observed 6 h after nutrient addition. The characteristics of the graph for the chemotactic response to leucine by *Vibrio* sp. strain S14 after addition of nutrients indicated the presence of a saturated system, unlike that displayed during starvation.

Recent results obtained in our laboratory by two-dimensional gel electrophoresis of pulse-labeled starved and recovered cells show the temporary synthesis of several proteins that are unique to the recovery period between nutrient

addition and onset of growth (3a). Components of the chemosensory system are apparently part of this unique class of temporary recovery proteins.

In contrast to results for the initial recovery period, *Vibrio* sp. strain S14 cells responded most strongly to the highest leucine concentration used (10^{-1} M) during energy and nutrient limitation. Thus, it appears that for S14 cells the kinetics of chemotaxis are different for starved and growing cells. It is possible that, as for substrate uptake systems, there may be two types of chemosensory systems in marine bacteria.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Adler, J.** 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. J. Gen. Microbiol. **74**:77–91.
2. **Adler, J., G. L. Hazelbauer, and M. M. Dahl.** 1973. Chemotaxis toward sugars in *Escherichia coli*. J. Bacteriol. **115**:824–847.
3. **Albertson, N. H., T. Nyström, and S. Kjelleberg.** 1990. Exoprotease activity of two marine bacteria during starvation. Appl. Environ. Microbiol. **56**:218–223.

3a. **Albertson, N. H., T. Nyström, and S. Kjelleberg.** 1990. FEMS Microbiol. Ecol. **70**:205–210.

4. **Alldredge, A. L.** 1989. The significance of suspended detrital aggregates of marine snow as microhabitats in the pelagic zone of the ocean, p. 108–112. *In* T. Hattori et al. (ed.), Recent advances in microbial ecology. Japan Scientific Society Press, Tokyo.
5. **Azam, F., and R. E. Hodson.** 1981. Multiphasic kinetics for D-glucose uptake by assemblages of natural marine bacteria. Mar. Ecol. Prog. Ser. **6**:213–222.
6. **Biddanda, B. A., and L. R. Pomeroy.** 1988. Microbial aggregation and degradation of phytoplankton-derived detritus in seawater. I. Microbial succession. Mar. Ecol. Prog. Ser. **42**:79–88.
7. **Chet, I., and R. Mitchell.** 1976. The relationship between chemical structure of attractants and chemotaxis by marine bacterium. Can. J. Microbiol. **22**:1206–1208.
8. **Chet, I., and R. Mitchell.** 1976. Ecological aspects of microbial chemotactic behaviour. Annu. Rev. Microbiol. **30**:221–239.
9. **Chet, I., and R. Mitchell.** 1976. An enrichment technique for isolation of marine chemotactic bacteria. Microb. Ecol. **3**:75–78.
10. **Dahl, M. K., and M. D. Manson.** 1985. Interspecific reconstitution of maltose transport and chemotaxis in *Escherichia coli* with maltose-binding protein from various enteric bacteria. J. Bacteriol. **164**:1057–1063.
11. **Davis, C. L., and F. T. Robb.** 1985. Maintenance of different mannitol uptake systems during starvation in oxidative and fermentative marine bacteria. Appl. Environ. Microbiol. **50**: 743–748.
12. **Eisenbach, M.** 1990. Functions of the flagellar modes of rotation in bacterial motility and chemotaxis. Mol. Microbiol. **4**:161–167.
13. **Geesey, G. G., and R. Y. Morita.** 1979. Capture of arginine at low concentrations by a marine psychrophilic bacterium. Appl. Environ. Microbiol. **38**:1092–1097.
14. **Hammond, S. A., P. A. Lambert, and A. N. Rycroft.** 1984. The bacterial cell surface. Kapitan Szabo Publishers, Washington, D.C.
15. **Hazelbauer, G. L., and J. S. Parkinson.** 1977. Bacterial chemotaxis, vol. 3, p. 61–91. Chapman & Hall, Ltd., London.
16. **Humphrey, B., S. Kjelleberg, and K. C. Marshall.** 1983. Responses of marine bacteria under starvation conditions at a solid-water interface. Appl. Environ. Microbiol. **45**:43–47.
17. **Jackson, G. A.** 1989. Stimulation of bacterial attraction and adhesion to falling particles in an aquatic environment. Limnol. Oceanogr. **34**:514–530.
18. **Komeda, Y., H. Suzuki, J. Ishidsu, and T. Ino.** 1975. Role of cyclic AMP in flagellation of *Salmonella typhimurium*. Mol. Gen. Genet. **142**:289–298.
19. **Kurath, G., and R. Y. Morita.** 1983. Starvation-survival physiological studies of a marine *Pseudomonas* sp. Appl. Environ. Microbiol. **45**:1206–1211.
20. **Lofgren, K. W., and F. Fox.** 1974. Attractant-directed motility in *Escherichia coli*: requirement for a fluid phase. J. Bacteriol. **118**:1181–1182.
21. **Malmcrona-Friberg, K., A. Tunlid, P. Mårdén, S. Kjelleberg, and G. Odham.** 1986. Chemical changes in the cell envelope and poly-β-hydroxybutyrate during short term starvation of a marine bacterial isolate. Arch. Microbiol. **144**:340–345.
22. **Malmcrona-Friberg, K., A. Valeur, G. Odham, and S. Kjelleberg.** 1987. A new system for estimating the bacterial chemotactic response applied to a model oligotrophic marine system. J. Microbiol. Methods **7**:45–55.
23. **Mårdén, P., T. Nyström, and S. Kjelleberg.** 1987. Uptake of leucine by a marine gram-negative heterotrophic bacterium during exposure to starvation conditions. FEMS Microbiol. Ecol. **45**:233–241.
24. **Mården, P., A. Tunlid, K. Malmcrona-Friberg, G. Odham, and S. Kjelleberg.** 1985. Physiological and morphological changes during short term starvation of marine bacterial isolates. Arch. Microbiol. **142**:326–332.
25. **Mesibov, R., and J. Adler.** 1972. Chemotaxis towards amino acids in *Escherichia coli*. J. Bacteriol. **112**:315–326.
26. **Mesibov, R., G. Ordal, and J. Adler.** 1973. The range of attractant concentrations for bacterial chemotaxis and the threshold and size of response over this range. J. Gen. Physiol. **62**:203–223.
27. **Miller, J. B., and D. E. Koshland, Jr.** 1977. Membrane fluidity and chemotaxis: effects of temperature and membrane lipid composition on the swimming behaviour of *Salmonella typhimurium* and *Escherichia coli* J. Mol. Biol. **111**:183–201.
28. **Nyström, T., P. Mården, and S. Kjelleberg.** 1986. Relative changes in incorporation rates of leucine and methionine during starvation survival of two bacteria isolated from marine waters. FEMS Microbiol. Ecol. **38**:285–292.
29. **Oliver, J. D., and W. F. Stringer.** 1984. Lipid composition of a psychrophilic marine *Vibrio* sp. during starvation-induced morphogenesis. Appl. Environ. Microbiol. **47**:461–466.
30. **Pointdexter, J. S.** 1981. Oligotrophy: feast and famine. Adv. Microb. Ecol. **5**:63–89.
31. **Poole, P. S., and J. P. Armitage.** 1988. Motility response of *Rhodobacter sphaeroides* to chemotactic stimulation. J. Bacteriol. **170**:5673–5679.
32. **Shanks, A. L., and J. D. Trent.** 1979. Marine snow: microscale nutrient patches. Limno. Oceanogr. **24**:850–854.
33. **Terracciano, J. S., and E. Canale-Parola.** 1984. Enhancement of chemotaxis in *Spirochaeta aurantia* grown under conditions of nutrient limitation. J. Bacteriol. **159**:173–178.
34. **Torrella, F., and R. Y. Morita.** 1982. Starvation induced morphological changes, motility and chemotaxis patterns in a psychrophilic marine vibrio. Publ. CNEXO (Actes Colloq. Fr.) **13**:45–60.
35. **Väätänen, P.** 1976. Microbiological studies in coastal waters of the northern Baltic Sea. I. Distribution and abundance of bacteria and yeasts in the Tvärminne area. Walter Andre de Nottbeck Found. Sci. Rep. **1**:1–56.