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Chemotaxis Mutants of *Spirochaeta aurantia*

KATHY FOSNAUGH† AND E. P. GREENBERG‡*

Department of Microbiology, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca, New York 14853-7201

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Five *Spirochaeta aurantia* chemotaxis mutants were isolated. One mutant (the *che-101* mutant) never reversed, one (the *che-200* mutant) flexed predominantly, two (the *che-300* and *che-400-1* mutants) exhibited elevated reversal frequencies, and one (the *che-400* mutant) exhibited chemotactically unstimulated behavior similar to that of the wild-type strain. The *che-101* and *che-400* mutants were essentially nonchemotactic, whereas the *che-200*, *che-300*, and *che-400-1* mutants showed impaired chemotactic responses. Protein methylation in response to attractant addition appeared normal in all of the mutants. Compared with the wild type, all of the mutants exhibited significantly altered membrane potential responses to the attractant xylose.

The spirochetes represent one of about ten major subgroupings of the eubacteria (12, 30). Although there is an enormous degree of metabolic diversity among the different spirochetes, they all share a unique type of motility (8, 9). The facultatively anaerobic *Spirochaeta aurantia* has been the model for studies of chemotaxis in spirochetes (18). *S. aurantia* cells measure about 0.3 μm in diameter by 15 to 30 μm in length and possess two periplasmic flagella (6). The hook-basal body complexes of each of the flagella are inserted through the peptidoglycan into the cytoplasmic membrane at opposite poles of the cell, and the filamentous portion of each is contained within the periplasmic space where it wraps around the peptidoglycan layer (8). Except for location, the *S. aurantia* flagella appear analogous to other flagella in that they propel a cell by rotation driven by a proton motive force (2, 13).

S. aurantia is attracted to a number of different sugars (19), is repelled by sulfide and a variety of acids and alcohols (C. Kämpf and E. P. Greenberg, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, I-73, p. 193), and is aerotactic (19). Methyl-accepting proteins from *S. aurantia* have been identified and are remarkably similar to the methyl-accepting chemotaxis proteins of *Escherichia coli* (22, 26). In *E. coli*, attractant binding to methyl-accepting chemotaxis proteins results in the rapid generation of an excitatory signal which affects the flagellar motors; this binding also results in a relatively slow increase in the level of methyl-accepting chemotaxis protein methylation, which corresponds with adaptation to a given concentration of attractant (17, 28). Chemotactic signaling in *E. coli* does not appear to involve membrane potential (25, 33). In contrast, the *S. aurantia* chemosensory mechanism has been reported to involve membrane potential in some as yet undefined manner (14–16).

S. aurantia cells generally run in relatively straight lines; occasionally they reverse swimming direction, the cell anterior becomes posterior, and the cells can also stop swimming and flex (11, 18, 19). Like *E. coli* (3, 4, 7), *S. aurantia* appears capable of making time-dependent comparisons of attractant concentrations. Addition of attractant results in increased running, decreased flexing, and suppression of reversals (11). Making three assumptions, Berg (2) proposed an explanation for motility of *S. aurantia*. The assumptions are 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. 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 because of the overall helical configuration. Reversals occur when both flagella switch direction of rotation synchronously. If switching is asynchronous, i.e., if only one flagellum switches, a smooth swimming cell will stop and flex as the cell ends twist in opposition to each other.

Presumably, to generate runs of smooth swimming in *S. aurantia*, the two flagellar motors which are identical to each other must rotate in opposite senses, one clockwise (CW) and one counterclockwise (CCW). If both motors switch direction of rotation synchronously, the cell will reverse. When both motors rotate CW or CCW a cell will flex. In a flexing cell, flexing should persist if both motors switch synchronously (11, 18). To account for the behavioral response to attractants (increased smooth swimming, decreased flexing, and suppression of reversals), we have postulated the existence of a mechanism which coordinates CW-CCW rotation (a mechanism for communication between the two flagellar motors) and of a signal that synchronizes switching of the two flagellar motors, i.e., a reversal generator (11, 18). Apparently, in response to attractants, motor coordination is enhanced and synchronous switching is suppressed.

In *E. coli* and *Salmonella typhimurium*, both peritrichously flagellated bacteria, runs of smooth swimming are correlated with CCW flagellar rotation and tumbles are correlated with CW flagellar rotation (23, 24). Addition of attractants biases the flagellar motors to rotate CCW, and thus run length is extended (7). Much of our current knowledge of the chemotactic responses of *E. coli* and *Salmonella typhimurium* has come from the examination of chemotaxis mutants (28). Therefore, to further understand the distinct chemosensory process in spirochetes, we have undertaken the isolation and characterization of chemotaxis mutants of *S. aurantia*. In this initial report, five mutants with distinct motility patterns are described.

Enrichment and isolation of the *che* mutants was based on

* Corresponding author.
† Present address: Department of Biology, University of California, San Diego, La Jolla, CA 92093.
‡ Present address: Department of Microbiology, College of Medicine, University of Iowa, Iowa City, IA 52242.

TABLE 1. *S. aurantia* strains

Strain or mutant type[a]	Relevant characteristics	Source (reference)
M1	Wild type; motility and chemotaxis well characterized	E. Canale-Parola (6)
NP-7	Nonmotile mutant derived from M1 after UV mutagenesis	E. Canale-Parola (29)
che-101	Derived from M1 after NTG[b] mutagenesis	This study
che-200	Spontaneously generated from M1	This study
che-300	Spontaneously generated from M1	This study
che-400	Derived from M1 after NTG mutagenesis	This study
che-400-1	Spontaneously occurring pseudorevertant derived from the *che-400* mutant	This study

[a] For details of isolation of *che* mutants, see text.
[b] NTG, 1-Methyl-3-nitro-1-nitrosoguanidine.

a procedure developed for *E. coli* (27). Five microliters of an *S. aurantia* M1 culture was used to inoculate the center of a xylose swarm agar plate. After 2 to 3 days, a sharp band of migrating chemotactic cells could be observed. At this time, a loop of agar from the center of the plate where only nonmigrating cells remained was used to inoculate a second swarm plate. This process was repeated through four to six cycles, after which cells from the center of the plate were transferred to glucose-trypticase-yeast extract (GTY) broth, grown to mid-logarithmic phase, and plated in 2.5 ml of xylose swarm agar overlaid on xylose swarm agar plates. Cells from colonies failing to form bands similar to those formed by strain M1 were saved for further studies. In some cases, before enrichment, cultures were mutagenized with 100 μg of 1-methyl-3-nitro-1-nitrosoguanidine per ml for 45 to 50 min at 25°C. This resulted in a 95 to 99% decrease in viability. The *che-400-1* mutant was isolated as a pseudorevertant of the *che-400* mutant. This was accomplished by inoculating the *che-400* mutant on a xylose swarm agar plate for 5 days, after which a migrating band from which the *che-400-1* mutant was isolated had arisen.

Each of the *che* mutants listed in Table 1 formed swarms that were distinct from that of the wild-type strain M1 in that the chemotactic ring migrated more slowly or the swarms did not form a sharp ring. The *che* mutants were also distinct from nonmotile mutants, which did not migrate from the point of inoculation on swarm agar plates (Fig. 1). The *che* mutants and the wild type grew equally well in GTY broth (the media and culture conditions have been described elsewhere [11, 14, 19]). The cell size and shape of each of the *che* mutants were indistinguishable from those of *S. aurantia* M1, and, as indicated by Western blot (immunoblot) analysis, the flagellar filament polypeptide compositions of the *che* mutants were identical to the composition of the biochemically complex filaments of *S. aurantia* M1 (5; B. Brahamsha, personal communication).

The four mutants isolated after enrichment on xylose swarm agar could be distinguished from each other on the basis of their motility patterns, and the pseudorevertant (the *che-400-1* mutant) could also be distinguished from its parent (the *che-400* mutant) on this basis, as discussed below. By using a variety of mutagens, other mutants which exhibited behaviors similar to those of one or another of the mutants used in this study were isolated independently. Thus, we consider the mutants characterized here representative of classes of behavioral mutants. Unstimulated cells of the *che-101* mutant exhibited runs of smooth swimming and periods of flexing but were never observed to reverse (Table 2). The frequency of computer-generated false reversals, primarily due to paths crossing, was exacerbated by the long runs of smooth swimming characteristic of this strain. We do not mean to imply that the anterior and posterior of *che-101* cells never switch. Rather, if switching did occur, it did so only after a relatively long (>0.7 s) period of flexing. The *che-200* mutant was characterized by cells that flex incessantly. Because runs of smooth swimming were rare, the paths generated by motion analysis of *che-200* cells could not be analyzed with our program for analysis of spirochete motility (SPMOT). SPMOT deletes paths with low mean speeds (11), a characteristic common to *che-200* paths. *che-300* cells exhibited a reversal frequency much higher than that of the wild-type strain, M1 (Table 2). In contrast to cells of strain M1, which rarely reversed more than once within the 5-s tracking period, nearly half of the *che-300* cells did so. Cells of the *che-400* mutant exhibited an unstimulated behavior which could not be distinguished readily from behavior of the wild type (Table 2). Curiously, the *che-400* pseudorevertant, *che-400-1*, which had the ability to form chemotactic rings on swarm agar (Fig. 1), exhibited a somewhat elevated reversal frequency, intermediate between that of the wild type and that of the *che-300* mutant (Table 2).

Initially tactic responses were assessed by using swarm agar plates. This assay is quite sensitive because the attract-

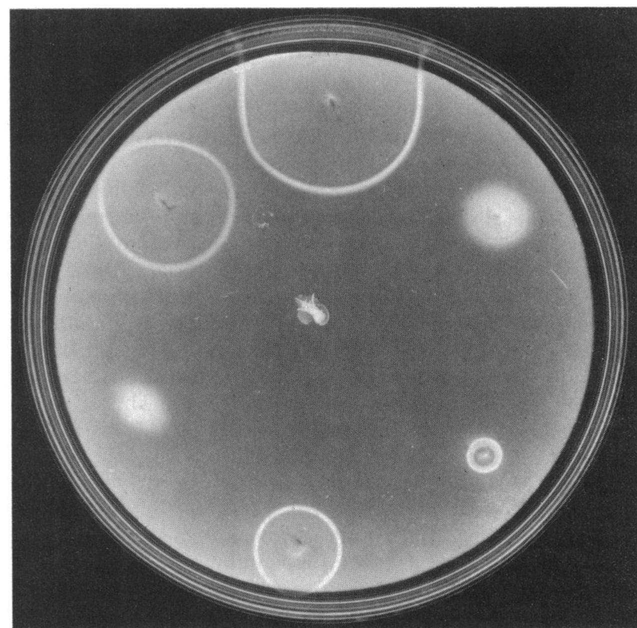

FIG. 1. D-Xylose swarm agar plate showing swarm phenotypes of wild-type and mutant strains. Swarms (clockwise from top) are of wild type and *che-101*, *che-200*, *che-300*, *che-400*, and *che-400-1* mutants. The nonmotile mutant, NP-7, was spotted in the center of the plate. Incubation was for 48 h at 30°C. Inocula were 2 μl of late-logarithmic-phase cultures.

TABLE 2. Quantitative analysis of behavior in *S. aurantia* strains[a]

Strain or mutant type	No. of paths analyzed	% of time[b]: Smooth swimming	% of time[b]: Flexing	Total no. of reversals	Population reversal frequency[c]
Wild type	99	65	34	31	0.31
che-101	125	80	20	11	<0.10[d]
che-300	110	73	24	83	0.75
che-400	91	66	33	23	0.25
che-400-1	71	73	25	41	0.58

[a] Behavior was analyzed by using the SPMOT program (11) and computer-assisted motion analysis.

[b] The first 5 s of each path was analyzed. Percent of time is that for all of the paths in the population analyzed.

[c] The sum of the reversals which occurred in a particular 5-s analysis divided by the number of cells analyzed.

[d] Reversal frequencies of 0.10 or less are considered background levels attributed to computer error.

ant gradient which forms as a result of cellular metabolism is very sharp and chemotactic cells accumulate within this gradient. On xylose swarm agar plates, the *che-101* and *che-400* mutants formed diffuse swarms with no obvious migrating rings of chemotactic cells, while the *che-200*, *che-300*, and *che-400-1* mutants formed rings of chemotactic cells that migrated more slowly than rings of the wild-type strain, M1 (Fig. 1). These results indicate that the *che-101* and *che-400* mutants did not respond to xylose gradients, that the *che-200* mutant responded slightly, that the *che-300* mutant responded better than the *che-200* mutant, and that the *che-400-1* mutant responded best of all the mutants but not as well as the wild type (Fig. 1). Results with glucose swarm agar were identical to those with xylose swarm agar (data not shown). Because glucose and xylose are detected by different chemoreceptors in *S. aurantia* (18), these findings indicate that none of the mutants had defects in specific receptors.

Responses to xylose and glucose were measured quantitatively by capillary assays (1, 19). With the exception of the pseudorevertant, none of the mutants exhibited a significant response compared with the wild-type strain (Fig. 2). The pseudorevertant exhibited a substantial response to glucose and a lesser response to xylose (Fig. 2F).

It has been demonstrated previously that addition of an attractant to wild-type cells results in a temporary modification of behavior. Initially, cell paths are smoother and reversals are suppressed, but a few minutes after attractant addition, behavior is indistinguishable from the prestimulus behavior (11). To define further each of the *che* mutants, the effects of xylose addition on reversal frequency were examined (Fig. 3). Addition of xylose did not affect reversal frequency of the *che-101* mutant (Fig. 3B). This was not surprising; even without the addition of xylose, this mutant did not reverse (Table 2). In fact, addition of xylose did not affect the extent of running or flexing appreciably in the *che-101* mutant (data not shown). The *che-101* mutant was one of two mutants that did not show even a slight response on swarm plates. The other such mutant was the *che-400* mutant, which appears more or less normal in terms of its unstimulated behavior (Fig. 1 and Table 2). Addition of xylose to *che-400* cells resulted in only a slight response (Fig. 3D). Both the *che-300* and *che-400-1* mutants formed chemotactic rings in swarm agar (Fig. 1), and addition of xylose to cells of these mutants resulted in suppression of reversals (Fig. 3C and E). *che-300* cells adapted to the added xylose similarly to the wild-type; however, the *che-400-1* mutant did

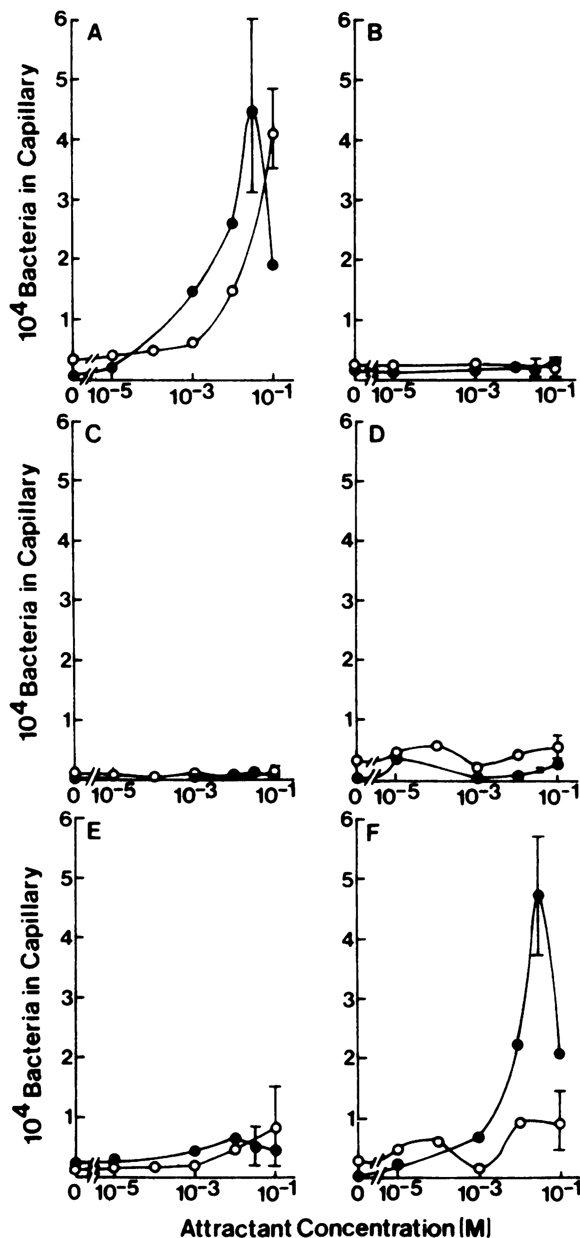

FIG. 2. Concentration response curves for D-glucose (●) and D-xylose (○) taxis, measured by a quantitative capillary assay. (A through F) Wild-type, *che-101*, *che-200*, *che-300*, *che-400*, and *che-400-1* cells, respectively. The marker bars indicate the standard deviations of responses in six assays performed on three different days with three separately grown cultures. Capillary assays were performed by the procedure of Greenberg and Canale-Parola (19) with the following modifications: the pH of the buffer was 5.5 rather than 7.0, and D-glucose was provided as the energy source in the D-xylose assays (14).

not adapt within the period of our experiments. As mentioned above, behavior of the mutant in which flexing predominates, the *che-200* mutant, could not be analyzed.

Previous investigations revealed methylation of specific *S. aurantia* polypeptides in response to the addition of an attractant (22). Furthermore, when an attractant such as xylose is added to cell suspensions, a transient membrane depolarization occurs (14). Thus, it was of interest to examine the effects of attractants on polypeptide methylation and

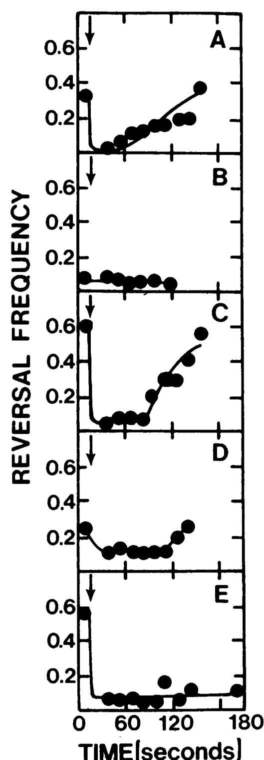

FIG. 3. The behavioral response of *S. aurantia* strains to D-xylose (10 mM) as indicated by the population reversal frequency (number of reversals per path per 5 s). (A through E) Wild-type, *che-101*, *che-300*, *che-400*, and *che-400-1* cells, respectively. Arrows indicate the time of xylose addition. The data represent averages from four experiments with separately grown cultures on four different days, except data for *che-300* (only three experiments). These experiments were performed as described previously (11).

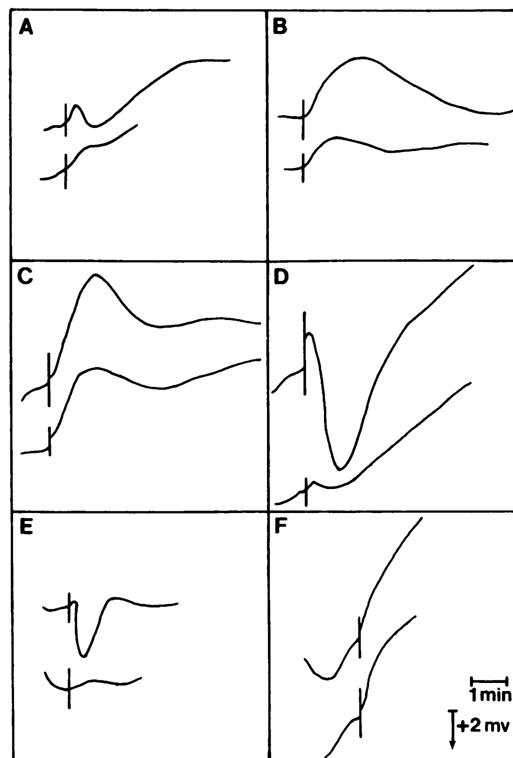

FIG. 4. Fluctuations in membrane potential upon addition of the attractant D-xylose (10 mM) (upper trace) or the nonattractant D-ribose (10 mM) (lower trace). The vertical lines indicate the time additions were made. (A through F) Wild-type, *che-101*, *che-300*, *che-400*, *che-400-1*, and *che-200*, respectively. To monitor $\Delta\psi$, cells were suspended at a density of 4.3×10^{10}/ml in 10 mM potassium phosphate (pH 5.5) plus 5 mM D-glucose and 2 μM TPP^+ in a water-jacketed stirring chamber at 25°C. External TPP^+ concentration was continuously monitored with a TPP^+ electrode (20). Values of $\Delta\psi$ were calculated by the Nernst equation (32) by using an intracellular volume of 3.2 μl/mg of protein. Intracellular volume was measured as described elsewhere (31). The calculated steady-state $\Delta\psi$ value was -150 ± 10 mV, which was in agreement with values obtained when intracellular [^{3}H]TPP^+ was measured as described by Kashket et al. (21).

$\Delta\psi$ in the *che* mutants. As measured by a previously described technique (10, 22), the polypeptide methylation patterns and the time course of methylation in wild-type strain M1 and all of the *che* mutants were indistinguishable. This suggests that all the *che* mutations affect a step distal to the protein methylation step in the *S. aurantia* sensory transduction pathway.

Our results obtained by using a tetraphenylphosphonium bromide (TPP^+) electrode to monitor $\Delta\psi$ in the wild type (Fig. 4) were comparable to previously reported results obtained by using a fluorescent probe to monitor $\Delta\psi$ (14). Upon addition of D-xylose there was an initial depolarization followed by a repolarizing phase with a slight overshoot (Fig. 4A). The nonattractant xylose analog, D-ribose, had only a slight affect on $\Delta\psi$ in *S. aurantia* M1 (Fig. 4A). For each mutant, $\Delta\psi$ values before stimulation with xylose were similar to the value for strain M1; however, in none of the mutants were $\Delta\psi$ responses to xylose the same as in strain M1 (Fig. 4). The $\Delta\psi$ in two of the mutants, the *che-101* and *che-300* mutants, showed a greater decrease than the $\Delta\psi$ of strain M1 upon addition of xylose, and the repolarizing phase was relatively slow (Fig. 4B and C). However, it should be noted that in these mutants the $\Delta\psi$ fluctuation in response to D-ribose, the nonattractant, was relatively large (Fig. 4B and C). Both the *che-400* mutant and the pseudorevertant derived from it, the *che-400-1* mutant, exhibited a greatly exaggerated repolarization and overshoot after xylose addition compared with strain M1 (Fig. 4). In the case of the *che-200* mutant, the flexing mutant, TPP^+ was taken up but a steady state was difficult to achieve. Furthermore, both the attractant and the nonattractant seemed to decrease the capacity of *che-200* cells to retain TPP^+ (Fig. 4).

To our knowledge this report represents the first description of any spirochete chemotaxis mutants. It should be pointed out that each of the mutants could well have multiple lesions and complex functional defects. An understanding of the genetics of *S. aurantia* chemotaxis will be facilitated by development of a gene transfer system for this organism.

One of the mutants described, the *che-101* mutant, had lost the ability to generate reversals. This mutant was unresponsive in each of the three chemotaxis assays (Fig. 1, 2B, and 3B). The existence of this mutant which did exhibit runs of smooth swimming and flexing (Table 1) fits with the hypothesis that in *S. aurantia* there is a signal which can synchronously switch the two flagellar motors (11, 18). It also suggests that the modulation of reversals is related to chemotaxis. In this regard, the other nonchemotactic mutant, the *che-400* mutant, exhibited reversals (Table 2) but the addition of an attractant had little effect on the frequency of reversals (Fig. 3D). In all of the mutants, methylation of

the methyl-accepting proteins appeared normal. Apparently, the methyl-accepting proteins and the enzymes required for methylation and demethylation constitute a step in the sensory transduction pathway proximal to the steps affected in the mutants we have isolated. One of the mutants, the *che-300* mutant, had an elevated reversal frequency (Table 1), and the interval between reversals was often short. In this mutant, addition of attractant evoked a behavioral response similar to that of the wild type (Fig. 3A and C). A reduced degree of chemotaxis was detected with the swarm agar assay (Fig. 1) and with capillary assays (Fig. 2). Evidently the defect in this mutant resulted in an overactivity of the signal which generated reversals, and this was detrimental to the efficiency of the chemotactic apparatus (Fig. 1 and 2D). In the *che-200* mutant, flexing behavior predominated. According to the model for spirochete motility (2, 11, 18), this behavior would be predicted of any mutant in which there was a severe CW or CCW bias on rotation of the flagellar motors. This mutant did show a slight tactic response in the most sensitive assay, the swarm plate assay (Fig. 1). Attractants must have increased smooth swimming to some degree, and although this was not quantitated, such a response was observed. The pseudorevertant, the *che-400-1* mutant, exhibited a somewhat impaired chemotactic response (Fig. 1 and 2F) and was abnormal with respect to adaptation, although chemotaxis-related polypeptide methylation did appear normal.

Previous studies have revealed a relationship between membrane potential and chemotaxis in *S. aurantia* (14, 15). Consistent with this conclusion, all the mutants exhibited $\Delta\psi$ responses to the attractant xylose which were distinct from the response of the wild type (Fig. 4). Aside from this, little else can be ascertained from the $\Delta\psi$ measurements with the *che* mutants. The type of $\Delta\psi$ response to xylose was not obviously correlated with the behavioral response (Fig. 4).

According to the model for *S. aurantia* motility (11, 18), cells swim smoothly when the two flagellar motors rotate in opposite senses, one CW and one CCW. If both motors switch direction of rotation synchronously, a cell will reverse. Flexing occurs when only one motor in a smooth swimming cell switches. Our analysis of *che* mutants, together with other available information on *S. aurantia* chemotaxis (11, 14, 18, 19), is at least consistent with the hypothesis that there is a signal that generates reversals. This could be a rapidly conducted impulse that signals motors to switch regardless of their direction of rotation. The fact that cells also flex can be explained if the signal has some probability of causing only one motor to switch. Chemoattractant-induced modulation of impulse signal generation would allow cells to respond to a tactic stimulus by decreasing the frequency of reversals and increasing smooth swimming, as has been observed (11).

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