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Enhancement of Chemotaxis in *Spirochaeta aurantia* Grown Under Conditions of Nutrient Limitation

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***Spirochaeta aurantia* M1 cells were grown in a chemostat under conditions of energy and carbon source limitation. The chemotactic responses of the chemostat-grown cells were compared with those of *S. aurantia* cells grown in batch culture in the presence of excess energy and carbon source. Chemotactic responses were measured by determining the number of cells that entered a capillary tube containing a solution of attractant. *S. aurantia* cells grown in the chemostat under energy and carbon source limitation exhibited enhanced chemotactic responses and detected lower concentrations of attractant, as compared with cells grown in batch culture. The chemotactic response toward an attractant was specifically enhanced when that attractant was the growth-limiting energy and carbon source. The medium used contained either D-glucose or D-xylose as the sole energy and carbon source. Cells had the greatest chemotactic response toward glucose when grown at a dilution rate (D) of 0.045 h^{-1} under glucose limitation and toward xylose when grown at D = 0.06 h^{-1} under xylose limitation. When cells were grown under glucose limitation (D = 0.045 h^{-1}), they sensed concentrations of attractant (glucose) ca. 1,000 times lower than those sensed by batch-grown cells. A similar enhancement of sensing ability (toward xylose) was observed in cells grown under xylose limitation. The results indicated that *S. aurantia* cells are able to regulate their chemosensory system in response to nutrient limitation. Maximum enhancement of chemotaxis occurs in cells growing at very low concentrations of energy and carbon source. Most likely, this property provides the spirochetes with competitive advantages when the availability of nutrients becomes severely limited in their habitats.**

In natural environments, nutrients essential for the growth of bacteria are frequently present at very low concentrations. In fact, nutrient insufficiency is a condition to which microorganisms are routinely exposed in their habitats (8, 17, 19). The physiological systems that are active in bacteria under conditions of nutrient limitation are often different from those that function when nutrients are present in excess (8, 17). Physiological systems expressed by bacteria in nutrient-poor environments can be studied with cells grown under nutrient limitation in a chemostat (17).

Chemostat studies have shown that exposure of bacteria to nutrient limitation elicits adaptive changes that have the main function of allowing the cells to utilize the limiting nutrient efficiently at very low concentrations (8). Adaptive changes have been observed in nutrient uptake systems and in the levels of catabolic and anabolic enzymes (8, 12, 13). We carried out the present study to determine whether growth under nutrient limitation elicited adaptations in the chemotactic behavior of *Spirochaeta aurantia* M1, a saccharolytic, facultatively anaerobic bacterium commonly present in aquatic environments. We compared the chemotactic behavior of *S. aurantia* cells cultured in a chemostat at growth-limiting concentrations of energy and carbon source with the chemotactic behavior of *S. aurantia* cells grown in batch culture in the presence of excess energy and carbon source.

All previous investigations on bacterial chemotaxis have been performed with cells grown in batch culture, and there are no reports of studies on the chemotactic responses of bacteria in nutrient-limited environments. It seemed likely that studies on bacterial chemotactic behavior as it is expressed under conditions of nutrient limitation would yield information of ecological significance, inasmuch as such conditions are common or prevalent in microbial habitats (8, 17, 19).

(A preliminary report of part of this work has appeared previously [J. S. Terracciano and E. Canale-Parola, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, I69, p. 151].)

MATERIALS AND METHODS

Bacterial strain and growth conditions. *S. aurantia* M1 (4) was grown in a chemically defined basal medium (basal medium CD), to which either D-glucose or D-xylose was added as the only carbon and energy source. Basal medium CD had the following composition (in grams per 100 ml of distilled water): KH_2PO_4, 0.03; Na_2HPO_4, 0.15; $MgSO_4 \cdot 7H_2O$, 0.02; $MnSO_4 \cdot H_2O$, 0.002; thiamine · HCl, 5×10^{-5}; biotin, 5×10^{-7}; L-cysteine · HCl, 0.05; L-glutamic acid, 0.05; and NH_4Cl, 0.1. L-cysteine was added to basal medium CD because it was required as an exogenous growth factor by *S. aurantia* M1, and L-glutamic acid was added to basal medium CD because it was stimulatory for growth. Neither amino acid served as an energy source for growth of *S. aurantia* M1. Basal medium CD did not support growth of *S. aurantia* M1. To grow *S. aurantia* in the chemostat, either D-glucose or D-xylose was added as the growth-limiting energy and carbon source to basal medium CD at a final concentration of 6 or 5 mM (0.1 or 0.08 g/100 ml), respectively. These were the sugar concentrations in the medium contained in the chemostat reservoir. The medium used to grow *S. aurantia* in batch cultures consisted of basal medium CD, to which 0.2 g of either D-glucose or D-xylose was added per 100 ml. This amount of sugar was in excess of that utilized for growth by the spirochetes. The sugars, vitamins, and phosphate salts were added separately to basal medium CD as filter-sterilized solutions. The pH of the medium (minus additions) was adjusted to 7.5 before autoclaving.

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After autoclaving and making all of the additions, the final pH of the medium was 7.2.

Batch-grown cells were cultured in test tubes (16 × 150 mm), each containing 10 ml of basal medium CD (plus sugar). These cultures were incubated on a rotary shaker (New Brunswick Scientific Co., New Brunswick, N.J.; model VS-100) operating at 120 rpm. Incubation was continued until the cultures were in late logarithmic phase of growth (usually 24 h), at which time they showed their peak motility.

A different basal medium (basal medium BC), to which 0.2 g of D-glucose per 100 ml was added, was used for viable cell counts performed for the enumeration of cells in chemotaxis assays (see below). This medium differed from basal medium CD in that it contained 0.5 g of vitamin-free casein hydrolysate (acid hydrolysis, U.S. Biochemical Corp.) per 100 ml, instead of L-cysteine, L-glutamic acid, and NH_4Cl. Furthermore, it contained 0.75 g of agar (Difco Laboratories) per 100 ml. The filter-sterilized solutions were added, and the pH was adjusted as described above for basal medium CD. Basal medium BC, without the addition of D-glucose, did not support growth of *S. aurantia* M1.

Periodically, the culture of *S. aurantia* M1 was enriched for actively motile cells as described previously (7). Basal medium BC, to which D-glucose (0.02 g/100 ml) and only 0.5 g of agar per 100 ml were added, was used for these enrichments.

All cultures were incubated at 30°C.

Continuous culture methods. The spirochetes were cultured in a chemostat (18) with a working volume of 200 ml (culture vessel volume, 300 ml). The culture was constantly stirred with a star-head Teflon magnetic stirring bar (35 × 12 mm; Fisher Scientific Co., Medford, Mass.) operating at 400 to 600 rpm. Filter-sterilized air was blown continuously over the surface of the culture by means of an aquarium pump (Star, model 33012; Hialeah, Fla.). The temperature in the water-jacketed culture vessel was maintained at 30°C with a Haake circulator bath (model FE2; Saddle Brook, N.J.). The flow rate of fresh medium into the culture vessel was controlled by means of a peristaltic pump (Buchler Multistaltic; Buchler Instruments, Inc., Fort Lee, N.J.). Steady-state conditions were verified by determining that culture absorbance (660 nm) readings were constant after allowing the cells to grow at a given dilution rate (*D*) for at least five volume changes. Glucose limitation was verified by measurements of glucose levels in steady-state cultures (STAT-ZYME glucose reagent; Worthington Diagnostics, Freehold, N.J.). Xylose limitation was determined by measuring proportionate increases in the optical density of cultures upon addition of xylose.

Cells for chemostat inoculation were grown as described above for batch-grown cells, except that basal medium BC was used.

Chemotaxis assays. Chemotactic responses were measured by determining the number of cells that accumulated in a capillary tube containing a solution of attractant. For these measurements, we used the Adler chemotaxis assay (2) as modified for *S. aurantia* M1 by Greenberg and Canale-Parola (7). Some of the assays were carried out for 1 h, others for 20 min. The 20-min assay was used to measure chemotactic responses to peak attractant concentrations (2, 7) of cells grown at various *D*s (dilution rates) in the chemostat. For the 20-min assay only, the cells were suspended in chemotaxis buffer (7) containing 5 mM D-glucose (5, 6). This low level of glucose was used as an energy source to minimize possible differences in the ATP pools of spirochetes grown at various *D*s. The chemotaxis assays were run for 1 h for measurements of threshold concentrations (2, 3) and to compare the chemotactic responses of cells grown under energy and carbon source limitation with those of batch-grown cells. In all chemotaxis assays, the bacterial suspension contained 1.4×10^8 cells per ml. The chemotaxis assays were carried out at 25°C.

Each measurement of chemotactic response or threshold concentration was obtained by carrying out three separate chemotaxis assays in duplicate and plating the cells in duplicate plates for each individual assay. The colony counts of these 12 plates were averaged to obtain the measurement.

Chemotactic response was expressed in terms of "relative response," defined as the number of cells that accumulated in the capillary containing the attractant divided by the number of cells that accumulated in the capillary without the attractant. By expressing the data in terms of relative response, it was possible to correct for differences in motility of cell populations grown under various conditions.

The cells were examined by phase-contrast microscopy before all chemotaxis assays. From 95 to 100% of the cells had translational motility in populations grown at all *D*s tested and in batch cultures.

Competition experiments were performed as described by Adler and coworkers (1, 3).

Rates of glucose metabolism. Cell suspensions were prepared as described for cells used in chemotaxis assays (7), except that the buffer solution included 10^{-2} M D-glucose and the final cell density was 1.25×10^9 cells per ml. The cell suspensions (15 ml, in a 250-ml Erlenmeyer flask) were incubated statically at 25°C for 1 h. Samples (2 ml) were taken at 0, 20, 40, and 60 min, and were filtered within 1.5 to 2 min through filters of 0.3-μm pore diameter. After assaying the filtrate for glucose, data were plotted, using linear regression analysis. The slope of the line was taken as the rate of glucose metabolism.

Chemicals. All chemicals used were of reagent grade. 2-Deoxy-D-glucose was from Eastman Kodak. All other sugars used were from Sigma Chemical Co., St. Louis. Mo. 2-Deoxy-D-glucose contained less than 0.01 mol% of glucose, and D-xylose contained less than 0.1 mol% of glucose.

RESULTS

Enhancement of chemotaxis. *S. aurantia* M1 cells grown in continuous culture under glucose limitation showed an enhancement in their chemotactic response toward glucose as compared with batch-grown cells (Fig. 1). The chemostat-grown cells showed their greatest enhancement when the glucose concentration in the capillary was 5×10^{-2} M, which is the peak concentration (2) of glucose for *S. aurantia* M1 (7). At this concentration, cells grown in the chemostat at a *D* of 0.05 h^{-1} had a relative response four times greater than that of batch-grown cells (Fig. 1). The relative response of chemostat-grown cells to other glucose concentrations tested was also higher than that of batch-grown cells.

Cells grown at different *D*s in glucose-limited continuous culture had different relative responses to the peak concentration of glucose (Fig. 2). The *D* at which the cells exhibited their greatest chemotactic response (referred to as the "peak dilution rate") was 0.045 h^{-1} (Fig. 2). When grown at *D*s higher or lower than the peak *D*, the cells showed a marked decline in their chemotactic response to glucose. At very low *D*s (0.02 h^{-1}), the chemotactic response to D-glucose was greatly decreased or absent. The steady-state glucose concentration (13, 15) in the culture at $D = 0.045$ h^{-1} was 3.5 μM (data not shown).

The chemotactic responses of cells grown in continuous culture under D-xylose limitation were studied to determine whether an enhancement similar to that observed with glucose would occur with other sugars. D-Xylose was selected because, in *S. aurantia* M1, this sugar is detected by a chemoreceptor(s) other than the glucose chemoreceptor(s), as demonstrated by competition experiments (Table 1).

Cells grown in a chemostat under xylose limitation showed an enhancement in their chemotactic response toward this pentose as compared with cells grown in batch culture (Fig. 3). The greatest enhancement was shown by the chemostat-grown cells when the xylose concentration in the capillary was 10^{-1} M (Fig. 3), which is the peak concentration for D-xylose chemotaxis (7). For cells grown at D = 0.045 h^{-1}, strongly enhanced responses were observed only when the concentration of xylose added to the capillary was higher than 10^{-3} M (Fig. 3). In comparison, the responses toward glucose were markedly enhanced when the concentration of glucose added to the capillary was higher than 10^{-6} M (Fig. 1). Cells grown in the chemostat under xylose limitation had their maximum chemotactic response to xylose when grown at D = 0.06 h^{-1} (Fig. 4). It may be expected that in cells grown at this D value, the enhancement of chemotactic responses toward the lower concentrations of xylose would be greater, as compared with cells grown at D = 0.045 h^{-1} (Fig. 3) or at other Ds. The peak D for xylose chemotaxis differed from the peak D for glucose chemotaxis. However, either with glucose or xylose, the cells expressed their maximum chemotactic response when grown at a distinct D (the peak D).

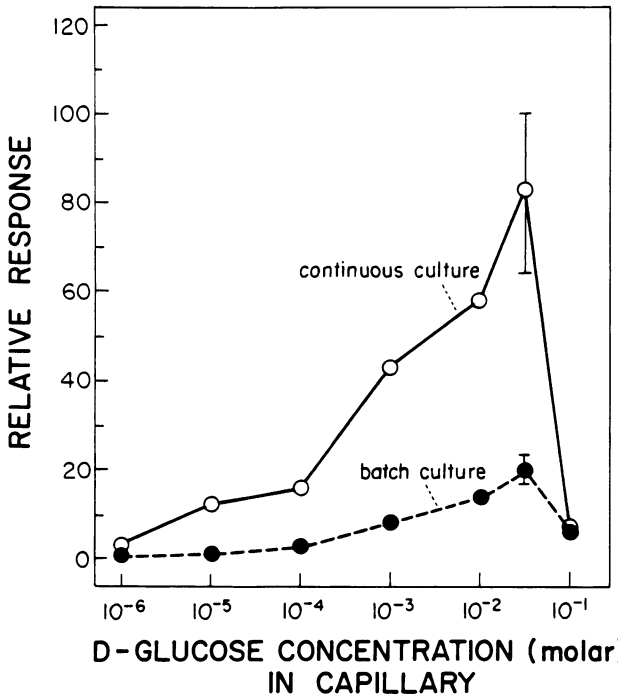

FIG. 1. Chemotactic response of *S. aurantia* M1 cells grown in glucose-limited continuous culture (D = 0.05 h^{-1}). Assays were run for 1 h. Figure shows response of cells grown in continuous culture (○) and batch culture (●). The marker bar indicates the standard deviation of six assays performed on 3 different days. Background accumulation (the capillary contained no attractant) per capillary was ca. 4,000 cells for batch-grown cells and ca. 2,500 cells for chemostat-grown cells.

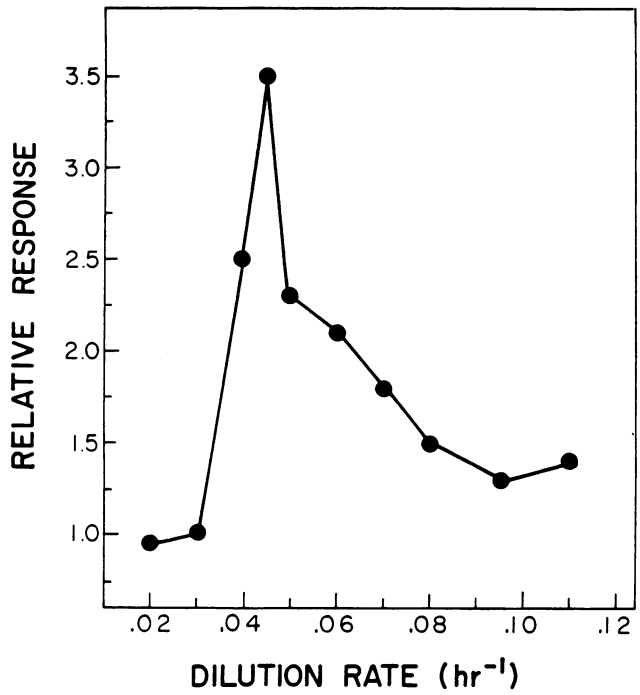

FIG. 2. Effect of D (dilution rate) on the chemotactic response of glucose-limited *S. aurantia* M1 cells. D-Glucose (5×10^{-2} M) was the attractant. Assays were run for 20 min. Background accumulation ranged from ca. 2,000 to 8,000 cells per capillary, depending on D at which the cells were grown. The average standard deviation for all assays was 11.5%.

The chemotactic response of *S. aurantia* cells to xylose was markedly greater than their response to glucose (Table 1; Fig. 2, 4).

Threshold concentration. To determine whether cells grown under glucose or xylose limitation in a chemostat responded to lower attractant concentrations than batch-grown cells, we studied the effect of glucose or xylose limitation on threshold concentrations (2). Cells of *S. aurantia* grown in batch culture with D-glucose as the energy and carbon source had a threshold concentration of 10^{-5} M glucose (Table 2). When cells were grown in glucose-limited continuous culture at the peak D for glucose chemotaxis (0.045 h^{-1}), they exhibited a threshold for D-glucose 1,000 times lower than that of batch-grown cells (Table 2). When grown at higher or lower Ds, the threshold concentration was higher than that of cells grown at the peak D but was still lower than that of batch-grown cells (Table 2). Likewise, when cells were grown in xylose-limited continuous culture,

TABLE 1. Competition experiments for D-glucose and D-xylose taxis [a]

Attractant	Competitor	Response[b]
D-Glucose	None	47,300
D-Glucose	D-Xylose	51,300
D-Xylose	None	190,000
D-Xylose	D-Glucose	180,000

[a] The attractant concentrations were: D-glucose, 5×10^{-2} M; D-xylose, 10^{-1} M. Competitor concentration was 10^{-2} M. The cells were grown in batch culture in basal medium BC, to which D-glucose (0.2 g/100 ml) was added.

[b] Number of cells attracted in capillary in 1 h. Corrected for background accumulation (ca. 3,000 cells).

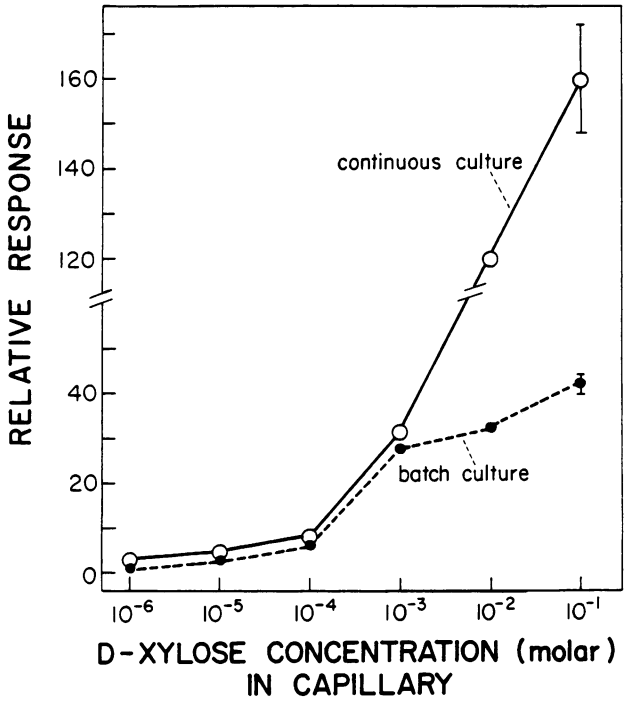

FIG. 3. Chemotactic response of *S. aurantia* M1 cells grown in xylose-limited continuous culture (D = 0.045 h^{-1}). Assays were run for 1 h. Figure shows response of cells grown in continuous culture (○) and batch culture (●). The marker bar indicates the standard deviation of six assays performed on 3 different days. Background accumulation per capillary was ca. 3,500 cells for batch-grown cells and ca. 3,000 cells for chemostat-grown cells.

they had lower threshold concentrations for xylose than cells grown in batch culture with xylose as substrate (Table 2). Cells grown at the peak D for xylose chemotaxis (0.06 h^{-1}) responded to concentrations as low as 3×10^{-9} M xylose. When cells were grown at Ds higher or lower than 0.06 h^{-1}, the threshold concentration for xylose was somewhat elevated but was still lower than that of batch-grown cells (Table 2).

Specificity of chemotaxis enhancement. *S. aurantia* M1 cells grown in the chemostat under xylose limitation had an enhanced relative response to xylose, as compared with cells grown under glucose limitation or in batch culture (Table 3). Analogous results were obtained with cells grown in the chemostat under glucose limitation. These cells exhibited an enhanced chemotactic response toward glucose, as compared with cells grown under xylose limitation or cells grown in batch culture with glucose as the energy and carbon source (Table 3). These experiments indicated that the chemotactic response toward an attractant was specifically enhanced when that attractant was the growth-limiting energy and carbon source and was not enhanced when the growth-limiting energy and carbon source was another attractant detected by a different chemoreceptor(s).

Selection for mutants. It was possible that highly chemotactic mutants were being selected when cells were grown under glucose or xylose limitation in the chemostat. Selection for these mutants may have caused the enhanced responses that were observed. Chemostat-grown cells which had an enhanced chemotactic response to the growth-limiting energy and carbon source were plated, cloned, and grown in batch culture. Their chemotactic response to the peak concentration of either glucose or xylose was not greater than that of batch-grown cells which had never been grown in continuous culture. Fifteen clones of chemostat-grown cells were tested in this manner, and all gave relative responses similar to those of batch-grown cells for glucose or xylose. These results indicated that selection for mutants was not responsible for the enhancement of chemotactic responses.

Rates of attractant metabolism. Threshold and peak concentration values may vary, depending on the rate at which the attractant is metabolized during the capillary assay (3). If *S. aurantia* cells grown at various D values or in batch culture metabolized the attractant at different rates, then the observed differences in chemotactic responses and threshold concentrations could have resulted from the different metabolic rates. To explore this possibility, we determined the rates of glucose utilization by suspensions of *S. aurantia* M1 cells grown with glucose as the energy and carbon source in batch culture and in the chemostat (Table 4). The rate of glucose utilization by cell suspensions increased moderately as the D value at which the spirochetes were grown was increased (Table 4). This pattern did not correspond to that observed when relative responses of cells grown at increasing D values were measured (Fig. 2). Also, it did not correspond to the pattern that resulted when threshold concentrations of cells grown at increasing D values were determined (Table 2). Finally, the rate of glucose utilization by batch-grown cells did not differ greatly from that of cells grown at the D value (0.045 h^{-1}) which elicited the most pronounced enhancement of chemotactic response (Table 4;

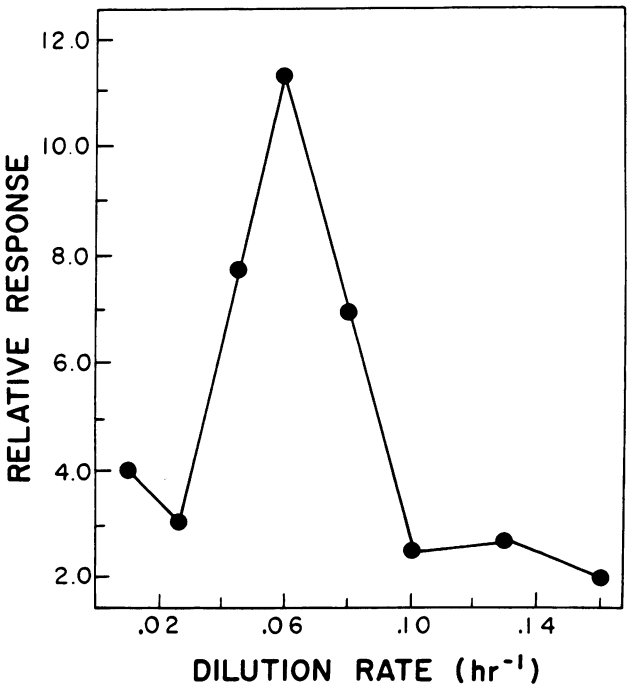

FIG. 4. Effect of D (dilution rate) on chemotactic response of xylose-limited *S. aurantia* M1 cells. D-Xylose (10^{-1} M) was the attractant. Assays were run for 20 min. Background accumulation ranged from ca. 4,000 to 12,000 cells per capillary, depending on D at which the cells were grown. The average standard deviation for all assays was 17%.

TABLE 2. Threshold concentrations for D-glucose and D-xylose

Growth condition	Threshold concentrations[a] (M) for:	
	D-Glucose[b]	D-Xylose[c]
Batch culture	1×10^{-5}	1×10^{-6}
$D = 0.02\ h^{-1}$	ND[d]	2×10^{-7}
$D = 0.025\ h^{-1}$	3×10^{-6}	ND
$D = 0.045\ h^{-1}$	1×10^{-8}	ND
$D = 0.06\ h^{-1}$	ND	3×10^{-9}
$D = 0.085\ h^{-1}$	1×10^{-7}	ND
$D = 0.10\ h^{-1}$	ND	1×10^{-7}

[a] Based on double-log plots of attractant concentration versus relative response. Assays were run for 1 h.
[b] Cells grown with glucose as energy and carbon source.
[c] Cells grown with xylose as energy and carbon source.
[d] ND, Not determined.

Fig. 2) and which corresponded to the lowest threshold concentration for glucose (Tables 2, 4).

The effect of metabolic rates on measurements of chemotaxis can be eliminated if an attractant is used which is not metabolized by the cells during the capillary assay. Thus, we measured the chemotactic responses of *S. aurantia* M1 to 2-deoxy-D-glucose, an attactant that is not metabolized by cell suspensions of this spirochete (data not shown) and that does not serve as a carbon and energy source for growth (7). First, we carried out competition experiments (1, 3), which showed that D-glucose abolished all chemotaxis toward 2-deoxy-D-glucose, whereas the latter compound abolished only 50% of chemotaxis toward D-glucose. These results indicated that *S. aurantia* M1 has at least one chemoreceptor that detects both D-glucose and 2-deoxy-D-glucose and that it has a glucose chemoreceptor(s) that does not detect 2-deoxy-D-glucose. Then, we determined the threshold concentrations and the chemotactic responses to 2-deoxy-D-glucose of (i) cells grown at various D values (D = 0.02, 0.045, 0.08 h^{-1}) under D-glucose limitation in the chemostat and (ii) cells grown in batch culture with D-glucose as the energy and carbon source. Chemostat-grown cells showed lower threshold concentrations and enhanced chemotactic responses to 2-deoxy-D-glucose, as compared with batch-grown cells. Batch-grown cells had a threshold concentration of 10^{-4} M for 2-deoxy-D-glucose, whereas cells grown at $D = 0.045\ h^{-1}$ had a threshold concentration of 10^{-6} M. Maximum chemotactic response to 2-deoxy-D-glucose was by cells grown at $D = 0.045\ h^{-1}$.

The above-mentioned studies on glucose utilization rates and on 2-deoxy-D-glucose chemotaxis indicated that the differences in chemotactic responses and in threshold concentrations observed in *S. aurantia* cells grown under different conditions were not due to differences in rates of attractant metabolism.

TABLE 3. Specificity of chemotaxis enhancement[a]

Growth condition	Energy and carbon source	Attractant[b]	Relative response[c]
Batch culture	D-Glucose or D-xylose	D-Xylose	3.95 (± 0.13)
$D = 0.045\ h^{-1}$	D-Glucose	D-Xylose	3.85 (± 0.44)
$D = 0.045\ h^{-1}$	D-Xylose	D-Xylose	7.74 (± 0.40)
Batch culture	D-Glucose	D-Glucose	2.14 (± 0.10)
$D = 0.045\ h^{-1}$	D-Xylose	D-Glucose	1.88 (± 0.05)
$D = 0.045\ h^{-1}$	D-Glucose	D-Glucose	3.50 (± 0.12)

[a] Assays were run for 20 min.
[b] Concentrations: D-glucose, 5×10^{-2} M; D-xylose, 10^{-1} M.
[c] Background accumulation: ca. 3,000 cells per capillary for batch-grown cells; 8,500 cells per capillary for glucose-limited cells; and 10,000 cells per capillary for xylose-limited cells. Data show mean (± standard deviation).

TABLE 4. Rates of D-glucose utilization by suspensions of *S. aurantia* M1 cells grown under different conditions

Growth condition[a]	Rate of D-glucose utilization[b]
$D = 0.02\ h^{-1}$	11.7
$D = 0.03\ h^{-1}$	33.0
$D = 0.045\ h^{-1}$	39.8
$D = 0.08\ h^{-1}$	42.5
$D = 0.10\ h^{-1}$	45.0
Batch culture	30.6

[a] The cells were grown in medium CD with D-glucose as energy and carbon source. After harvesting them by centrifugation, the cells were washed once and suspended in chemotaxis buffer (10^9 cells per ml) containing 10^{-2} M glucose.
[b] Rates were determined by measuring D-glucose disappearance for 1 h. Linear regression analysis was used to calculate slopes. Rates are expressed as nanomoles of D-glucose utilized per 10^9 cells per min.

DISCUSSION

When cells of *S. aurantia* were grown under energy and carbon source limitation, they exhibited enhanced chemotactic responses and detected lower concentrations of attractant, as compared with *S. aurantia* cells grown in the presence of excess energy and carbon source. These results indicate that *S. aurantia* cells are able to regulate their chemosensory system when exposed to growth substrate limitation. This type of regulation had not been previously observed in bacteria. The enhancement of chemotactic response exhibited by *S. aurantia* toward a given attractant is specific, inasmuch as it occurs when that attractant is the growth-limiting energy and carbon source, but does not occur when the growth-limiting energy and carbon source is another attractant recognized by a different chemoreceptor(s). On the other hand, our work with 2-deoxy-D-glucose (see above) indicates that when cells are grown in media in which an attractant such as glucose is the growth-limiting energy and carbon source, there occurs as enhancement of chemotaxis not only toward glucose but also toward other attractants recognized by the glucose chemoreceptor(s).

S. aurantia cells detected the lowest attractant concentrations and exhibited maximum enhancement of chemotactic response when grown at a D (peak D) that was characteristic of the growth-limiting energy and carbon source. At peak D, the steady-state concentration of growth-limiting energy and carbon source (13, 15) in the chemostat culture was very low (e.g., 3.5 μM when D-glucose was the substrate) (data not shown). These results indicate that *S. aurantia* exhibits maximum enhancement of chemotaxis when growing under conditions of extreme energy and carbon source limitation. Most likely, this property equips the spirochetes with an important competitive advantage in nature.

When *S. aurantia* cells were grown in the chemostat at D values lower than the peak D, the steady-state energy and carbon source concentration was so low that apparently the cells could not maintain a fully functional chemotactic system. Under these near-starvation conditions, cell chemotactic responses declined dramatically.

We have no information on the mechanisms by which *S. aurantia* cells regulate the magnitude of their chemotactic responses and threshold concentrations under conditions of nutrient limitation. It is possible that when the spirochetes

are present in environments containing very low concentrations of energy and carbon source, they activate regulatory processes which increase the number of chemoreceptors per cell or the effectiveness of the stimulus-receptor complex or both. These are likely possibilities, inasmuch as it has been reported that the magnitude of response to a chemoattractant is a function of either or both of these factors (3, 9–11, 14, 16).

Probably, the spirochetes we studied are not unique among bacteria in being able to enhance their chemotactic responses under conditions of energy and carbon source limitation. However, it may be expected that maximum enhancement would occur at the lowest substrate concentrations in bacteria adapted to nutrient-poor natural environments.

ACKNOWLEDGMENT

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