JOURNAL OF BACTERIOLOGY, Apr. 1977, p. 485-494
Vol. 130, No. 1

Printed in U.S.A.

Chemotaxis in *Spirochaeta aurantia*

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Received for publication 20 September 1976

Cells of *Spirochaeta aurantia* M1 suspended in isotropic buffer solution swam in nearly straight lines and appeared to spin around their longitudinal axis. Occasionally, cells stopped and flexed, and then resumed translational motility, usually in a different direction. The average cell velocity was 26 μm/s. A quantitative assay for chemotaxis was used to test various chemicals for their ability to attract *S. aurantia* M1. The cells exhibited a tactic response toward 5 × 10^{-2} M D-glucose between 10 and 35°C; the optimum response was at 25°C. At 5°C motility was not impaired, but D-glucose taxis was abolished. Chemotaxis toward D-glucose was stimulated by L-cysteine (2 × 10^{-4} M). D-Glucose, 2-deoxy-D-glucose, α-methyl-D-glucoside, D-galactose, D-fucose, D-mannose, D-fructose, D-xylose, maltose, cellobiose, and D-glucosamine were effective attractants for *S. aurantia* M1. D-Galactose taxis and D-fucose taxis were induced by the presence of D-galactose in the growth medium. The amino acids tested did not serve as attractants. Growing cells of *S. aurantia* M1 exhibited an aerotactic response.

Many flagellated bacteria are chemotactic, that is, they move towards, or away from, chemical stimulants (2, 16, 17, 38, 39). Specific chemoreceptors, which serve to identify chemicals acting as attractants or repellents, are present in these bacteria (3). In response to a chemical stimulus, the bacterial cells alter their normal pattern of motility. For example, cells of *Escherichia coli* or *Salmonella typhimurium* normally swim in smooth lines that are occasionally interrupted by tumbles (7, 30). The tumbles are followed by smooth swimming in a new, randomly chosen direction (7, 30). Cells moving up a concentration gradient of a chemoattractant tumble less frequently than cells moving down the gradient (7, 30). Thus, more time is spent swimming up a gradient than down a gradient of attractant. It has been shown that tumbling results from reversal of the normal direction of flagellar rotation (28).

Spirochetes are motile bacteria that do not have flagella. Spirochetal cells are usually helical and possess a coiled "protoplasmic cylinder" consisting of the protoplasmic and nuclear regions, surrounded by a membrane-cell wall complex. Wrapped around the protoplasmic cylinder are structures called "axial fibrils" or "axial filaments," which vary in number from 2 to more than 100 in different spirochetes (11). One end of each axial fibril is inserted near one pole of the protoplasmic cylinder, whereas the other end is not inserted. Inasmuch as each axial fibril is wrapped around most of the length of the protoplasmic cylinder, axial fibrils inserted near one end of the protoplasmic cylinder overlap, in the central region of the cell, with axial fibrils inserted near the opposite end. Both the protoplasmic cylinder and the axial fibrils are enclosed by a three-layered membrane called the "outer sheath" or "outer cell envelope." Thus, axial fibrils differ significantly from bacterial flagella in that they are entirely endocellular organelles. It has been suggested that axial fibrils may play a role in spirochetal motility (6, 21, 23, 32), because they are similar in fine structure and chemical composition to bacterial flagella (8, 9, 20–23, 29, 32). Some investigators have referred to these structures as "endoflagella" or "flagella" (21, 40). Direct experimental evidence for the involvement of axial fibrils in motility is lacking.

Spirochetes perform flexing movements and appear to rotate around their longitudinal axis as they swim in liquids. It has been reported that the number of translating cells and the velocity of translation of the spirochete *Leptospira interrogans* (*biflexa*) are enhanced in highly viscous solutions (24). In contrast, solutions of similar viscosity greatly impair the motility of *E. coli* and *Spirillum serpens* (34). In addition to swimming in liquids, at least some spirochetes perform "creeping" or "crawling" movements on solid surfaces (10, 15). Attempts to interpret the mechanisms of motility in spirochetes have been made, but there is little experimental evidence in support of the proposed theories (6).

The purpose of the investigation reported here was to study the motility and especially the chemotactic behavior of *Spirochaeta auran-*

tia, a facultatively anaerobic spirochete commonly found in aquatic environments (12). In a previous paper, it was reported that growing cells of *S. aurantia* exhibited chemotaxis towards glucose (12). Except for that report, essentially no information on chemotaxis of spirochetes was available at the time we began our studies. We believed that such information, as well as experimental observations on the motility of spirochetes, would be of value in gaining insights into the ecology and evolutionary history of these bacteria.

(A preliminary account of this work was presented at the 76th Annu. Meet. Am. Soc. for Microbiol., Atlantic City, N.J., 1976.)

MATERIALS AND METHODS

Bacteria and media. The organism used was *S. aurantia* M1 (12). As determined by electron microscopy, this organism had the morphological features typical of spirochetes (11), that is, it possessed axial fibrils and a protoplasmic cylinder both surrounded by an outer sheath (Fig. 1). Generally, cells had two axial fibrils, each inserted near one end of the protoplasmic cylinder and overlapping the other fibril in a 1-2-1 arrangement. A small percentage of cells exhibited a 2-4-2 arrangement, with two axial fibrils inserted at two distinct locations near each end of the cell.

As recommended by Adler (2), the culture of *S. aurantia* M1 was enriched at the onset of this investigation, for actively motile cells. This was accomplished by allowing cells to migrate on plates of CT medium (12), to which 0.02% (wt/vol) D-glucose was added, and then picking cells from the edge of the migrating population (12).

Unless otherwise specified, *S. aurantia* M1 was cultured routinely in glucose-Trypticase-yeast extract (GTY) medium, which contained 0.2 g of D-glucose, 0.5 g of Trypticase (BBL), and 0.2 g of yeast extract (Difco) per 99 ml of distilled water. After the pH had been adjusted to 7.5 with KOH, the medium was autoclaved and then allowed to cool to room temperature. Finally, 1 ml of sterile 1 M potassium phosphate buffer (pH 7.0) was added to the medium.

Cultural conditions. Cells for motility and chemotaxis experiments were grown in stationary tube cultures (5 ml of GTY medium per test tube [16 by 150 mm]). Unless otherwise specified, incubation was in air atmosphere at 30°C, and cells were harvested from cultures in the late-logarithmic phase by centrifugation (3,000 × *g*) at room temperature (22 to 24°C) after 44 h of incubation (final culture density; 5×10^8 cells/ml). The inoculum (0.1 ml) for these cultures was from similar 5-ml cultures that had been inoculated with cells grown on a plate of GTY agar medium (GTY medium plus 0.75%, wt/vol agar [Difco]) and then incubated for 24 h.

Sugars used to supplement GTY medium or sugars replacing D-glucose in GTY medium were

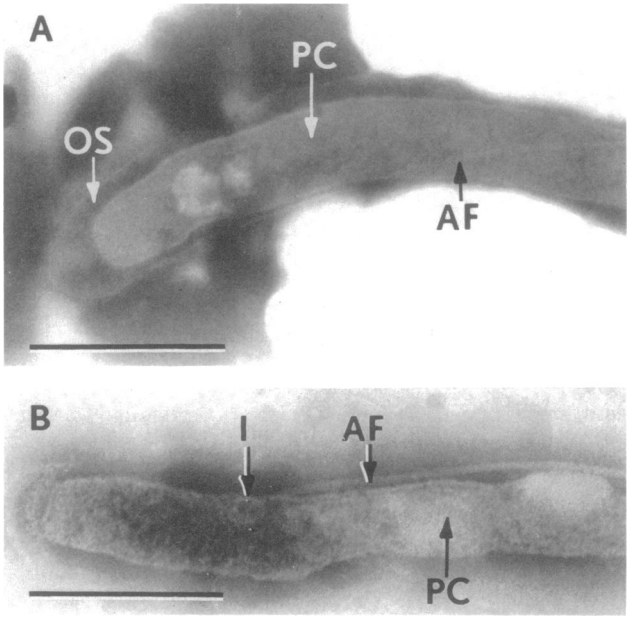

FIG. 1. *Electron micrographs of portions of cells of S. aurantia M1. Negative stain preparations. (a) The stain employed was ammonium molybdate (2%, wt/vol, pH 7.5). Note the outer sheath (OS), the protoplasmic cylinder (PC), and the axial fibril (AF). (b) The cell was stained with potassium phosphotungstate (1%, wt/vol, pH 7.0). This treatment destroys the OS and reveals the subterminal insertion disk (I), which anchors the axial fibril (AF). (Bars represent 0.5 μm).*

present at a final concentration of 0.2% (wt/vol). Cultures in which D-mannitol replaced D-glucose were incubated for 72 h before harvesting.

S. aurantia M1 was maintained by growing the cells on plates of GTY agar medium and then storing the plate cultures at 5°C. These cultures were transferred monthly.

Preparation of cell suspensions. Cells harvested from 3-ml batches of cultures were washed once by suspending them in 3 ml of chemotaxis buffer (0.01 M potassium phosphate buffer, pH 7.0, containing 2×10^{-4} M L-cysteine-hydrochloride). This buffer was prepared shortly before it was needed, with distilled water. After centrifugation, the washed cells were suspended to a density of approximately 1.4×10^8 cells/ml, unless otherwise specified, for use in motility and chemotaxis studies.

Motility measurements. Cell suspensions were used to fill chambers prepared by placing a glass cover slip over two other glass cover slips spaced a few millimeters apart on a microscope slide. Motility of cells in these chambers was observed at room temperature by phase-contrast microscopy. Velocities were measured by determining the time required by individual cells to travel 45 μm. This was accomplished using an eyepiece micrometer and a stopwatch graduated in 0.1 s. Average velocities were calculated by the method of Kaiser and Doetsch (24). Slides and cover slips for these experiments were soaked in fuming nitric acid (90%) for 1 h or longer and then thoroughly rinsed in distilled water.

Chemotaxis assay. The method for measuring chemotaxis was similar to that described by Adler (2). The U-shaped tubes, microscope slides, and cover slips used were acid-washed by soaking them in fuming nitric acid as described above. Unless otherwise specified, the assays were carried out at 25°C for 1 h, and the cell suspensions contained 1.4×10^8 cells/ml. The chemotaxis buffer is described above. In all experiments, a blank (no attractant present), and a positive control (5×10^{-2} M D-glucose in capillary) were included.

After incubation for 1 h (or for different time periods when specified), the contents of the capillaries were transferred to GTY medium from which the potassium phosphate had been omitted. Appropriate dilutions were prepared, and then 0.1-ml samples were mixed with 2-ml volumes of melted GTY agar medium at 45°C and poured onto GTY agar medium plates. Colonies were counted after the plates had been incubated at 30°C for 5 days. Results are based on averages of duplicate plate counts on each of two separate capillary assays.

The following terms, used in this paper, were defined by Mesibov and Adler (31): response, blank, concentration response curve, peak response, peak concentration, and threshold concentration. The term "effective attractant" is used to describe a chemical with a threshold concentration of 10^{-4} M or lower (38).

Microscopy. Negatively stained specimens of *S. aurantia* M1, washed and suspended in chemotaxis buffer, were prepared and examined by electron microscopy by using techniques described previously (14). Dark-field micrographs of aerotactic bands of *S. aurantia* M1 were taken on photographic film (Kodak 35-mm Tri-X) using a light microscope (Leitz Labolux) equipped with a darkfield condenser (Leitz).

Chemicals. Sugars, sugar analogues, and amino acids were obtained from various commercial sources. Most of the commercial sugar and sugar analogue preparations were analyzed for glucose contamination by means of glucose oxidase assays (Glucostat, Worthington Biochemical Corp., Freehold, N.J.). Commercial preparations that contained less than 0.01 mol% glucose included D-fructose, D-mannitol, D-ribose, 2-deoxy-D-glucose, and D-fucose. Commercial preparations that contained between 0.01 and 0.1 mol% glucose included cellobiose, D-xylose, D-sorbitol, D-glucosamine, and D-gluconate. Commercial preparations that were contaminated with more than 0.1, but less than 0.2, mol% glucose included α-methyl-D-glucoside, β-methyl-D-glucoside, D-galactose, and D-mannose. The commercial source of maltose contained approximately 1 mol% contaminating glucose.

RESULTS

Motility of *S. aurantia* M1. Microscopic examination revealed that cells grown in GTY medium were motile throughout the logarithmic phase of growth, an indication that motility of *S. aurantia* M1 is not subject to glucose repression. After being washed and suspended in chemotaxis buffer, or chemotaxis buffer without cysteine, over 99% of the cells used in our experiments retained translational motility for at least 2 h. The average velocity of cells translating through chemotaxis buffer was 26 μm/s. The range of velocities was from 23 to 30 μm/s. Translational motility was not enhanced by increasing the viscosity of the chemotaxis buffer with either methylcellulose or polyvinylpyrrolidone (E. P. Greenberg and E. Canale-Parola, manuscript in preparation).

The behavior of *S. aurantia* M1 cells suspended in isotropic chemotaxis buffer may be described as follows. The organisms generally swim in straight lines or nearly straight lines, and they appear to spin rapidly about their longitudinal axis as they translate through the buffer. Occasionally, a cell stops momentarily and flexes, and then resumes spinning and translational motility. However, when translation resumes, the direction of movement is usually altered, and often the previously anterior cell end becomes the posterior end of the cell. The duration of a flexing motion (a flex) varies from a fraction of a second for some flexes to several seconds for other flexes.

Conditions for chemotaxis. Accumulation of cells in capillaries containing 5×10^{-2} M D-glucose was greatest when *S. aurantia* M1 was

harvested from late-exponential-phase cultures. However, average cell velocity and percentage of motile cells in these populations were similar to those of populations harvested from early-exponential-phase cultures, as judged by microscopic examination and by performing motility assays (1).

When suspensions containing 1.4×10^8 cells/ml were used for chemotaxis experiments, after a short lag period the rates of accumulation of cells in the capillaries were linear for at least 60 min, whether no attractant was present or D-glucose (5×10^{-2} M or 10^{-3} M) was the attractant (Fig. 2).

The tactic response to 5×10^{-2} M D-glucose was directly proportional to the bacterial concentration over the range tested (Fig. 3). Likewise, the number of bacteria in the capillary was directly proportional to the bacterial concentration when the attractant was absent. When the concentration of D-glucose was lowered to 10^{-3} M, the response was proportional over a limited range only (Fig. 3), and a plateau was reached at cell concentrations above 1.5×10^8 cells/ml. Similar plateaus have been observed with various flagellated bacteria and different attractants, but in all of these cases, the response leveled off at substantially lower bacterial concentrations (2, 38, 39). In view of the results of these experiments, our chemotaxis assays were carried out using cell suspensions containing 1.4×10^8 cells/ml. It should be noted that, with other attractants, the relationships between bacterial concentration, time, and the size of the tactic response might be different from those observed with D-glucose.

The optimum response of *S. aurantia* M1 to 5×10^{-2} M D-glucose was at 25°C (Fig. 4). This temperature was adopted as the standard temperature for all other chemotaxis assays. At 5°C, the chemotactic response was completely inhibited, but motility was not impaired at this temperature (Fig. 4).

When *S. aurantia* M1 was washed and suspended in 0.01 M phosphate buffer (pH 7.0) with no L-cysteine added, D-glucose taxis could be demonstrated, but results were erratic (Fig.

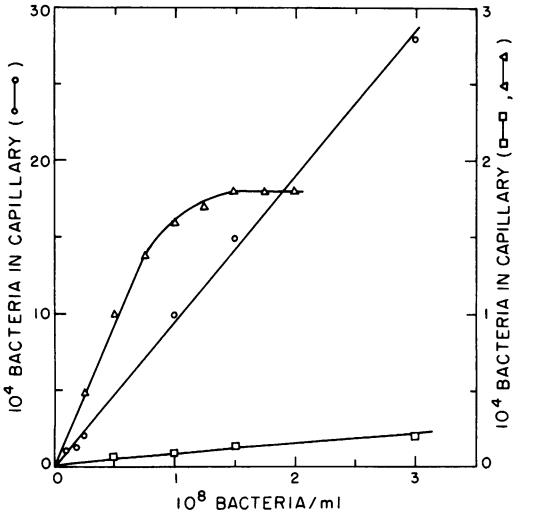

FIG. 3. *Effect of bacterial concentration on chemotaxis with attractant: 5×10^{-2} M D-glucose* (○); *10^{-3} M D-glucose* (△); *no attractant present* (□).

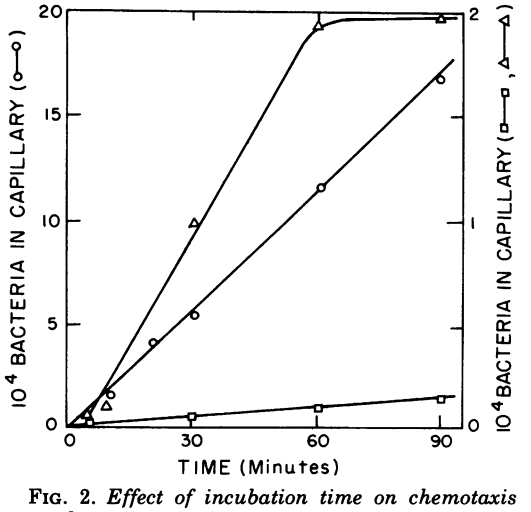

FIG. 2. *Effect of incubation time on chemotaxis towards 5×10^{-2} M D-glucose* (○) *and 10^{-3} M D-glucose* (△); *no attractant* (□).

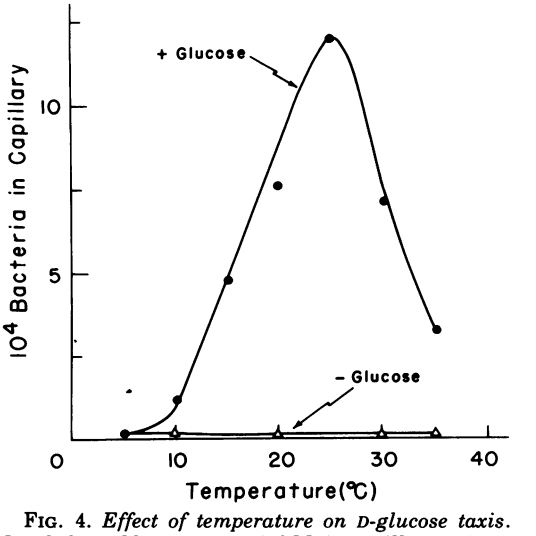

FIG. 4. *Effect of temperature on D-glucose taxis. Symbols: D-Glucose, 5×10^{-2} M, in capillary* (●); *no attractant* (△).

5). For example, when the attractant was 10^{-3} M D-glucose, several of the responses were not significantly different from responses observed in the absence of attractant. Addition of 2×10^{-4} M L-cysteine to the wash buffer, as well as to the buffer in the cell suspensions and the capillary, resulted in greater accumulations of *S. aurantia* M1 in capillaries containing D-glucose (Fig. 5). The typical response was 2 to 15 times greater than the response obtained with cells from the same culture in the absence of L-cysteine (Fig. 5). Apparently, this effect was not due to a stimulation of motility by L-cysteine, since the average velocity of the cells was similar in buffer with or without cysteine. The reproducibility of the chemotaxis assay was markedly improved when L-cysteine was added to the buffer solution. Thus, the range of responses to 5×10^{-2} M D-glucose was between 0.8×10^5 and 2.1×10^5 bacteria per capillary, and the standard deviation was 30%, in 20 assays performed in the presence of L-cysteine using 10 separate cultures on 10 different days. In similar assays, using 10^{-3} M D-glucose as attractant, the standard deviation was 28%. In contrast, when L-cysteine was not included in the buffer, the standard deviation was 70% whether 5×10^{-2} M or 10^{-3} M D-glucose was the attractant. L-Methionine, L-cystine, or glycine, at various concentrations, did not replace L-cysteine.

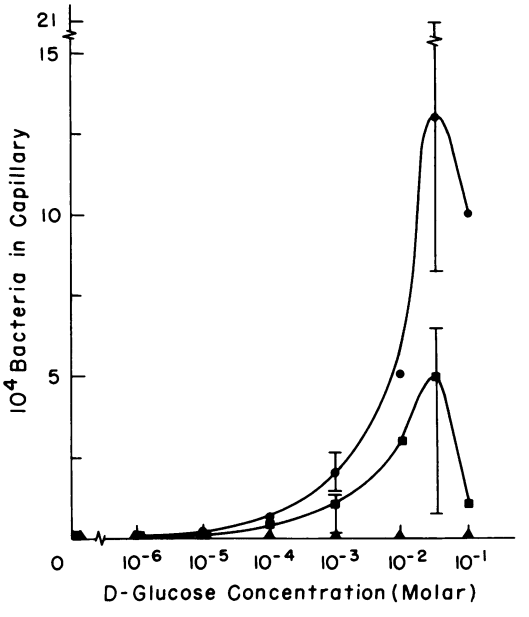

FIG. 5. *Stimulation of D-glucose taxis by L-cysteine. Response to D-glucose in the presence of* 2×10^{-4} *M L-cysteine in the chamber and capillary (●); background accumulation was ca. 1,200 bacteria per capillary. Response to D-glucose in the absence of L-cysteine from the chamber and capillary (■). Response in the presence of D-glucose at equal concentration in the cell suspension and in the capillary (▲), with L-cysteine (*2×10^{-4} *M) added. The marker bars indicate the range of responses in 10 assays performed on 5 different days using five separately grown cultures.*

In one experiment in which six capillaries were used to measure the response to 5×10^{-2} M D-glucose the range of the number of cells accumulating per capillary was 1.16×10^5 to 1.38×10^5, with a mean of 1.29×10^5 and a standard deviation of 12%.

When D-glucose was present in both the capillary and bacterial suspension at equal concentration, there was no accumulation of *S. aurantia* M1 over the background accumulation (Fig. 5). These experiments showed that the response to D-glucose was a response to a concentration gradient of this attractant and not merely a consequence of stimulation of cell motility in the presence of D-glucose.

Attraction to sugars and other compounds. Various compounds were surveyed for their ability to elicit a tactic response from *S. aurantia* M1 (Table 1). Peak responses, peak concentrations, and threshold concentrations for each of the compounds screened were determined from concentration response curves such as those shown in Fig. 5 and 6. In addition, threshold concentrations were more accurately determined by extrapolating double logarithmic plots of concentration versus response (5).

D-Glucose served as an effective attractant, whereas L-glucose did not (Table 1). The striking difference in the response to these two sugars demonstrates that D-glucose taxis is not due to an osmotic gradient. The D-glucose analogues, 2-deoxy-D-glucose and α-methyl-D-glucoside, which are not used by *S. aurantia* M1 as energy sources for growth, served as effective attractants (Table 1). This indicates that, in *S. aurantia* M1, metabolism of the attractant is not required for chemotaxis, as has been shown previously with *E. coli* (1, 5).

Cells for the D-galactose and D-fucose experiments (Table 1) were grown in GTY medium in which 0.2% (wt/vol) D-galactose replaced D-glucose. Both D-galactose and D-fucose, which is an analogue of D-galactose, were effective attractants (Table 1). However, tactic responses to D-galactose and D-fucose were negligible when D-glucose was the sole energy source added to the medium. To determine whether the responses to D-galactose and D-fucose were induced by growth in the presence of D-galactose, or repressed by growth in D-glucose, cells were

TABLE 1. *Survey of sugars, sugar analogues, and amino acids*[a]

Sugars, sugar analogues, and amino acids[b]	Growth (h for doubling)	Chemotaxis		
		Threshold(M)	Peak(M)	Peak response (no. of bacteria attracted)[c]
D-Glucose*	6	10^{-5}	5×10^{-2}	130,000
L-Glucose	NG[d]	5×10^{-4}	5×10^{-2}	20,000
2-Deoxy-D-glucose*	NG	10^{-4}	10^{-1}	40,000
α-Methyl-D-glucoside*	NG	10^{-4}	10^{-1}	40,000
β-Methyl-D-glucoside	40	10^{-3}	10^{-1}	15,000
D-Galactose[e]*	24	10^{-5}	5×10^{-2}	140,000
D-Fucose[e]*	NG	5×10^{-5}	10^{-1}	120,000
6-Deoxy-D-glucose	NG	2×10^{-4}	10^{-1}	40,000
6-Deoxy-D-glucose[e]	NG	2×10^{-4}	10^{-1}	40,000
D-Mannose*	30	4×10^{-6}	10^{-2}	240,000
L-Sorbose	NG	5×10^{-4}	10^{-2}	30,000
D-Fructose*	32	2×10^{-7}	5×10^{-2}	22,000
D-Xylose*	14	10^{-6}	10^{-1}	280,000
D-Ribose	NG	2×10^{-3}	10^{-1}	12,000
Maltose*	14	5×10^{-6}	5×10^{-2}	70,000
Cellobiose*	6	5×10^{-7}	10^{-4}	21,000
D-Mannitol	20	10^{-2}	10^{-1}	7,000
D-Sorbitol	NG	2×10^{-4}	10^{-1}	20,000
D-Glucosamine*	NG	5×10^{-6}	10^{-2}	250,000
D-Gluconate		10^{-1}	10^{-1}	7,000
Glycine	NG			1,700
L-Serine	NG	10^{-2}	10^{-1}	4,000
L-Aspartate	NG			2,000
L-Asparagine	NG			2,000
L-Tryptophan	NG			1,500
L-Valine	NG			2,000
L-Methionine	NG			1,800

[a] Chemicals were tested over the range 10^{-7} to 10^{-1} M (except for L-tryptophan, which was tested over the range 10^{-7} to 10^{-2} M).

[b] Effective attractants are indicated with an asterisk (those attractants with threshold values of 10^{-4} M or lower).

[c] Values are not corrected for background (ca. 1,800).

[d] NG, No detectable growth as determined turbidimetrically.

[e] Growth medium was GTY in which D-galactose replaced D-glucose as the energy source.

grown in the presence of D-glucose, D-galactose, or both sugars together. There was no appreciable response to D-galactose when the cells were grown with D-glucose as the sole energy source (Fig. 6). Cells grown in the presence of D-galactose, or both D-galactose and D-glucose, recognized D-galactose as an attractant (Fig. 6). Thus, it appears that D-galactose, or a metabolite of D-galactose, serves to induce D-galactose taxis in *S. aurantia* M1. In similar experiments, the addition of D-galactose to the growth medium resulted in induction of D-fucose taxis.

In *E. coli*, 6-deoxy-D-glucose is recognized by the D-galactose chemoreceptor and is an effective attractant (5). In contrast, the response of *S. aurantia* M1 to 6-deoxy-D-glucose is minimal (Table 1) and may be attributed to D-glucose contamination of the commercial 6-deoxy-D-glucose preparation (see Materials and Methods). Furthermore, 6-deoxy-D-glucose taxis is not induced by growth of *S. aurantia* M1 in the presence of D-galactose (Table 1). Apparently, the D-galactose receptor in *S. aurantia* M1 has a specificity different from that of *E. coli*.

D-Mannose, D-fructose, D-xylose, maltose, cellobiose, and D-glucosamine were effective attractants (Table 1). Tactic responses to these attractants were not stimulated when cells were grown in media containing the attractant as the sole source of energy (for those attractants that served as energy sources), or when cells were grown in the presence of both the attractant and D-glucose.

D-Ribose taxis was not induced by growth of *S. aurantia* M1 in media supplemented with D-ribose. It is possible that the slight attraction to D-ribose (Table 1) was due to small quantities of D-xylose contaminating the commercial preparations. D-Mannitol was not an effective attractant (Table 1), even when *S. aurantia* M1

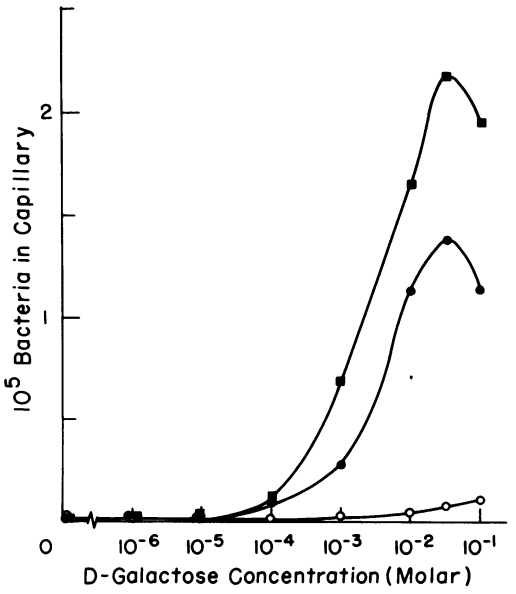

FIG. 6. *Concentration response curves for* D-*galactose taxis. Symbols: Cells grown in the presence of* D-*glucose* (○); *cells grown in the presence of both* D-*galactose and* D-*glucose* (■); *cells grown in the presence of* D-*galactose* (●).

was grown in the glucose medium (GTY) supplemented with D-mannitol or in a medium in which D-mannitol replaced D-glucose. This is a situation in which a growth substrate does not seem to attract the cells. The opposite situation was found with D-glucosamine, which did not support growth of *S. aurantia* M1, but served as an effective attractant (Table 1).

Several amino acids were surveyed, but none attracted *S. aurantia* M1 in our assays (Table 1). Both L-methionine and L-aspartate were screened, not only in the standard chemotaxis buffer but also in buffer from which L-cysteine had been omitted. Under either condition, these amino acids did not serve as attractants for *S. aurantia* M1. The slight attraction observed in the L-serine experiment (Table 1) is probably due to contamination of the commercial preparation of this amino acid.

Aerotaxis in *S. aurantia* M1. It was observed that *S. aurantia* M1 cells, grown statically in GTY medium tubes, accumulated approximately 1 cm from the air-medium interface, forming a distinct band. After the cultures were shaken to disperse the cells, a new band formed in the same location within 1 h. Similarly, narrow bands of cells accumulated at some distance from air bubbles trapped in wet-mount preparations of 40-h cultures of *S. aurantia* M1 (Fig. 7). Cells within these narrow bands appeared to be constantly flexing. Possibly, the cells were repelled by oxygen concentrations higher than those found in the band (e.g., oxygen concentrations near the air bubble), but attracted by the oxygen concentrations present in the region where the band was located. Analogous observations have been made with spirilla (27). It should be noted that the banding of *S. aurantia* M1 was not observed when cultures were very young (24 h), or when cells were suspended in chemotaxis buffer or in chemotaxis buffer without L-cysteine.

DISCUSSION

The behavioral repertoire of *S. aurantia* M1 cells suspended in isotropic chemotaxis buffer includes smooth swimming in nearly straight lines and flexes of variable duration. Inasmuch as flexes are followed by changes of translational direction, it is possible that they are analogous to the tumbles (twiddles) of flagellated bacteria (7, 30). Likewise, the smooth swimming of the spirochetes may correspond to the runs of flagellated bacteria (7, 30). It remains to be demonstrated experimentally whether the frequency of flexes is modulated as are the tumbles of flagellated bacteria in gradients of attractants or repellents (7, 30). The observation that cells in aerotactic bands of *S. aurantia* M1 were constantly flexing supports the possibility that alterations in the frequency of flexing occur in response to stimuli.

Chemotaxis of *S. aurantia* M1 towards D-glucose was stimulated by L-cysteine (Fig. 5). We have not determined whether the stimulation by L-cysteine is specific for D-glucose taxis or whether it extends to taxes for other compounds. Furthermore, the biochemical bases for this stimulation are not yet clear. This effect was not observed when other amino acids replaced L-cysteine. Thus, the sulfhydryl group of cysteine may be important in maintaining the D-glucose chemoreceptor (and possibly other chemoreceptors) in a reduced state, or some component involved in all chemotactic responses of *S. aurantia* M1 may require the reducing potential provided by L-cysteine. *S. aurantia* M1 appears to be repelled by atmospheric tensions of O_2 (Fig. 7). Possibly, a relationship exists between this repelling effect of O_2 and the observed stimulation of D-glucose taxis by L-cysteine. This seems unlikely, however, since the aerotactic response was not observed when cells were suspended in chemotaxis buffer or chemotaxis buffer without L-cysteine. Another possibility is that the role of L-cysteine in chemotaxis of *S. aurantia* M1 is similar to that of L-methionine in chemotaxis of

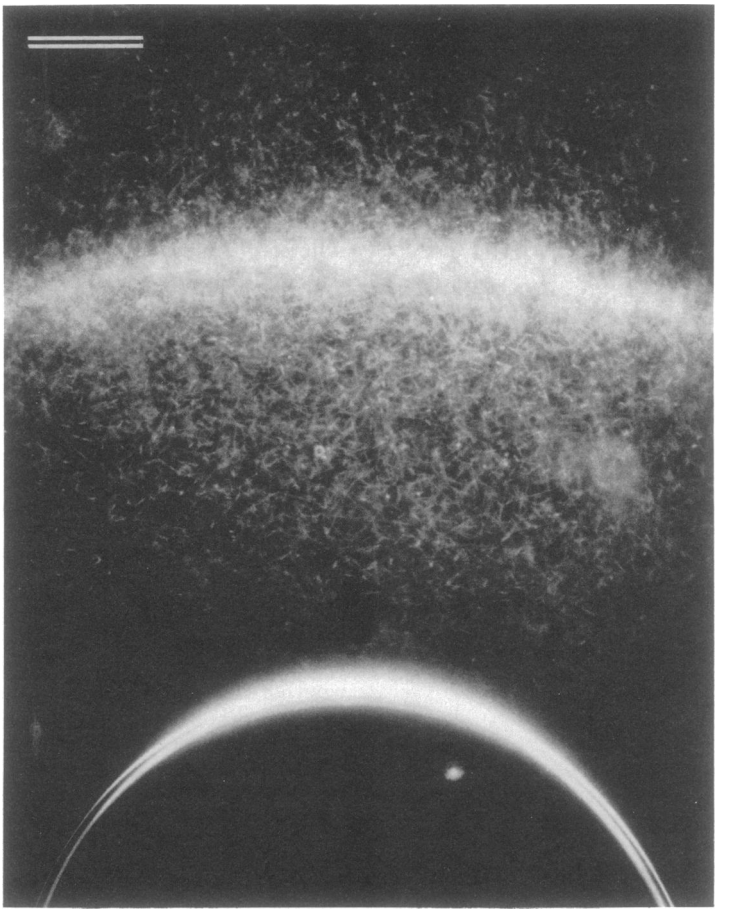

FIG. 7. *Dark-field photomicrograph of a band of S. aurantia M1 cells that had accumulated approximately 2 mm from an air bubble trapped under a glass cover slip. The wet mount was prepared by adding a drop of a 40-h GTY culture to a microscope slide, covering with a no. 1 cover slip, and sealing the edges with wax. (Bar indicates 0.5 mm.)*

E. coli (2, 4, 26, 37). It should be noted that chemotaxis of *Bacillus subtilis* towards amino acids (18), as well as transport of amino acids by this species (25), are inhibited by L-cysteine. Inhibition of transport is probably due to interaction between the sulfhydryl group of L-cysteine and a membrane component needed for active transport (25).

It was demonstrated that D-glucose, 2-deoxy-D-glucose, α-methyl-D-glucoside, D-galactose, D-fucose, D-mannose, D-fructose, D-xylose, maltose, cellobiose, and D-glucosamine served as attractants for *S. aurantia* M1 (Table 1). These attractants are either used by *S. aurantia* M1 as energy sources for growth or are closely related, structurally, to energy sources for growth of *S. aurantia* M1 (Table 1). However, not all energy sources (e.g., D-mannitol) were effective attractants for *S. aurantia* M1 (Table 1). The amino acids tested did not serve as attractants for *S. aurantia* M1 (Table 1). *S. aurantia* is an eminently saccharolytic organism; it does not derive energy for growth by dissimilating amino acids (12).

The threshold and peak concentrations of cellobiose were strikingly lower than those of other attractants (Table 1). The only compound with a comparable threshold concentration, but with a much higher peak concentration, was D-fructose (Table 1). In addition to being a strong attractant for *S. aurantia* M1, cellobiose supports high growth yields of this spirochete (J.A. Breznak, Ph.D. dissertation, University of Massachusetts, Amherst, 1971), and the doubling time of *S. aurantia* M1 is relatively short when this sugar is the energy source (Table 1). It has been found that numerous anaerobic and facultatively anaerobic spirochetes use cello-

biose as a growth substrate (13). This is interesting, because spirochetes thrive in anaerobic environments in which cellulose is degraded, e.g., in the rumen, in guts of termites and wood-eating cockroaches, in salt water marshes, and in the muds of lakes and ponds (11). Spirochetes have not been reported to degrade cellulose, but during biological cellulose breakdown in natural environments, cellobiose released by the action of other organisms' extracellular cellulases may be utilized by the spirochetes. Presumably, the levels of free cellobiose present in environments in which cellulose is degraded are quite low (36). The possession of a strong tactic response to very low cellobiose concentrations would offer spirochetes an advantage in competing successfully with other cellobiose-utilizing organisms. Furthermore, since low levels of cellobiose inhibit the activity of at least some cellulases (36), spirochetes may play a role in enhancing the rate of cellulose degradation in natural environments. Inasmuch as decomposition of cellulose seems to be the limiting step in many ecosystems (33, 35), enhancement of cellulose digestion would be important in the functioning of microbial food chains that depend on cellulose degradation.

ACKNOWLEDGMENTS

We are grateful to C.D. Cox for help with dark-field photomicroscopy and S.C. Holt for assistance with electron photomicroscopy.

This investigation was supported by Public Health Service training grant GM-02168 from the National Institute of General Medical Sciences, and by Public Health Service grant AI12482 from the National Institute of Allergy and Infectious Diseases.

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