

Motility, Chemokinesis, and Methylation-Independent Chemotaxis in *Azospirillum brasilense*

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Observations of free-swimming and antibody-tethered *Azospirillum brasilense* cells showed that their polar flagella could rotate in both clockwise and counterclockwise directions. Rotation in a counterclockwise direction caused forward movement of free-swimming cells, whereas the occasional change in the direction of rotation to clockwise caused a brief reversal in swimming direction. The addition of a metabolizable chemoattractant, e.g., malate or proline, had two distinct effects on the swimming behavior of the bacteria: (i) a short-term decrease in reversal frequency from 0.33 to 0.17 s⁻¹ and (ii) a long-term increase in the mean population swimming speed from 13 to 23 μm s⁻¹. *A. brasilense* therefore shows both chemotaxis and chemokinesis in response to temporal gradients of some chemoeffectors. Chemokinesis was dependent on the growth state of the cells and may depend on an increase in the electrochemical proton gradient above a saturation threshold. Analysis of behavior of a methionine auxotroph, assays of *in vivo* methylation, and the use of specific antibodies raised against the sensory transducer protein Tar of *Escherichia coli* all failed to demonstrate the methylation-dependent pathway for chemotaxis in *A. brasilense*. The range of chemicals to which *A. brasilense* shows chemotaxis and the lack of true repellents indicate an alternative chemosensory pathway probably based on metabolism of chemoeffectors.

Azospirillum brasilense is a soil bacterium living in close association with plant roots. It stimulates plant growth, probably because of its ability to fix nitrogen and to produce phytohormones (33). *A. brasilense* is capable of aerotaxis (8) and chemotaxis towards different organic compounds (7, 38, 49). Although there have been some studies of the chemosensory pathways in this organism (17, 37), very little is known about its mechanism of motility and chemotaxis. *A. brasilense* has a mixed pattern of flagellation; a single polar flagellum is synthesized during growth in liquid media, whereas lateral flagella are synthesized in addition to the polar flagellum during growth on solid media. The polar and lateral flagella are structurally and immunologically different, and they perform different motility functions. The polar flagellum is responsible for swimming motility, and the lateral flagella are responsible for swarming (18). In natural environments, migration of *A. brasilense* towards plant roots is limited by soil moisture (9), suggesting that free swimming through the water space rather than swarming plays the major role in chemotactic behavior in natural environments.

Oxygen and other electron acceptors are recognized by *A. brasilense* as chemoeffectors via a sensory pathway based on the functioning redox chain (17, 37). The aerotactic responses in *Escherichia coli* and *Salmonella typhimurium* also operate as a result of changes in electron flow through the redox chain and/or consequent changes in proton motive force (41, 42). It is possible that some chemicals which are also effective electron donors, such as pyruvate and proline, are also sensed via this pathway (for a review, see reference 29). One of the major chemosensory pathways in *E. coli* and

S. typhimurium, however, involves a family of methyl-accepting chemotaxis proteins (MCPs) rather than electron transport. These serve as specific chemoreceptors and/or signal transducers and change their methylation level in response to the binding of chemoeffectors (for recent reviews, see references 3 and 22). This methylation-dependent pathway allows a very sensitive response to a range of different chemical stimuli. In almost all bacteria studied so far, MCPs have been identified by the use of antibodies raised against an *E. coli* MCP (1, 43) and by *in vivo* methylation assays (14, 19, 24, 40, 41, 46). Only one bacterium, *Rhodobacter sphaeroides*, has been shown to lack MCPs (43) and to possess metabolism-dependent chemotaxis (36; for a review, see reference 4).

The aims of this study were to characterize the basic swimming behavior of *A. brasilense* and to determine whether this bacterium uses an MCP-based chemosensing system and, if so, how the methylation-dependent pathway integrates with the redox chain-dependent pathway for aerotaxis. We failed to identify an MCP system in *A. brasilense*, and it seems probable that the main chemosensory pathway in this bacterium is methylation independent.

MATERIALS AND METHODS

Bacterial strains. *A. brasilense* Sp7 (ATCC 29145), a wild-type strain, was used throughout this study. Its derivative 7007, a methionine auxotroph, was a gift from C. Elmerich (Institut Pasteur, Paris, France). *S. typhimurium* 19, without known mutations, was one of our laboratory strains. *E. coli* RP437, which is wild type for motility and taxis, was a gift from J. S. Parkinson (University of Utah, Salt Lake City).

Growth conditions. *A. brasilense* was grown on defined malate medium at 30°C and prepared for chemotaxis experiments as described previously (17). The medium was supplemented with 100 μg of methionine ml⁻¹ for growth of the

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methionine auxotroph. *E. coli* and *S. typhimurium* cells were grown in L broth at 37°C.

Chemotaxis measurements. Cultures were harvested and washed twice in 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.2) with 50 µg of chloramphenicol ml⁻¹ to prevent cell growth. For long-term starvation, the methionine auxotroph was resuspended in 10 mM HEPES as described above but with 10 mM malate as a carbon source. After 16 h of starvation, cells were harvested and resuspended in HEPES buffer without malate to measure the chemotactic response to malate and other chemicals.

Chemotaxis to a spatial gradient of chemoeffectors was measured in chemotaxis wells (5), where two 0.6-ml chambers were separated by a polycarbonate Nuclepore membrane (Costar Corp.) (8-µm pore size). The bacteria were placed in the bottom chamber, and a chemoeffector solution was placed in the top chamber. After 90 min, the number of bacteria which had moved from the lower chamber into the upper chemoeffector-containing chamber was determined with a Coulter Counter, and the chemotactic response was calculated relative to buffer controls (23). To discount the possibility of a false-positive response caused by chemokinesis to the chemoeffectors, controls were carried out with the effector added to the cells in the bottom chamber and with chemoeffector on both sides of the membrane. Five replicates of each experiment were performed.

Motion analysis. For videomicroscopy, free-swimming cells were sealed in optically flat microslides (flat capillaries) (Camlab, Cambridge, United Kingdom). Antiflagellin antibody-tethered cells were prepared as previously described (6, 12) and were viewed by looking down from the tethered filament towards the rotating cell body. Samples of free-swimming and tethered cells were observed with a Nikon Optiphot phase-contrast microscope. The images were transferred via a Panasonic Vidicon video camera to a Sony Umatic video recorder. Rotating and free-swimming cells were tracking by using an image analysis system (Seescan, Cambridge, United Kingdom) as described previously (35). Manual frame-by-frame analysis involved tracing individual tracks of free-swimming cells on acetate sheets directly from the monitor screen. All temporal gradient assays were conducted in pairs by first tracking free-swimming cells in a buffer and then tracking cells plus chemoeffector. For the manual analysis, 20 cells were tracked in one assay, typically for 6 to 10 s each. At least three replicates were performed.

In vivo methylation. Cells from actively motile cultures were harvested, washed twice, and then resuspended at 10⁸ cells per ml in 10 mM potassium phosphate (pH 7.0) containing 10 mM sodium succinate and chloramphenicol (200 µg ml⁻¹). The cells were then labelled by the method of Kort et al. (26). After incubation for 15 min, 0.5-ml samples were placed in microcentrifuge tubes, and the cells were labelled for 30 min by addition of 6 µl of L-[methyl-³H]methionine (85 Ci mmol⁻¹; Amersham) to each tube. The incubation time with chloramphenicol before labelling varied between 10 and 90 min. The reaction was stopped by addition of ice-cold trichloroacetic acid to a final concentration of 10%. The cells were then harvested, washed in acetone, and solubilized by being boiled for 90 s in 60 µl of Laemmli sample buffer (27). Samples were electrophoresed on 10% polyacrylamide Laemmli slab gels, soaked in Amplify (Amersham), dried, and exposed to X-ray film at -70°C for 3 to 7 days.

Western immunoblot analysis. A crude membrane fraction was obtained by passing the cells through a French press, removing whole cells by low-speed centrifugation, and then

pelletting the membrane fraction by high-speed centrifugation (13). The total protein concentration was determined with a Bio-Rad Protein Assay kit. The membranes were stored at -70°C until required. Actively motile cells were harvested and treated with acetone, and the extract was resuspended in Laemmli sample buffer to provide a total cell sample. The samples, containing about 10⁹ cells ml⁻¹ for the total cell extract and 3 mg of protein ml⁻¹ for the membrane extract, were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 10% polyacrylamide slab gels, and the polypeptides were then transferred to Hybond-C nitrocellulose sheets with Bio-Rad Transblot equipment. The Western blots were incubated with polyclonal antisera raised against the MCP Tar of *E. coli* (a gift from D. E. Koshland, Jr., University of California, Berkeley). Visualization of antibody-antigen complexes was achieved with goat anti-rabbit secondary antiserum conjugated to alkaline phosphatase and with a Nitro Blue Tetrazolium-5-bromo-4-chloro-3-indolyl phosphate indicator system (Sigma Chemical Co.).

RESULTS

Tethered cells. *A. brasilense* cells tethered by antiflagellar antibody rotated in both counterclockwise (CCW) and clockwise (CW) directions. CW rotation of a cell corresponded to a CCW rotation of a flagellar filament when viewed from behind a swimming cell. There was a wide variation in speed and reversal frequency from cell to cell as well as within individual cells. Tethered cells often appeared to rotate faster in a CW direction than in a CCW direction. This may, however, simply be the result of physical constraints on the cell body caused by being tethered by a complete flagellum rather than by a shorter hook. Tethered cells also showed more frequent changes in the direction of flagellar rotation than free-swimming cells. The differences in the behavior of free-swimming and tethered cells could have been the result of the viscous load on the tethered cell body altering motor activity or an unidentified effect of the antibody on rotation. Because of this observation, the effect of chemoeffectors on the behavior of free-swimming cells only was investigated.

Motile behavior of free-swimming cells. By inference from the tethered behavior, CCW flagellar rotation probably caused forward movement in free-swimming cells, presumably pushing the cells. Free-swimming cells spent about 80% of the time swimming forward. A change in the direction of flagellar rotation to CW caused a brief reversal. Reversals occurred about every 3 s, and the cells reversed for several body lengths before returning the CCW flagellar rotation and another period of forward swimming.

The flagellum of *A. brasilense* was inferred to be a left-handed helix rotating mainly in a CCW direction, as swimming bacteria moved in curved trajectories (Fig. 1) which were CW at the lower surface of the microslide and CCW at the upper surface of the microslide. This was probably the result of frictional drag at the glass surface on the cell body rotating in a CW direction with the flagellum rotating in a CCW direction (45).

Chemokinesis and chemotaxis: a temporal gradient assay. Individual free-swimming cells of *A. brasilense* Sp7 showed speeds of up to 100 µm s⁻¹ with a mean population speed of 13 µm s⁻¹ when speeds were determined by motion analysis. Manual frame-by-frame measurement gave an average swimming speed of 23 µm s⁻¹. Motion analysis, however, measured all bacterial cells in a population, whether stopped or swimming, and also included the time taken for reversals;

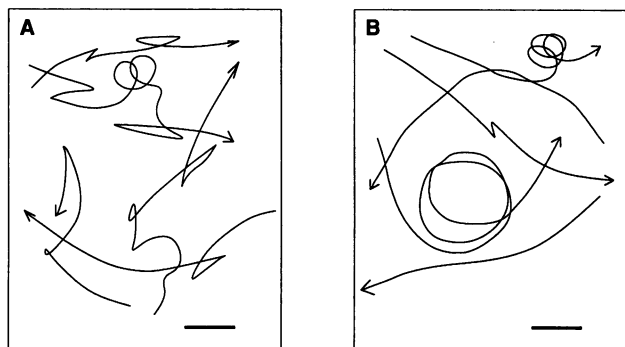


FIG. 1. Examples of paths of *A. brasilense* cells in HEPES buffer traced within 20 s of addition of buffer (A) or 1 mM malate (B). Bars, 50 μm .

manual measurements were only of periods of active free swimming.

Addition of the strong attractant malate to cells had two independent effects on swimming behavior: (i) it caused a brief suppression of direction changing, i.e., chemotaxis, and (ii) it caused a long-term increase in swimming speed, i.e., chemokinesis. It has been shown that for a chemotactic response to occur in bacteria, a change in the direction of flagellar rotation followed by adaptation is essential (28). The reversal frequency of unstimulated cells was 0.33 s^{-1} as measured by manual frame-by-frame analysis. Addition of 1 mM malate to a cell suspension resulted in a decrease in reversal frequency to 0.17 s^{-1} , with almost all of the cells swimming smoothly (Fig. 1). After 60 to 90 s, cells returned to their initial prestimulus reversal frequency. This behavior is characteristic of a chemotactic response. No true repellents were found for *A. brasilense* (7, 38, 49); however, the addition of 50 mM sodium acetate (pH 7.0) did result in a 30-s stop response in the whole population. There was a correlation between acetate concentration and adaptation time similar to that seen for *E. coli* (25) (data not shown).

In addition to the short-lived chemotactic response, the cells showed long-term chemokinesis, with the mean population speed increasing from 13 to $23\text{ }\mu\text{m s}^{-1}$ (Fig. 2A). The same effect has been described for *R. sphaeroides* stimulated with some chemoeffectors (34). As with *R. sphaeroides*, the change in the mean population speed of *A. brasilense* cells was not due to a decrease in the number of direction changes in the population but the result of a real increase in the swimming speed of individual cells. Manual analysis (excluding stopped and nonmotile cells) revealed the same relative increase in the actual mean swimming speed, from $23\text{ to }32\text{ }\mu\text{m s}^{-1}$, after addition of either 1 mM malate or 1 mM proline. There was no short-term adaptation to the chemokinetic stimulus, with the increase in the mean speed being sustained for at least 30 min after addition of the chemoeffector. The effect was strong in a freshly harvested population but weakened over several hours of starvation in buffer, being completely lost after 8 h of starvation (Fig. 2B). Interestingly, the mean swimming speed of the population increased from 13 to $25\text{ }\mu\text{m s}^{-1}$ after 10 h of starvation in buffer.

Chemotaxis in a spatial gradient of chemoeffectors. Wild-type *A. brasilense* showed a strong attractant response towards malate (Fig. 3A) and other substrates which served as effective electron donors, such as succinate, citrate, and proline. The peak concentration for all these compounds was

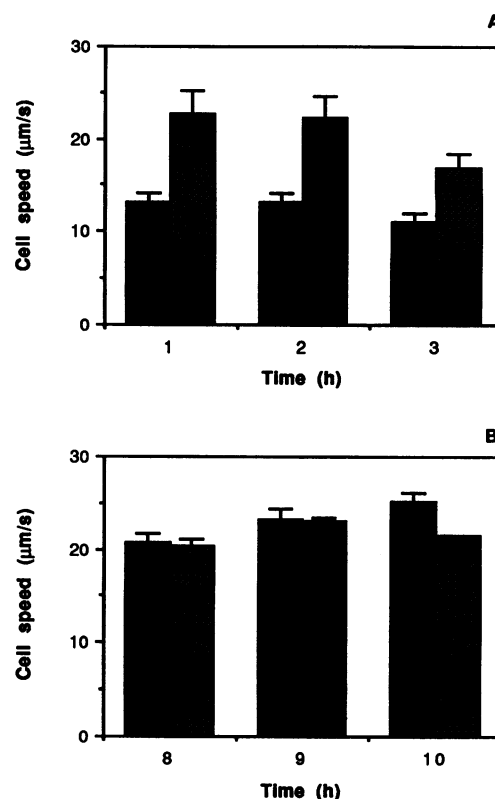


FIG. 2. Effect of malate addition on the speed of free-swimming cells of *A. brasilense* starved in HEPES buffer for 1 to 3 h (A) or 8 to 10 h (B). Data were obtained by a computerized motion analysis system within 1 min after addition of stimuli. ■, HEPES buffer; ■, 1 mM (final concentration) malate. Bars represent standard errors.

1 mM when measured with the chemotaxis wells. This is in good agreement with the results obtained by a capillary assay (38). Chemotaxis towards these compounds has been demonstrated previously with both swarm plates and plug plates (49). Compounds which caused a strong positive response in *E. coli* by signalling via MCPs were weak attractants for *A. brasilense*; some such compounds were aspartate (Fig. 3B), serine, ribose, and maltose (49). Controls with malate added to the cells in the bottom chamber or to both sides of the membrane showed that the response was the result of chemotaxis, not chemokinesis.

The methionine auxotroph *A. brasilense* 7007, starved of methionine for 16 h, showed the same pattern of chemotactic response towards a strong attractant, malate, and a weak attractant, aspartate, as did the wild type (Fig. 3), both in the presence and in the absence of $50\text{ }\mu\text{g}$ of chloramphenicol ml^{-1} .

In vivo methylation. Figure 4 shows the methylation pattern of polypeptides after stimulation with aspartate in the presence of L-[methyl- ^3H]methionine. The increase in label incorporated into *S. typhimurium* membranes after 15 min of stimulation showed the expected methylation-dependent adaptation to an attractant (15, 26). *A. brasilense* showed uptake of labelled methionine, but no changes in methylation pattern were observed in cells stimulated with either malate or aspartate. An increase in the incubation time with chloramphenicol to 60 min resulted in only one band being labelled; this band corresponded to a protein of about 43 kDa. This indicated that minor bands labelled after 10 min of

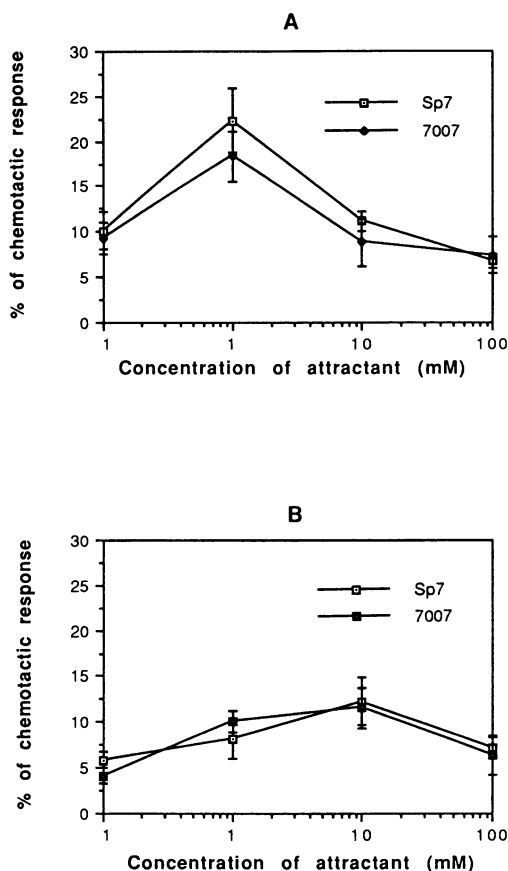


FIG. 3. Chemotaxis of *A. brasilense* Sp7 and 7007 to malate (A) and aspartate (B). Cells were starved in 10 mM HEPES-chloramphenicol ($50 \mu\text{g ml}^{-1}$) buffer, pH 7.2, for 16 h. The control results (given as percentages of chemotactic response) were as follows: buffer control, 4.26 ± 0.57 for Sp7 and 4.09 ± 0.42 for 7007; positive control (1 mM malate in bottom chamber), 5.91 ± 0.74 for Sp7 and 5.11 ± 0.05 for 7007; and chemokinesis control (1 mM malate in both chambers), 6.62 ± 1.38 for Sp7 and 5.88 ± 1.05 for 7007 (see Materials and Methods for details). Bars represent standard errors.

incubation with chloramphenicol (Fig. 4, lanes 3 to 5) probably corresponded to nonspecific incorporation of labelled methionine into proteins. No changes in the methylation pattern of the 43-kDa protein were seen in cells stimulated with malate or aspartate (Fig. 4) or with succinate, citrate, proline, or serine (data not shown). The period of stimulation was varied between 5 and 30 min, as was the concentration of chemoeffector (0.1 to 50 mM final concentration), but no change was seen in the labelling pattern or intensity under any conditions. Under these conditions ($200 \mu\text{g}$ of chloramphenicol ml^{-1} , 60-min incubation), cells remained motile and were still capable of chemotaxis towards all of the compounds mentioned above. The 43-kDa protein was probably related to the 43-kDa nutrient-dependent, chemotaxis-independent, methylated protein recently identified in *E. coli* and *Bacillus* species (11, 47).

Anti-Tar antibody hybridization assay. The MCP Tar mediates chemotaxis to aspartate in *E. coli*. The antibody raised against one of the MCPs has been shown to recognize structural features common to all other transducers in *E. coli* and is therefore an appropriate probe for conserved structures in methyl-accepting proteins of more distantly related

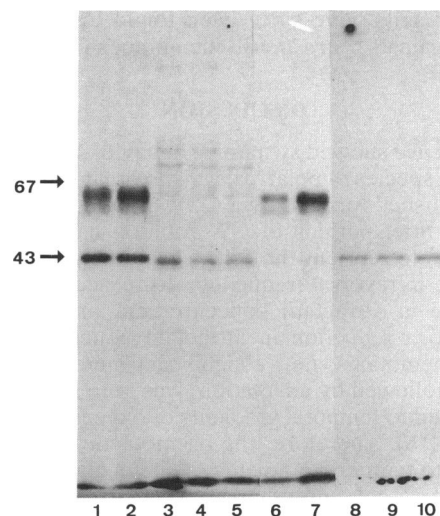


FIG. 4. In vivo methylation of *S. typhimurium* (lanes 1, 2, 6, and 7) and *A. brasilense* (lanes 3 to 5 and 8 to 10). The cells were incubated with chloramphenicol for 10 min (lanes 1 to 5) or 60 min (lanes 6 to 10). Lanes: 1, 3, 6, and 8, control (buffer); 2, 4, 7, and 9, 15-min stimulation with 10 mM sodium aspartate; 5, 10- to 15-min stimulation with 10 mM sodium malate. Arrows represent molecular weight markers (in thousands).

species (32). Figure 5 shows the proteins from *E. coli* and *A. brasilense* whole cells and *A. brasilense* cell membranes (Fig. 5a) and the pattern of hybridization with anti-Tar antibody (Fig. 5b). The antibody showed a strong interaction with a family of 60-kDa proteins from *E. coli* cells corresponding to MCPs (lane 5). No hybridization bands were found with proteins from either whole *A. brasilense* cells (lane 7) or *A. brasilense* membranes (lane 6), even when the gels were heavily overloaded (lane 3).

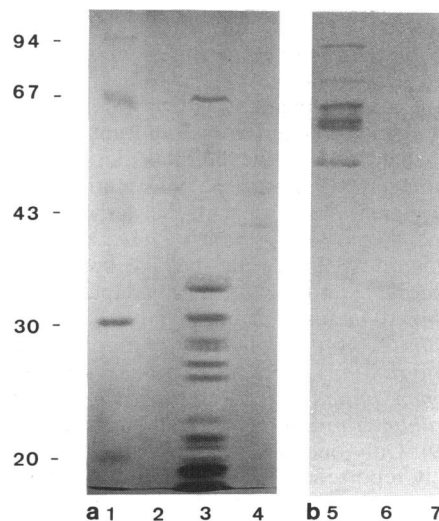


FIG. 5. Anti-Tar antibody hybridization. (a) Sodium dodecyl sulfate-polyacrylamide gel showing total proteins; (b) Western blot of proteins transferred from part of the same gel. Lanes: 1, molecular weight markers (indicated at left in thousands); 2 and 5, *E. coli* cells; 3 and 6, *A. brasilense* membranes; 4 and 7, *A. brasilense* cells. The arrow indicates a 60-kDa band.

Similar results have since been found by using anti-Trg antibody against *A. brasilense* membranes (21).

DISCUSSION

A. brasilense showed swimming behavior similar to that of some other species of polarly flagellate organisms (45), being generally pushed forward by a CCW-rotating flagellum with occasional brief periods of CW rotation causing reversals which serve to reorient the cell. Chemotaxis was the result of a change in reversal frequency, decreased in response to an increase in attractant concentration, and increased in response to a reduction in attractant concentration. True negative chemotaxis, i.e., a significant increase in reversal frequency followed by adaptation, was seen in *A. brasilense* cells exposed to temporal gradients of oxygen under specific conditions (48). Therefore, the chemotactic response of *A. brasilense* to temporal gradients of chemoattractants resembled that of *E. coli* and other bacteria (10, 16, 20, 30). The attractants tested were all metabolites, and they caused an additional chemokinetic response. This response may be the result of increased metabolism increasing the electrochemical proton gradient ($\Delta\mu\text{H}^+$).

The chemokinetic response appeared to be dependent on the metabolic state of the population, being strongest in populations freshly harvested into buffer and being lost after about 8 h of starvation in buffer. *A. brasilense* has been shown to start utilizing the endogenous storage substrate poly- β -hydroxybutyrate (PHB) after a few hours of starvation (44). It is possible that the $\Delta\mu\text{H}^+$ fell below the saturation level for flagellar rotation immediately after the bacteria were resuspended in buffer because of the lack of respiratory substrates, causing a consequential decrease in swimming speed. Subsequent utilization of the substrate PHB, which involves NADH dehydrogenase and electron transport, may have increased the $\Delta\mu\text{H}^+$ and therefore restored the swimming speed. It is therefore possible that the increase in swimming speed seen after the addition of malate or proline was the result of increased electron transport activity increasing the driving force for flagellar rotation, the $\Delta\mu\text{H}^+$ (31). The loss of a chemokinetic response after several hours of starvation and the induction of PHB metabolism may therefore reflect a $\Delta\mu\text{H}^+$ above the saturation value for flagellar rotation.

The methylation-dependent pathway is the central chemotactic system in *E. coli* and *S. typhimurium* (22, 29). Chemo-effector ligands bind to the periplasmic side of the membrane-spanning MCPs, and the information is transduced across the membrane to change the activity of a series of cytoplasmic chemotaxis proteins: CheA, a histidine kinase, and its substrates; CheY, the flagellar switch protein; and CheB, a methyl esterase involved in adaptation. MCPs have been identified in many other bacterial species, such as *Bacillus subtilis* (46), *Halobacterium halobium* (2, 41), *Spirochaeta aurantia* (24), *Pseudomonas aeruginosa* (14), *Pseudomonas putida* (19), *Caulobacter crescentus* (40), *Rhodospirillum rubrum* (43), and *Agrobacterium tumefaciens* (39). Only one bacterium, *R. sphaeroides*, has been conclusively reported to lack a methylation-dependent pathway for chemotaxis (36, 43). This microorganism shows a strong positive response to electron donors (organic acids), phosphotransferase system-independent sugars, and electron acceptors (oxygen and dimethyl sulfoxide) (4).

A. brasilense shows chemotaxis towards a range of different nutrients, but the results presented here show that the dominant chemotactic system in this bacterium is not based

on the methylation-dependent pathway. We employed two direct methods to probe for MCPs in *A. brasilense*. Changes in protein methylation after chemotactic stimulation have identified MCPs in many different bacterial species, but none were found in *A. brasilense*. We also failed to find any protein in *A. brasilense* cell membranes which showed homology with MCPs from *E. coli* by using anti-MCP antibody. This method has revealed MCPs in bacteria distantly related to *E. coli* e.g., *B. subtilis*, *S. aurantia* (32), and *H. halobium* (1).

The results suggesting that the main chemosensory pathway in *A. brasilense* does not involve an MCP system can be summarized as follows. (i) Strong attractants for *A. brasilense* were effective substrates for growth under aerobic conditions, i.e., electron donors to the redox chain. These compounds are known to be attractants for *E. coli*, but they may also be sensed via an MCP-independent pathway probably involving the functioning redox chain (29). Taxis towards the main electron acceptor, oxygen, was so strong in *A. brasilense* that it often masked responses to other chemicals (8), suggesting a dominant role for redox-dependent responses in this organism. (ii) No repellents were identified among the compounds tested; repellents of *E. coli* (leucine, Ni^{2+} , and Co^{2+}) have been shown to be sensed by a general MCP-dependent response. (iii) The methionine auxotroph showed the same behavior as the wild-type strain even after prolonged methionine starvation. Methionine-dependent adaptation is a necessary prerequisite for a chemotactic response via MCP in *E. coli* (26). (iv) In vivo methylation and anti-Tar hybridization did not identify MCPs. We cannot exclude the possibility that *A. brasilense* has MCPs at a concentration which was not detectable by the methods used, but the results obtained suggest that even if MCPs are present, they do not play a major role in the chemotactic behavior of *A. brasilense*. The chemotactic pathway and the redox-dependent pathways involved in, for example, aerotaxis, must integrate to produce a balanced response. As yet, there are no data on the mechanisms of integration.

These findings show that the chemotactic behavior of *A. brasilense* is similar to that of *R. sphaeroides* despite the difference between the motility patterns of these bacteria (4, 6, 34). It is possible that the type of metabolism and the type of natural environment in which a species is found, rather than the type of flagellation and motility, dictate which chemosensory system is dominant. *A. brasilense* is found in nutrient-limiting environments (such environments are also typical for *R. sphaeroides*) where its main requirement is to find plant roots excreting organic substrates. Migration of *A. brasilense* towards plant roots in soil has been demonstrated in laboratory and field experiments (9); therefore, chemotaxis in natural environments would be expected to provide a real advantage for bacteria in search of a source of nutrients and in competition with other microorganisms. The chemotactic response of *A. brasilense*, like that of *R. sphaeroides*, seems to be dependent on uptake and/or metabolism of chemicals, supporting the idea that metabolism-dependent chemotaxis might be a dominant system in bacteria living in natural environments under growth-limiting conditions (4). Under these conditions, it may be an advantage to respond positively to any compounds which increase the metabolic rate rather than to rely on the more limited range of chemicals sensed via the dedicated MCP pathway.

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