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Motility and Chemotaxis of *Spirochaeta aurantia*: Computer-Assisted Motion Analysis

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A computer program has been designed to study behavior in populations of *Spirochaeta aurantia* cells, and this program has been used to analyze changes in behavior in response to chemoattractants. Three kinds of behavior were distinguished: smooth swimming, flexing, and reversals in direction of swimming after a short pause (120 ms). Cell populations exposed to chemoattractants spent, on average, 66, 33, and 1% of the time in these modes, respectively. After the addition of a chemoattractant, behavior was modified transiently—smooth swimming increased, flexing decreased, and reversals were suppressed. After addition of D-xylose (final concentration, 10 mM), the adaptation time (the time required for the populations to return to the unmodified behavior) for *S. aurantia* was 1.5 to 2.0 min. A model to explain the behavior of *S. aurantia* and the response of cells to chemoattractants is described. This model includes a coordinating mechanism for flagellar motor operation and a motor switch synchronizing device.

Spirochetes are relatively long, slender, helical bacteria that have flagella contained within the outer membrane, where they wrap around the peptidoglycan layer of the cell cylinder (9, 10, 18). *Spirochaeta aurantia* has two flagella which are inserted through the peptidoglycan into the cytoplasmic membrane at opposite poles of the cell (6; see Fig. 1). Except for location, the *S. aurantia* periplasmic flagella appear analogous to other bacterial flagella; they propel the cell by rotation driven by a proton motive force (2, 13). *S. aurantia* cells generally swim in relatively straight lines (runs), but occasionally they reverse swimming direction, the cell anterior becoming posterior, and often they stop running and flex. The duration of a flex can vary from a fraction of a second to several seconds (16, 17).

Berg (2) proposed a model to explain the behavior of *S. aurantia*. This model requires three assumptions for which there is strong supportive evidence: that the peptidoglycan-bound cell cylinder is semirigid, that the flagella rotate, and that the outer membrane is flexible and not fixed to the protoplasmic cylinder. According to this model, when the flagella rotate in concert they slip against the cylinder and roll against the outer membrane, causing the two to move in opposite directions. The cell rotates about its longitudinal axis and moves along it, due to the helical configuration. Reversals occur when both flagella switch their direction of rotation synchronously. If switching is asynchronous, i.e., if only one flagellum switches, the ends twist in opposition to each other and a flex is generated (Fig. 1).

According to the model for motility of *S. aurantia*, runs occur when the flagellar motor at one cell end is rotating counterclockwise (CCW) and the motor at the other cell end is rotating clockwise (CW). Synchronous switching of these two motors generates a run in the reverse orientation. When both motors rotate CW or CCW, the spirochetal cell flexes. Synchronous switching of the motors in flexing cells results in continued flexing (Fig. 1). In contrast, *Escherichia coli* and *Salmonella typhimurium*, both peritrichously flagellated bacteria, run when their flagella rotate CCW and tumble when they rotate CW (19, 20).

Studies of *E. coli* cell behavior in response to additions of chemoattractants and repellents have demonstrated that this organism overcomes environmental sampling problems by comparing stimulus concentrations over time (3). When a high concentration of attractant is added to a cell suspension, tumbling is rapidly suppressed (the frequency of CW flagellar rotation is decreased). Cells recover at the new attractant concentration; over time they return from the excited state to the original tumbling frequency. Thus, *E. coli* cells run for extended periods when traveling up an attractant gradient (3, 4). The response of motor rotation to stimuli appears to be mediated by one or more signaling proteins which interact with a switch at the base of each flagellum (24, 26, 30). Although sensory transduction in *E. coli* has not been fully elucidated, most published data indicate that electrical signals are not involved (23, 26).

For *S. aurantia*, it appears that chemosensory transduction does involve an electrogenic component (14, 15). In part because the motility patterns of *S. aurantia* are relatively complex (16), information on the behavioral responses of this organism to chemical stimuli is not available. We describe a method that can be used to analyze the behavior of *S. aurantia*. The pattern of *S. aurantia* motility is described, and the effects of attractants on this pattern are presented. The evidence presented in this article indicates that *S. aurantia*, like *E. coli* (4, 5, 8), *S. typhimurium* (22), and *Bacillus subtilis* (12), can use a temporal sensing mechanism to respond to environmental stimuli.

MATERIALS AND METHODS

Bacterial strain and culture conditions. The organism used was *S. aurantia* M1 (6). Unless otherwise specified, cultures were grown at 30°C in glucose-Trypticase (BBL Microbiology Systems, Cockeysville, Md.)-yeast extract broth as described previously (14). As recommended by Adler (1), the culture used was initially enriched for motility and chemotaxis. This was achieved by allowing cells to migrate

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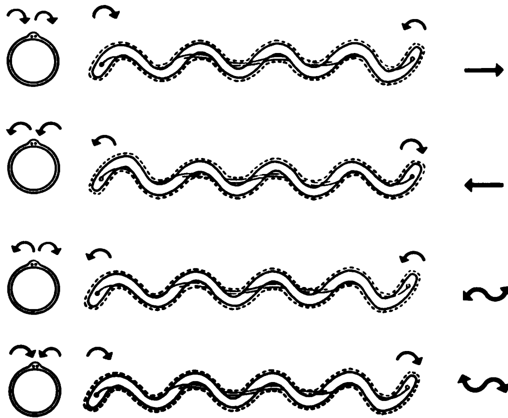

FIG. 1. Diagram of the sense of flagellar rotation and the resulting behavior of *S. aurantia* as described in the text. As viewed in cross section, the flagellar filaments rotating in concert generate a run. With respect to motor rotation, this occurs in either a CW-CCW or a CCW-CW configuration. When both motors switch in synchrony, a reversal occurs. When a single motor switches to give a CW-CW or a CCW-CCW orientation, the two flagellar filaments work against each other (as viewed in cross section). The resultant flexing persists until one of the motors switches independently of the other.

on plates of xylose swarm agar and then picking cells from the edge of the migrating population (17). Xylose swarm agar consisted of 0.5% agar in 0.01 M potassium phosphate (pH 7.6) to which the following separately sterilized ingredients were added (per 100 ml): 20 mg of D-xylose, 50 mg of L-cysteine, 8 mg of L-glutamic acid, 50 μg of thiamine, 5 μg of biotin, 100 mg of NH_4Cl, 20 mg of $MgSO_4$, and 2 mg of $MnSO_4 \cdot H_2O$.

Preparation of cell suspensions. For videomicroscopy, cells from late-logarithmic-phase cultures (5×10^8 cells per ml) were diluted in 10 mM potassium phosphate buffer (pH 5.5) to a final density of approximately 2.5×10^7 cells per ml.

Analysis of motility. Cells in a 10-μl suspension placed on a glass cover slip were observed by using an IM-35 inverted dark-field microscope fitted with a ×40 neofluar objective (Carl Zeiss, Inc.). Illumination was with an HBO 100-W/2 high-pressure mercury lamp; the light was passed through a VG9 green interference filter and a heat reflection filter (Carl Zeiss, Inc.). The microscope was coupled to a high-resolution video camera (RCA closed circuit model TC1005), and images were recorded with a Panasonic PV8950 video recorder.

The motility of videorecorded cells was analyzed with a computerized cell-tracking system (Motion Analysis Systems Inc., Santa Rosa, Calif.) in conjunction with a program designed for studying spirochete motility (SPMOT). Unless otherwise specified, tape segments of 15 s were input at a rate of 10 frames per s, and the path of each motile cell remaining in the field of focus for at least 5 s was determined (Fig. 2A). A motile cell was defined as one displaying an average speed of >3 μm/s. Thus, the small percentage of cells that were attached to the cover slip or that were immotile were eliminated from further analysis. The behaviors of individual motile cells during the initial 5 s of tracking were differentiated on the basis of linear speed and rate of change of direction (the absolute value of the angular velocity) of their paths. Smooth swimming is defined as a speed of >5 μm/s and a rate of change of direction <630°/s (Fig. 2B), flexing is indicated by a speed <5 μm/s and a rate of change of direction <630°/s (Fig. 2D), and a reversal is defined as a speed of <5 μm/s and a rate of change of direction >630°/s (Fig. 2C). These values were established empirically by comparing computer-generated data with visual observations. Except for infrequently generated false reversals, SPMOT accurately depicted cell behavior. False reversals were sometimes generated when two paths crossed. To minimize the frequency of paths crossing, it was important that cell density not exceed that used.

To examine reversing behavior more closely, cells exhibiting reversals were chosen by repeated viewing of videotapes and analyzed as described above except that the input rate was 60 frames per s and input of data was <15 s. The effect of flexing on swimming direction was studied by using SPMOT to determine the duration of individual flexing events and by subsequently measuring the angle between the run prior to a flexing event and the run after the event.

Behavioral response of *S. aurantia* to chemoattractants. By using SPMOT, the effects of attractants on the behavior of *S. aurantia* were analyzed. Chemical stimuli (in 1-μl volumes) were added rapidly to *S. aurantia* cell suspensions during videotaping. Successive 15-s tape segments were then analyzed, and the behavior was compared with prestimulus behavior. Unless otherwise specified, the results represent the averages of three separate experiments.

RESULTS

Analysis of *S. aurantia* motility. Computer-assisted motion analysis generated a qualitative picture of *S. aurantia* behavior similar to previous descriptions for behavior of this organism (16, 17; see Introduction). Within the time frame of our analyses, the behavior of individual cells cannot be predicted. This point is illustrated in Fig. 2B to D. Three cell paths are displayed; one cell exhibited an extended run of smooth swimming (Fig. 2B), one cell exhibited smooth swimming interrupted by a single reversal (Fig. 2C), and one cell exhibited several seconds of smooth swimming followed by several seconds of flexing (Fig. 2D). When populations of *S. aurantia* cells were analyzed, a reproducible behavioral pattern emerged (Table 1). A population of *S. aurantia* cells spent about 66% of the time swimming and about 33% of the time flexing. The average frequency of reversals in cell

TABLE 1. Quantitative analysis of *S. aurantia* behavior[a]

Expt. no.[b]	No. of paths analyzed	% of time[c] spent: Smooth swimming	Flexing	Reversing	Total no. of reversals	Reversal frequency (reversals/5 s)[d]
1	58	49.03	50.24	0.72	16	0.28
2	195	68.95	30.12	0.92	61	0.31
3	141	71.87	27.37	0.75	35	0.25
4	155	76.34	22.53	1.14	57	0.37
5	25	69.92	29.12	0.96	7	0.28
6	118	66.36	32.44	1.20	46	0.39
7	129	63.86	35.36	0.78	31	0.24
8	66	61.06	37.79	1.15	24	0.31
Avg[e]		65.93	33.12	0.95		0.31

[a] Behavior was analyzed with the SPMOT program and computer-assisted motion analysis as described in Materials and Methods.
[b] The data for each experiment were collected by using separately grown cultures on different days.
[c] As described in Materials and Methods, the first 5 s of each path was analyzed. Percent of time is that for all of the paths in the population analyzed.
[d] Sum of the reversals which occurred in a particular analysis divided by the number of cells analyzed.
[e] The mean when each experiment is equally weighted.

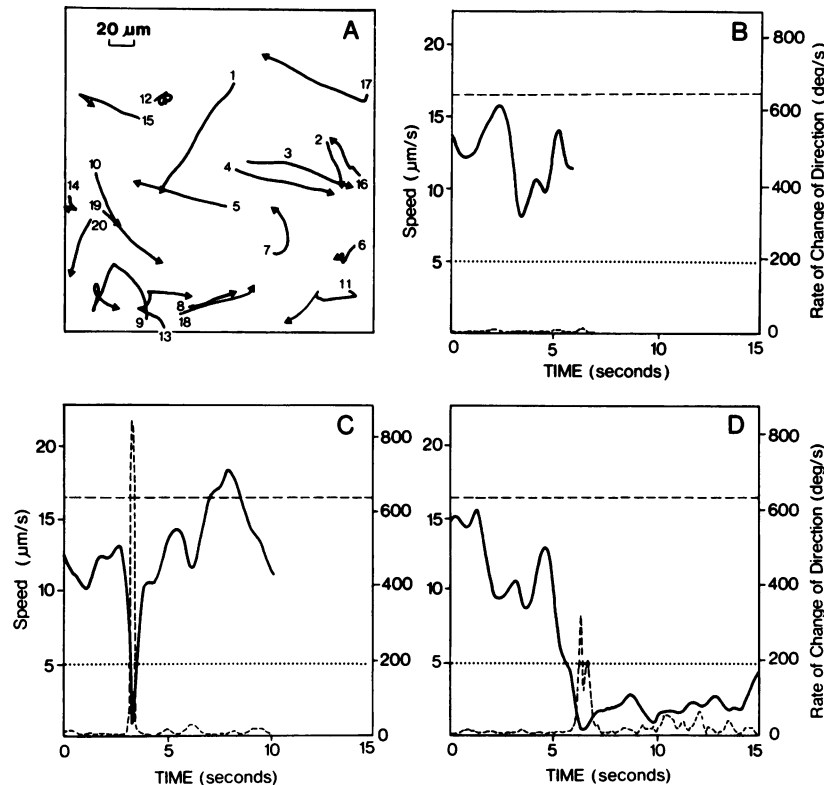

FIG. 2. Computer-assisted motion analysis of *S. aurantia*. (A) Paths of 20 cells in a microscope field for 5 to 15 s. Each cell is numbered where tracking is initiated, and the arrowheads denote the direction of motion at the end of the path. (B to D) The speed (——) and rate of change of direction (---) of three representative paths from panel A: (B) path 5, that of a cell which exhibited smooth swimming (speed, >5 µm/s; rate of change of direction, <630°/s); (C) path 3, that of a cell which exhibited smooth swimming interrupted by a single reversal (speed, <5 µm/s; rate of change of direction, >630°/s). (In panel A, the numeral 3 marks the origin of the path. Thus, this cell first ran in a direction opposite the arrowhead and then reversed.); (D) path 4, that of a cell smooth swimming for several seconds, followed by flexing (speed, <5 µm/s; rate of change of direction, <630°/s) for the remainder of the tracking period. As a reference, 5 µm/s and 630°/s are demarcated by dotted and dashed lines, respectively.

populations was 0.31/5 s. Within the 5-s tracking period, some cells reversed twice, some cells reversed once, and many cells did not reverse at all. Because many cells did not reverse at all within the period of analysis, an average interval between reversals cannot be calculated, nor can an average run length be calculated (because many cells exhibited smooth swimming for the complete duration of the tracking period). The average linear speed of smooth swimming cells was 16 µm/s, approximately 1 body length per s.

Reversals and flexing activity. Reversals can be subdivided into three behavioral groups: (i) reversals which interrupt two long runs, (ii) events in which a brief period of flexing occurs followed by a very short run (<1 s) in the original direction and then the reversal, and (iii) reversals immediately preceded by relatively long periods of flexing. For the first group, after the reversal the cell resumed running in a direction within roughly 180° of the previous direction (Fig. 3A). In the second group, there was more variability in the direction of the resultant run (Fig. 3B). The behavior in the third group was not considered further in our analysis of reversals because, according to the model for *S. aurantia* motility, it does not represent true reversals (synchronous switching of the two flagellar motors). Rather, this type of behavior represents two asynchronous switches. Usually SPMOT did not recognize this behavior as a reversal owing to relatively low rates of change of direction.

An analysis of nine reversals exhibited by seven cells indicated that the average time between two runs that delineated a reversal was 120 ms, with a standard deviation of 60 ms. This value may be a slight overestimate. On the basis of an average swimming speed of 16 to 17 µm/s and a cell length of 15 to 30 µm, a smooth-swimming cell moves about 0.1 body length in 120 ms. Smaller changes in the actual position of a cell would be difficult, if not impossible, to detect.

Flexing events lasting only a few tenths of a second caused little or no change in the direction of running. Longer periods of flexing (>0.5 to 1.0 s) appeared to randomize swimming direction (Fig. 4).

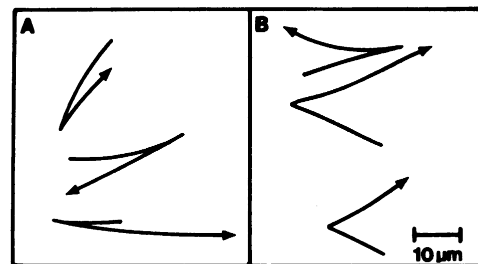

FIG. 3. Selected paths of cells exhibiting a reversal during the tracking period. (A) Group I reversals. (B) Group II reversals. Arrowheads indicate the direction of motion at the end of the tracking period.

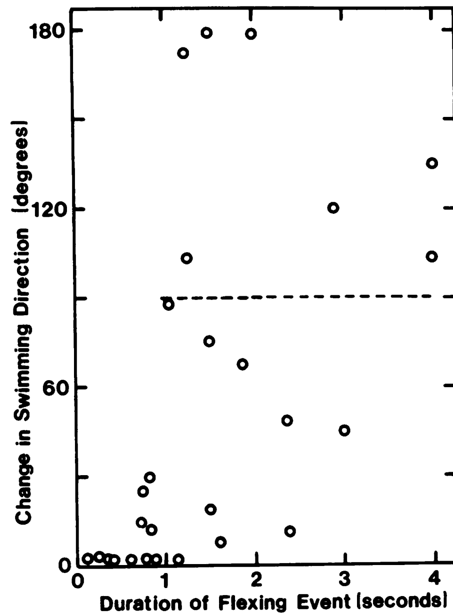

FIG. 4. Relationship between flexing duration and the change in direction of smooth swimming. The line was drawn arbitrarily at 90°; the predicted average change in direction assuming reorientation is random. The average for all flexes of duration >1 s was 84°.

Response to chemoattractants. Addition of the attractant D-xylose to *S. aurantia* cell suspensions resulted in a temporary modification of behavior. Initially, cell paths were smoother, but 1.5 to 2.0 min after xylose addition the paths were indistinguishable from the prestimulus cell paths (Fig. 5). A quantitative analysis of motility demonstrated that cells excited with 10 mM xylose exhibited increased smooth swimming, decreased flexing, and a complete suppression of reversal activity (Fig. 6). The average speed during runs of smooth swimming was unaffected by the stimulus. This response resembles the responses of *E. coli* and *S. typhimurium* to chemoattractants. For these enteric bacteria, excitation is rapid; within 200 ms, the excited cells exhibit smooth swimming, and adaptation is complete within a few minutes (3, 22, 27, 28).

If the response of *S. aurantia* to temporal gradients of attractants is related to chemotaxis, then, like the *S. aurantia* response to spatial gradients of xylose (17), there should be a concentration dependence. Such a concentration response was demonstrated by measuring the reversal frequency in populations of cells after stimulation by different concentrations of xylose (Fig. 7). Reversal frequency is reported because it was the most sensitive indicator of excitation. Below 10^{-4} to 10^{-5} M xylose, behavior was indistinguishable from that after buffer addition, for which no obvious change in behavior was detected. Lower concentrations of xylose may have induced a response with an adaptation period <15 s, but, using our procedure, this could not be detected.

Other attractants elicit a behavioral response, but nonattractants do not. D-Mannose is an effective attractant for *S. aurantia* (17) and is recognized by a chemoreceptor distinct from the xylose receptor (16). Addition of 10 mM D-mannose resulted in a clear response (Fig. 8), but the response was less pronounced than that to 10 mM D-xylose (Fig. 6). D-Ribose is not effective as an attractant for *S. aurantia* (17); addition of this sugar (10 mM) resulted in only a slight response (Fig. 8).

DISCUSSION

S. aurantia exhibits three kinds of behavior: runs of smooth swimming, flexes, and reversals (16, 17). It has been difficult to analyze the behavior of *S. aurantia* quantitatively because of the considerable variability in run lengths and in the duration of flexes and because reversals are brief events that occur relatively rarely (17). Therefore, we developed a computer program (SPMOT) for use in conjunction with an automated motion analysis system, and we quantitatively analyzed behavior in populations of cells. A somewhat similar computer-assisted motion analysis has previously been used to study the phototactic behavior of *Halobacterium halobium*, a bacterium which exhibits runs and reversals (29).

S. aurantia, like *E. coli* (3), appears capable of making time-dependent comparisons of stimulus concentrations

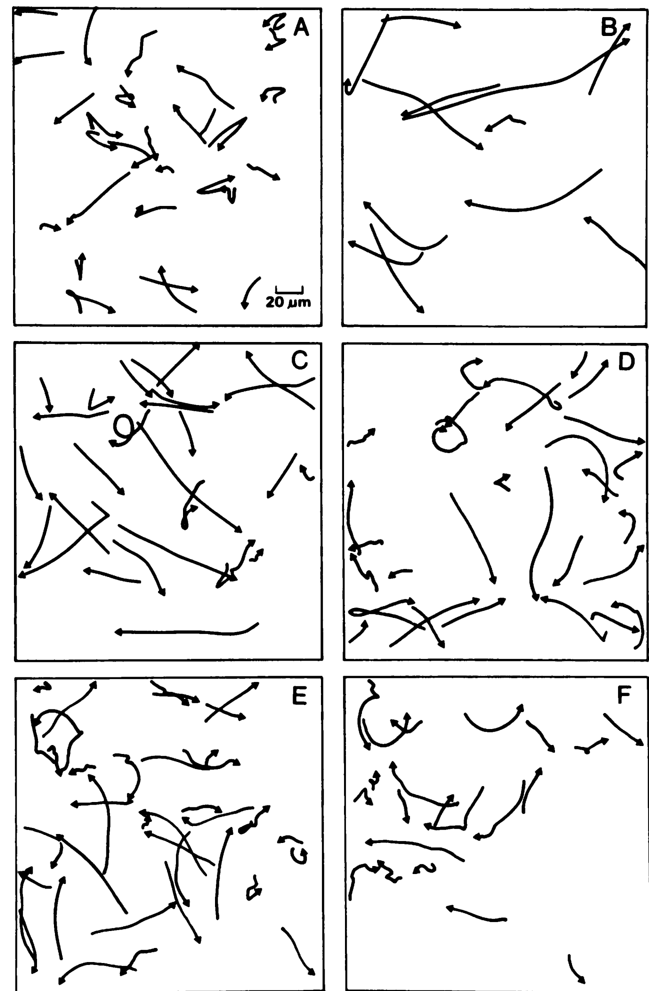

FIG. 5. Response of *S. aurantia* to the addition of D-xylose (final concentration, 10 mM). (A) Paths prior to stimulation with xylose. (B) Paths 15 to 30 s after xylose addition. (C) Paths 30 to 45 s after addition. (D to F) Paths 45 to 60 s, 60 to 75 s, and 75 to 90 s after addition of xylose, respectively. Each path represents a cell which was tracked for a minimum of 5 s and a maximum of 15 s.

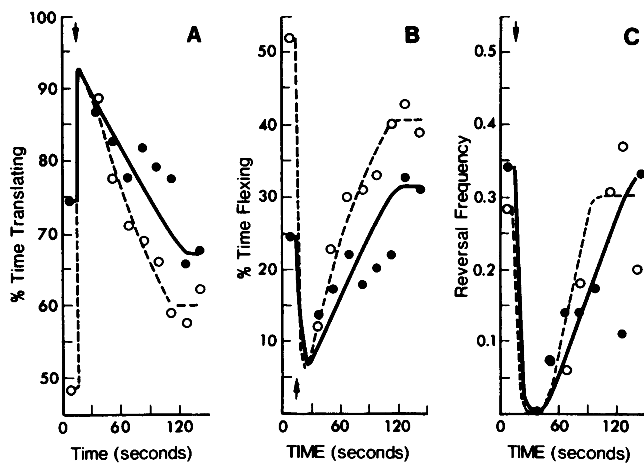

FIG. 6. Quantitative analysis of the behavioral response to D-xylose (10 mM). Percent of the time that cell populations exhibited (A) smooth swimming, (B) flexing, and (C) the population reversal frequency (number of reversals per 5 s). The arrow indicates the time of xylose addition. Symbols: ○, quantitative data from the experiment shown in Fig. 5; ●, average values of three separate experiments.

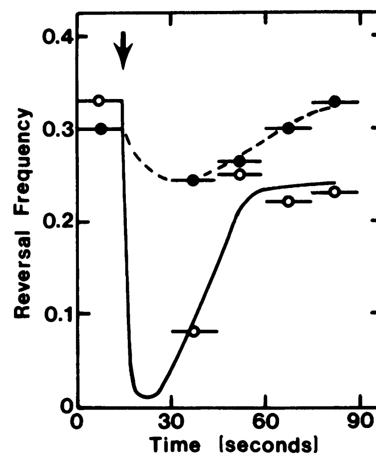

FIG. 8. Effects of the attractant D-mannose (○) and the nonattractant D-ribose (●) on the population reversal frequency (reversals per 5 s) of *S. aurantia*. The horizontal bars indicate the interval (15 s) for each analysis. Each sugar was added to a final concentration of 10 mM at the time indicated by the arrow. Cells for the D-mannose assay were grown in medium in which D-xylose (0.2 g/100 ml) replaced D-glucose. D-Glucose is a competitive inhibitor of D-mannose taxis (16). The data represent the average of three separate experiments, as described in Materials and Methods.

(Fig. 5 to 8). When an attractant was added to a cell suspension, running was increased, flexing decreased, and reversals were suppressed. Cells adapted to the new attractant concentration within about 2 min, returning to their prestimulus behavior. As in *E. coli* (3), the magnitude of the response is dependent on the attractant and upon its concentration (Fig. 5 to 8). Thus, it seems probable that *S. aurantia* responds to spatial concentrations of attractants by using a biased-random-walk mechanism analogous to that of *E. coli* (3).

According to the model for *S. aurantia* motility (2, 16; Fig. 1), cells swim smoothly when the motors of the two periplasmic flagella are in a CW-CCW mode. Assuming a cell has no head, a flagellum will have no rotational bias in either the CW or CCW orientation. Runs of smooth swimming were the predominant behavior of unstimulated cells (Table 1), and running increased upon excitation (Fig. 5 to 6). This

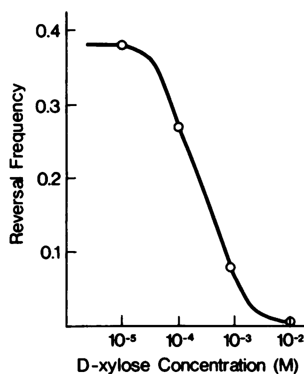

FIG. 7. D-Xylose concentration-response curve. The reversal frequencies (reversals per 5 s) are for the period 15 to 30 s after addition of xylose. The datum points represent average values collected from three series of experiments using separately grown cultures on three different days.

indicates that there is coordination of CW-CCW rotation; i.e., that there is a mechanism for communication between the two flagellar motors. It can be hypothesized that coordination is enhanced upon addition of attractants, resulting in the increased level of smooth swimming. In *E. coli*, runs result from CCW flagellar rotation, and tumbles result from CW rotation. Addition of attractants leads to generation of a signal which increases the CCW bias so that runs are extended.

For populations of unstimulated *S. aurantia* cells, the frequency of reversals is about 0.31/5 s of tracking (Table 1), although the time between any two reversals in a single cell appears to be variable. *S. aurantia* mutants specifically altered in reversal frequency have been isolated (K. Fosnaugh and E. P. Greenberg, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, I121, p. 185), indicating that reversals have a genetic and biochemical basis. Synchronization of switching of the flagellar motors in a reversal is within about 0.1 s (the average time between the two runs delineating a reversal). This is similar to the time for synchronous switching of the flagella in members of the genus *Leptospira* (11) and for reversals in members of the genus *Cristispira* (P. Dunlap and E. P. Greenberg, unpublished data). Leptospira cells are rather small (6 to 20 μm in length) with a single flagellum inserted at each cell end (7, 11). *Leptospira* flagella are relatively short and do not overlap in the central region of the cell (7); hence, in cells of *Leptospira*, reversals cannot result from direct mechanical interactions of the two flagella. Members of the genus *Cristispira* are extraordinarily long, >200 μm, with bundles of flagella inserted at the cell ends (10). For *Cristispira* cells, diffusion of a cytoplasmic signal from flagellar motors at one end to the motors at the other end would be too slow to account for synchronization of flagellar switching.

Assuming a nominal length of 15 μm for an *S. aurantia* cell, a signal with characteristics similar to those described by Segall et al. (26) for *E. coli* flagellar switching would require over 10 s to diffuse from the flagellar motor at one

end of the cell to the motor at the other end. Apparently, such a diffusible polypeptide does not account for the synchronization of motor switching required for reversals in *S. aurantia* or for the coordination of CW-CCW rotation. Even a small molecule, such as Ca^{2+}, would require approximately 0.5 s for diffusion from one end of a 15-μm-long cell to the other end (assuming a diffusion coefficient of 2.3×10^{-6} cm²/s for Ca^{2+} in axoplasm [26]). Particularly, for *Cristispira* cells, it is evident that diffusion of a signal from one end to the other cannot account for synchronous motor switching. A transmembrane impulse would be rapid enough to serve as a synchronizing signal. Consistent with this, it has been reported that a valinomycin-induced voltage clamp inhibits *S. aurantia* chemotaxis (14, 15) but not *E. coli* chemotaxis (23). However, it is also possible that in *S. aurantia* there is an intracellular oscillator similar to that proposed for generation of reversals in *H. halobium* (25). Such an oscillator could simply signal both flagellar motors to switch regardless of their orientation whenever its concentration reaches a critical level. Were this correct, then suppression of reversals in excited cells could result from a damping of the oscillations in the concentration of such a signal. Thus, reversals do not have to involve transmembrane impulses, and valinomycin-induced voltage clamping may affect some other aspect of sensory behavior.

Flexing events were quite variable in duration, and large changes in direction were not incurred by brief periods of flexing. Longer periods of flexing did appear to randomly reorient cells (Fig. 4). The relationship of flexing to chemotaxis is not clear at present. Perhaps brief periods of flexing allow a cell to make corrections in the direction of smooth swimming through a gradient of attractant, or perhaps they are related to randomizing the direction of cells in conjunction with reversals. This is suggested by the fact that group II reversals (those preceded by a brief period of flexing and a short run) give rise to a greater variability in the direction of a resultant run than do group I reversals (those which punctuate two long runs) (Fig. 3). Periods of flexing greater than a few seconds appear rather inefficient with respect to reorientation.

According to the model for motility of *S. aurantia* (2, 16), cells swim smoothly when the two flagellar motors rotate in the opposite senses, one CW and one CCW. If both motors switch direction of rotation synchronously, the cell reverses. Flexes occur when only one motor switches, resulting in either a CW-CW or a CCW-CCW orientation. To account for the observed behavior of motile *S. aurantia* cells and for their behavioral response to attractants, we have postulated the existence of a mechanism which coordinates CW-CCW flagellar rotation (a mechanism for communication between the two flagellar motors) and a signal that synchronizes switching of the two flagellar motors, thus generating reversals. With the development of a method to analyze *S. aurantia* behavior quantitatively, the motility of chemotaxis mutants (Fosnaugh and Greenberg, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986) can be studied in detail and should advance our understanding of chemosensory signaling in this bacterium.

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