Chapter 6
Chemotaxis in *Azospirillum*

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Abstract The ability of *Azospirillum* to colonize the roots of plants depends on motility and chemotaxis. *Azospirillum* cells are motile and capable of chemotaxis toward organic acids, sugars, and some aminoacids. *Azospirillum* is also able to navigate gradients of oxygen, alternative electron acceptors, and redox active compounds. Most attractants and repellents described thus far for this bacterial genus include compounds that affect intracellular metabolism, leading to the suggestion that most taxis responses correspond to energy taxis in *Azospirillum* spp. Several spatial and temporal gradient assays that can be implemented as quantitative methods are available to characterize taxis responses in *Azospirillum* species. The analysis of complete sequence genomes of several *Azospirillum* species reveals that taxis responses are coordinated by multiple chemotaxis pathways. All genomes also possess a chemotaxis pathway that is predicted to regulate alternative cellular functions other than flagellar motility. Genome analyses indicate that all *Azospirillum* spp. sequenced to date encode for an extremely large repertoire of putative chemotaxis receptors, which is likely contributing to explaining their ubiquitous distribution.

6.1 Chemotaxis: An Overview of Paradigm

Microorganisms have evolved a plethora of strategies to adapt to spatial and temporal changes in the environment. Some of these strategies involve changes in patterns of gene expression, and thus the implementation of long-term adaptive responses. In addition, many bacteria are able to rapidly and transiently respond to changes in the environment. In motile bacteria, these transient adaptive responses include the ability to navigate toward more favorable conditions or away from deleterious ones. This behavior is referred to as chemotaxis when motile bacteria respond to gradients of chemical effectors, aerotaxis when they respond to gradients of oxygen,

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phototaxis when they respond to gradients of light, pH taxis when they respond to gradients of pH, etc. (Wadhams and Armitage 2004). Because of their small sizes, bacteria experience low Reynolds number environments, where inertia is negligible and where viscosity is a major force that constrains movement of particles and bacterial cells. As a result, motile bacteria use an undulatory behavior via helical flagella that rotate as propellers to move the cell forward or backward. Furthermore, bacteria behave as particles in a solution and they are subjected to physical forces, such as Brownian motion, that do not impact the behavior of larger organisms (Berg 1993). Brownian motion is essential to randomly reorient swimming bacteria in new directions, and this physical force is particularly significant for the reorientation of singly polarly flagellated bacteria (Mitchell 2002), such as bacteria of the genus *Azospirillum*. Furthermore, because of their small size, motile bacteria do not detect gradients spatially. Instead, they use temporal sensing in which current conditions are compared with the ones encountered a few milliseconds before. In the absence of a gradient, motile bacteria move about randomly and thus not in any particular direction. When moving up a gradient of an attractant, motile bacteria suppress the changes in swimming direction in the direction of the attractant and swim longer toward the attractant. As a result, their net movement is up the attractant gradient. Conversely, in a gradient of a repellent, bacteria tend to change swimming directions more frequently in the direction of the repellent but less when moving away from it. This behavior allows them to run longer away from the repellent (Sourjik and Wingreen 2012).

The molecular mechanism of bacterial chemotaxis has been studied in great detail in the model organism *Escherichia coli*, a peritrichously flagellated bacterium (Wadhams and Armitage 2004; Sourjik and Wingreen 2012). The *E. coli* proteins that comprise the chemotaxis signal transduction pathway are encoded within a single operon, with additional chemotaxis receptors located elsewhere on the genome. Environmental signals are detected by chemotaxis receptors (chemoreceptors or the so-called methyl accepting chemotaxis proteins or MCP) that are arranged in large arrays of allosterically coupled proteins located at the cell poles (Hazelbauer et al. 2008). *E. coli* possesses five different chemotaxis receptors: Tar, Tsr, Trg, Tap, and Aer. Trimers of receptor dimers are bound on their cytoplasmic side by a ring of CheA-CheW proteins (Briegel et al. 2012). CheA is the dedicated histidine kinase, while CheW is a docking protein that anchors CheA and the chemoreceptors within the array. Reception of a signal triggers a conformational change in chemoreceptors, thought of as a piston-like movement, that in turn modulates the activity of the CheA histidine kinase. Upon receiving a repellent signal, CheA becomes phosphorylated on a conserved histidine residue. Phospho-CheA then phosphorylates two response regulators: CheY and CheB. Phospho-CheY, but not unphosphorylated CheY, is able to bind to the flagellar motors with high affinity to trigger a change in the swimming direction. CheB is a methylesterase, which demethylates conserved residues in the cytoplasmic domains of receptors and counteracts the methylation of these residues by a constitutively expressed CheR methyltransferase. Changes in the methylation status of receptors reset them in a sensing mode. Because methylation and demethylation are catalytically slower than phosphorylation, CheB and CheR

affect receptors at a slower rate compared to the rate of phosphorylation of CheA and CheY. This time delay confers a short "memory" in bacterial chemotaxis. *E. coli* also possesses a phosphatase, CheZ which enhances CheY dephosphorylation and thus contributes to signal termination (Hazelbauer et al. 2008).

This mode of signal transduction represents a blueprint for bacterial chemotaxis. The molecular principles that govern chemotaxis in *E. coli* appear to be conserved in other bacterial and archaeal species, albeit with several differences (Wadhams and Armitage 2004; Briegel et al. 2009; Wuichet and Zhulin 2010). The *E .coli* chemotaxis system is also very simple in comparison to other bacterial genomes in terms of number of chemotaxis operons and additional chemotaxis genes, as well as the greater number of chemoreceptors present (Wuichet and Zhulin 2010). There is a need to characterize a broader diversity of chemotaxis systems to gain an accurate perspective of the role of this behavior in the ability of bacteria to respond and adapt to changes in their surroundings.

6.2 Types of Taxis Responses in *Azospirillum* spp.

Bacteria of the genus *Azospirillum* are motile by means of a single polar flagellum in liquid media (swimming) and several lateral flagella when moving across surfaces (swarming). The polar and lateral flagella are not only functionally different, but they also comprise a distinct set of structural proteins (Moens et al. 1995, 1996). While chemotaxis has been described to regulate polar flagellum mediated motility, i.e., swimming, in liquid environments, experimental evidence for chemotaxis regulating swarming motility has yet to be obtained.

Taxis behaviors have long been suggested to contribute to the ability of *Azospirillum* spp. to colonize the roots of plants, and experimental evidence in support of this assumption has been obtained (Reinhold et al. 1985; Bashan and Holguin 1994; Vande Broek et al. 1998; Greer-Phillips et al. 2004). Motility and chemotaxis have been best described in *A. brasilense* strain Sp7, which is thus the model for understanding taxis behaviors in this bacterial genus.

6.2.1 Chemotaxis

A. brasilense is capable of chemotaxis toward a large set of organic acids, sugars, sugar alcohols, and to a lesser extent, amino acids (Okon et al. 1980; Alexandre et al. 2000). By testing a range of chemoeffector concentrations in different types of chemotaxis assays (see below), it is possible to determine the relative strength of a chemoeffector. The chemotaxis response toward a particular chemical usually correlates with its concentration. True chemotaxis is optimum at a certain concentration and declines proportionally at higher and lower concentrations. A strong chemoeffector elicits a maximum chemotaxis response at low concentrations, while a weak

chemoeffector elicits a peak chemotaxis response at relatively high concentrations. The stronger attractants for *A. brasilense* are also the best growth substrates, and starving cells display an enhanced chemotaxis response (Alexandre et al. 2000). Organic acids that are also intermediates of the citric acid cycle, such as malate and succinate, are very strong attractants for *A. brasilense*. Pyruvate, citrate, oxalate, and fumarate also represent good attractants for this species.

Amongst the sugars, fructose is one of the strongest attractants but ribose, arabinose, and galactose are also good attractants for *A. brasilense*. As in many other bacterial species, chemotaxis to galactose is inducible, and it is not detected unless the cells are grown in the presence of galactose prior to measuring chemotaxis (Alexandre et al. 2000). Galactose metabolism is inducible in *A. brasilense* and includes expression of a specific ABC transport system, which uses a periplasmic binding protein named SbpA. In *A. brasilense*, galactose chemotaxis is mediated by a chemotaxis receptor that also uses SbpA and a mutant lacking SbpA is specifically impaired in galactose chemotaxis and metabolism (Van Bastelaere et al. 1999). It is noteworthy to indicate that *A. brasilense* is unable to display chemotaxis toward glucose. This observation makes sense when one considers that glucose is not a carbon source used by *A. brasilense*.

Amino acids such as glutamate, aspartate, alanine, glutamine, and asparagine are weak attractants for *A. brasilense*. The weak propensity of these amino acids to elicit a chemotaxis response likely results from the diazotrophic metabolism of *Azospirillum* spp. and thus the limited reliance on external organic nitrogen sources such as amino acids for nutrition. Glycine, histidine, isoleucine, threonine, valine, arginine, lysine, and methionine are not chemoeffectors for *A. brasilense* (Okon et al. 1980; Alexandre et al. 2000).

Chemotaxis toward phenolic compounds such as benzoate, catechol, and hydroxybenzoate was reported (Lopez-de-Victoria and Lovell 1993) but could not be reproduced by Alexandre et al. (2000). This discrepancy may be related to the different assays used by these authors. In particular, Lopez de Victoria and Lovell (1993) used the capillary assay, which is prone to false-positive results due to the strong aerotaxis response of *Azospirillum* in capillary tubes assays, which can mask the chemotaxis response measured. Several studies analyzing chemotaxis in *Azospirillum* spp. have concluded that the capillary assay was inadequate to assess chemotaxis in this species (Okon et al. 1980; Barak et al. 1982; Alexandre et al. 2000).

6.2.2 Aerotaxis

The strongest taxis response in *Azospirillum* is aerotaxis or the directed movement of bacteria in gradients of oxygen (Barak et al. 1982; Zhulin et al. 1996).

Azospirillum spp. have a microaerophilic oxidative metabolism that is optimum at about 0.4 % dissolved oxygen (Zhulin et al. 1996). *Azospirillum* thus grow best under low aeration conditions and when exposed to a spatial oxygen gradient, motile cells move away from elevated oxygen concentrations and toward low oxygen

concentrations. The physiological consequences of this behavior are readily observed when motile *A. brasilense* cells are inoculated in a tube containing a low agar concentration to permit motility: cells concentrate as a band that grows below the surface but never on top of the agar. Note that the formation of a dense area of growth in this assay does not solely represent an aerotaxis response. Indeed, the formation of this zone of growth below the surface depends not only on the motility of cells but also on their ability to navigate in oxygen gradients (i.e., aerotaxis) as well as to grow under these conditions. Aerotaxis requires that the cells have an active respiratory chain, suggesting that it is not sensing oxygen itself that triggers the movement in the oxygen gradients and that cells are sensing oxygen indirectly, by monitoring another parameter related to respiration with oxygen (Alexandre et al. 2000).

6.2.3 Taxis to Alternative Electron Acceptors

In the absence of oxygen, *Azospirillum* spp. are able to use alternative terminal electron acceptors, including nitrate, nitrite, and DMSO, for respiration. It is thus not surprising that chemotaxis in gradients of alternative electron acceptors has also been described in *A. brasilense* (Alexandre et al. 2000). These taxis responses are much weaker than the aerotaxis response. Consistent with the bacteria sensing nitrate, nitrite, or DMSO as terminal electron acceptors and not to fulfill other nutritional needs, taxis responses toward these compounds is only observed under anaerobic conditions and they are abolished when cells are incubated with oxygen. The taxis response is also not observed if cells are not provided with an electron donor in a form of a carbon substrate for growth.

6.2.4 Redox Taxis

Redox taxis is a motility behavior first described in *E. coli* (Bespalov et al. 1996) and it corresponds to the ability of bacteria to navigate gradients of redox active compounds. During redox taxis, bacteria monitor changes in redox, instead of change in the concentration of a specific compound. Redox taxis is determined by using substituted quinones, which are small lipophilic compounds able to diffuse through the membrane. Redox taxis can be convincingly established by analyzing the taxis responses of cells in gradients of quinones with different redox potentials (Bespalov et al. 1996; Alexandre et al. 2000). In order to be redox active, the quinones are maintained in an oxidized state using potassium ferricyanide. Redox taxis is best established using oxidized quinones which have redox potential values within the range of redox potentials that permit electron transfer in the electron transport chain. One such example is 1,4-benzoquinone (Bespalov et al. 1996). Quinones differ in chemical structure as well as redox potential; the lower the redox

potential of the quinone tested, the greater the affinity of the oxidized quinone for electrons, and thus the more likely it is this oxidized quinone will effectively compete for electrons. *A. brasilense* motile cells demonstrate a repellent response to oxidized quinones: the repellent response increases with the decreasing redox potential of the quinone tested (Alexandre et al. 2000). Redox taxis can be inferred when the repellent response displayed by motile *Azospirillum* cells correlates with the redox potential, rather than the chemical structure of the quinones tested.

6.2.5 *Energy Taxis as the Dominant Mode of Environmental Sensing*

Most but not all chemotaxis and other taxis responses in *A. brasilense* require a functional electron transport system, and these responses are enhanced when cells are starved (Alexandre et al. 2000). Further, the strength of the taxis response correlates with the extent to which chemoeffectors affect the integrity and efficiency of the electron transport system. Electron donors and electron acceptors are excellent attractants for *A. brasilense* while chemicals that block electron transport within the electron respiratory chain, such as quinones or myxothiazol trigger repellent responses. Taxis responses toward particular chemicals are also abolished if structural analogs of these chemicals, that cannot be metabolized, are used. Most taxis responses of motile *A. brasilense* cells appear to originate within the electron transport system, an observation that has led to the proposition that energy taxis dominates motile behavioral responses in this species (Alexandre et al. 2000). Energy taxis refers to the ability of motile cells to navigate in gradients of physicochemical parameters that affect the energy-generating cellular processes such as the electron transport chain (Alexandre 2010). Dedicated chemotaxis receptors proposed (Tlp1; (Greer-Phillips et al. 2004)) or demonstrated (AerC; (Xie et al. 2010)) to sense redox-related parameters have indeed been characterized in *A. brasilense* Sp7. While most taxis responses are related to energy taxis in *A. brasilense*, metabolism-independent taxis also functions in this species as suggested by the role of the periplasmic binding protein SbpA in chemotaxis toward galactose (Van Bastelaere et al. 1999)

6.3 Assays Suitable for Measuring Chemotaxis, Aerotaxis, and Redox Taxis in *Azospirillum* spp.

Several quantitative and qualitative assays to monitor all forms of taxis responses have been developed and optimized in *A. brasilense* and can be used in any of the *Azospirillum* spp. (Fig. 6.1).

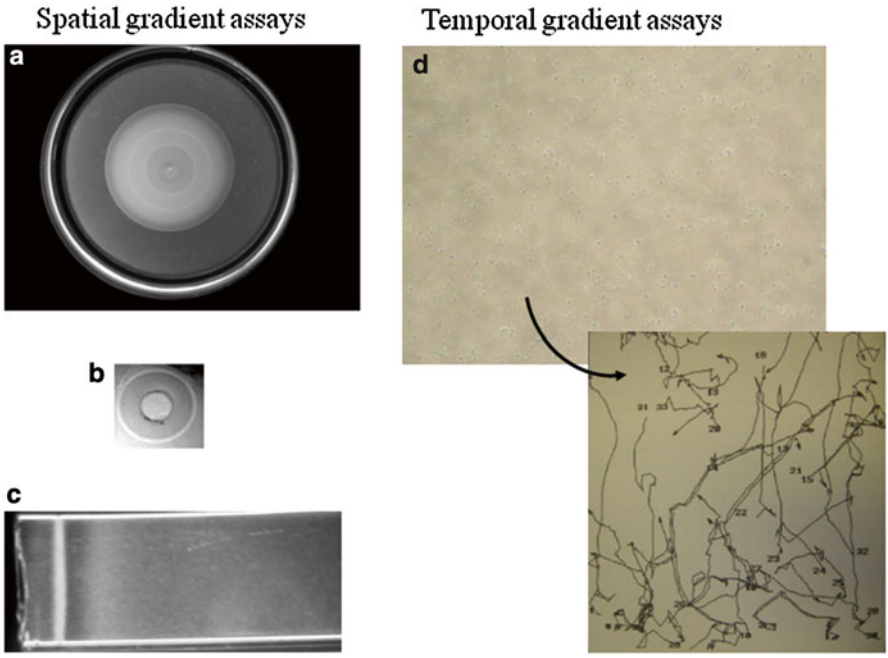

Fig. 6.1 Spatial and temporal gradient assays used to analyze taxis responses in *Azospirillum* spp. (**a**) Soft-agar plate assay; (**b**) mini-plug assay; (**c**) spatial gradient assay for aerotaxis; (**d**) temporal gradient assay. *Top*: example of a still image of a video captured to analyze by computational motion analysis. *Bottom*: example of tracks of individual cells detected by a computerized motion analysis system. See text for details

Regardless of the taxis response measured, using cell populations where at least 90 % of cells are motile and preferably grown in minimal medium to low density, is preferred to ensure homogenous results and avoid the potential interfering effect of the accumulation of intracellular granules of polyhydroxybutyrate (Alexandre et al. 2000). In addition, some taxis responses are inducible in *A. brasilense*. When testing taxis to compounds for which no previous experimental record exists, it is best to measure the taxis responses toward this compound in parallel assays that use cells induced with the compound to be tested as well as without the compound to be tested to ensure an accurate interpretation of the results.

Assays suitable for measuring taxis responses in *Azospirillum* spp. include spatial gradient assays (soft-agar plate, chemical-in-plug, aerotaxis assays) and temporal gradient assays. In spatial gradient assays, cells' metabolism interferes with the responses observed and thus, controls must be included to insure that taxis responses per se are measured. The most sensitive chemotaxis assays for *Azospirillum*, i.e., those able to detect the weakest behavioral responses are the temporal gradient assays while the least sensitive is the soft agar plate assay. Regardless of whether a spatial or temporal gradient assay is used, taxis responses are dose dependent and

show a maximum under specific conditions. A spatial or temporal gradient assay may thus be used in quantitative characterization of taxis behaviors by establishing a dose-dependent response. In this case, the stronger the chemoeffector, the lower the concentration at which it triggers a taxis response.

6.3.1 Soft Agar Plate Assay

The soft agar plate assay is carried out in media solidified with a low concentration of agar to allow cells swimming. This assay is often erroneously called a "swarm plate assay." Swarming is a form of motility across a surface and it requires different flagella and cell surface characteristics. The soft agar plate assay measures the ability of motile cells to swim down attractant gradients created via cellular metabolism. In this assay, cells from an overnight culture or a colony are inoculated in the center of a soft agar plate containing 0.3 % agar and a single carbon and nitrogen source that the cells can use for growth (Fig. 6.1, panel A). For this assay to be successful, the soft agar medium prepared with 0.3 % agar (w/v) must be boiled before autoclaving or pouring the plates to ensure uniform distribution and consistent results. Due to the sensitivity of *Azospirillum* spp. to oxygen gradients, it is also recommended that separate plates contain equivalent volumes of medium (e.g., 25 mL molten medium/plate).

Cells growing at the point of inoculation deplete the local environment of carbon and nitrogen sources. If these cells are motile and able to navigate chemical gradients (i.e., able of chemo-taxis responses), they will move away from this inoculation zone toward higher concentrations of nutrients and thus will spread outward. The presence of a chemotaxis ring, seen as a dense zone of cells with the cell density dropping before and after this zone, is indicative of chemotaxis or taxis to alternative electron acceptors, when performed under anaerobic conditions.

A variation of this assay consists of inoculating the cells in a soft agar plate made with a minimum buffer, and providing chemoeffectors "spots," distant to the inoculation point. The gradient of the chemoeffector tested will be established by diffusion, and the cells will preferentially move in the direction of the chemoeffector if it functions as an attractant. This latter assay has been used to demonstrate chemotaxis of *Azospirillum* toward the root exudates of various plants (Okon et al. 1980).

6.3.2 Chemical-in-Plug Assay

The chemical-in-plug assay is a spatial gradient assay that was first developed by Tso and Adler to detect chemotaxis responses toward repellents (Adler and Tso 1974). In *Azospirillum* spp., this assay can be used to measure chemotaxis, and it is also particularly well suited to analyze taxis responses to alternative electron

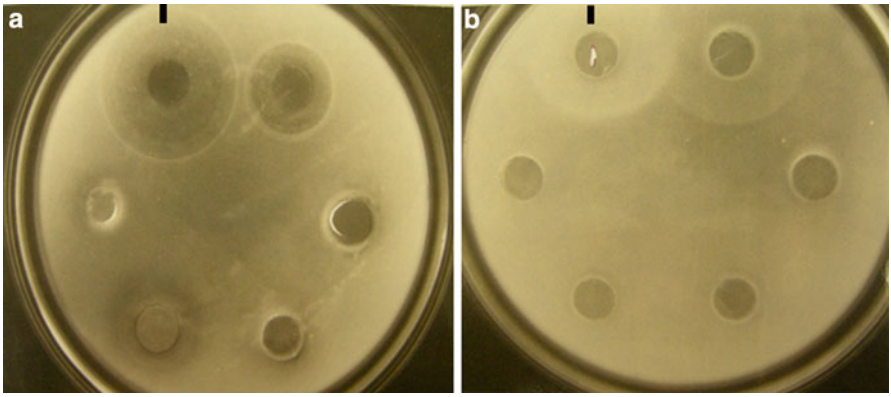

Fig. 6.2 Mini-plug assay used to detect repellent responses (**a**) and attractant responses (**b**). The observations were photographed after 1 h incubation at 28 °C. (**a**) Plugs contain the following described in clockwise direction from the mark on the plate: 1,4-benzoquinone (10 mM) and ferricyanide (2 mM), 1,4-benzoquinone (1 mM) and ferricyanide (2 mM), 1,4-benzoquinone (0.1 mM) and ferricyanide (2 mM), ferricyanide (2 mM) alone, acetate (10 mM), chemotaxis buffer. Note the clearing zone around the plugs in the repellents (1,4 benzoquinone and acetate). The size of the clearing zone depends on the concentration of the effector tested as seen with 1,4-benzoquinone. (**b**) Plugs contain the following described in clockwise direction from the mark on the plate: Malate (10 mM), Fructose (10 mM), Galactose (10 mM), Aspartate (10 mM), Proline (10 mM); Isoleucine (10 mM). Note the accumulation of cells around the plugs. The zone of accumulation is faint around Aspartate and Proline, and there is no accumulation around Isoleucine or Galactose. Cells were not induced with galactose prior to this assay

acceptors and to assess redox taxis (Figs. 6.1, panel B and 6.2). In this assay, the gradient of the chemoeffector to be tested is established spatially by diffusion from a plug of agar solidified with 2 % agar (w/v) placed into a soft agar (0.3 % agar) medium that also contains motile cells. The cells will form a ring around the plug if they respond tactically to it (Fig. 6.2). In the case of a repellent response, the ring will form around the plug but a clearing zone will be seen between the plug and the ring of cells. Confirmation of a taxis response and exclusion of growth as being responsible for the formation of the ring can include performing the assay in the presence of chloramphenicol or tetracycline to inhibit growth and by omitting essential nutrients (e.g., a carbon source) to prevent growth. The latter conditions may be achieved by using plugs and soft agar plates prepared in chemotaxis buffer (10 mM phosphate, 0.1 mM EDTA). Typically, a very dense cell suspension is mixed with an equal volume of 0.6 % agar in chemotaxis buffer to yield a soft agar at a final concentration of 0.3 %. Plugs of 2 % agar also prepared in chemotaxis buffer are cut out using large pipette tips as tools and placed in the still molten soft agar medium. Results are usually obtained between 1 and 4 h post-inoculation. A key parameter to consider in this assay is to use a dense suspension of motile cells which may be prepared from cultures grown to low density and concentrated

by centrifugation at low speed (to prevent shearing of the flagella). Different concentrations of chemoeffectors, ranging from 1 to 20 mM, can be introduced into the plugs in order to determine the threshold for the attractant or repellent response, which also makes the assay semiquantitative.

6.3.3 Mini-Plug Assay

A variation on the chemical-in-plug assay for use with a light microscope is the mini-plug assay. In this version of the assay, a small plug of 1.5 % low melting point agarose in chemotaxis buffer containing the chemical to be tested as a chemoeffector is placed in a microchamber. The microchamber is constructed using silicon grease to delineate a small volume the size of the coverslip to be used. A 100 μL aliquot of motile cell suspension, washed prior to being placed in chemotaxis buffer, is introduced into the microchamber and covered with a coverslip. A control plug containing only chemotaxis buffer must also be prepared. The formation of a chemotactic band away from (repellent effect) or near (attractant effect) the plug can be observed in real time. Chemoeffectors are tested in a wide concentration range, typically from 1 μM to 1 mM. Compared to the chemical-in-plug assay, the mini-plug yields results within a few minutes and there is no possible confounding effect of growth.

6.3.4 Aerotaxis Assay

Aerotaxis in *Azospirillum* can be analyzed using a spatial gradient assay where the oxygen gradient is established by diffusion of from air inside a suspension of highly motile cells. This assay is performed in optically flat microcapillaries (e.g., inner dimensions, 0.1 by 2 by 50 mm; Vitro Dynamics Inc., Rockaway, N.J.) and a light microscope. The microcapillaries are filled by capillarity by dipping them into a suspension of motile cells prepared by gentle washes and centrifugation at low speed and resuspension into fresh medium containing a carbon source and possibly, a nitrogen source. The formation of the aerotactic band is exclusively observed at the end of the microcapillary that was dipped into the cell suspension and not at the opposite end since conditions there are not controlled and consistent. Under these conditions, microscopic observations indicate that motile cells move from locations within the microcapillaries of higher and lower concentrations of oxygen toward a zone of preferred low oxygen concentration, forming an aerotactic band (Fig. 6.3). This aerotactic band forms within 2 min and remains stable over at least 30 min. The area or distance between the aerotactic band and the air-liquid meniscus can be measured for a semiquantitative use of this technique. However, the exact location of the aerotactic band with respect to the meniscus depends on cell motility, cell density, carbon source used as a substrate under these conditions, the respiration rate, and the stage of growth. Experimental conditions must therefore be calibrated to ensure reproducibility.

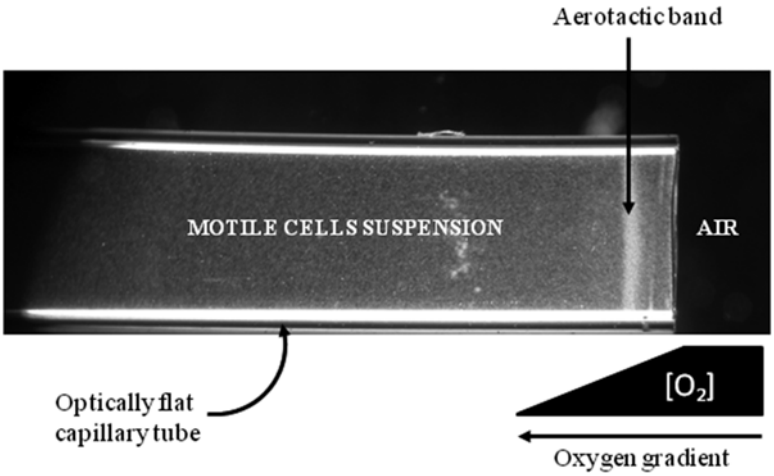

Fig. 6.3 Temporal gradient assay for aerotaxis in *Azospirillum* spp. using an optically flat capillary tube. This is a typical image taken 2 min after the capillary tube was filled with the cell suspension

6.3.5 Temporal Gradient Assay

The temporal gradient assay is the most direct assay to measure taxis responses in *Azospirillum* or any other motile bacterium because the ability of cells to temporally adjust their swimming motility bias (number of changes in swimming direction or reversals per unit of time) upon stimulation with an effector is measured (Fig. 6.1; panel D). Both attractant (transient suppression of reversals) and repellent (transient increase in the number of swimming reversals) responses can be measured. Furthermore, the response time (the time it takes for 50 % of the cell population to respond to the stimulus imposed) and adaptation time (the time it takes for the population to return to the pre-stimulus swimming bias) can also be measured using this method. Adaptation is the hallmark of bacterial taxis behaviors, and it cannot be measured in any of the other assay. In the temporal gradient assay, the chemoeffector (oxygen, air, or any chemicals) to be tested is directly added to a suspension of motile cells. Changes in the motility bias upon addition of the effector is recorded in real time and analyzed using a computerized motion analysis system (e.g., CellTrack, Hobson Tracker) to determine the reversal frequency or average number of changes in swimming direction per unit of time (Fig. 6.1; panel D). Video recording of the entire assay is required. A 9-μL drop of a diluted bacterial suspension (~10^7 cells/ml to facilitate tracking and downstream computerized motion analysis) in chemotaxis buffer is first placed on a microscope slide and equilibrated for at least 5 min. Next, the chemical compound to be tested (1 μL) is added to the suspension and video recording must be allowed for at least 5 min post-stimulation. Temporal assays can be performed under fully aerated conditions or under anaerobic conditions in a

microchamber (Zhulin et al. 1996; Alexandre et al. 2000), which allows the response in a temporal gradient of alternative electron acceptors or oxygen to be measured.

6.4 Chemotaxis in *Azospirillum* spp. Genomes

In contrast to the model organism *E. coli* which possesses a single chemotaxis pathway encoded within a single operon and only five different chemotaxis receptors, the genomes of *Azospirillum* species sequenced thus far indicate the presence of several chemotaxis signal transduction pathways encoded in operons and a very large number of receptors (Kaneko et al. 2010; Sant'Anna et al. 2011; Wisniewski-Dye et al. 2011, 2012).

The genome of *A. amazonense* Y2 encodes three chemotaxis pathways and 40 chemotaxis receptors, that of *A. brasilense* Sp245 encodes four chemotaxis pathways and 51 chemotaxis receptors, that of *A. lipoferum* 4B encodes five chemotaxis pathways and 63 chemotaxis receptors and the genome of *Azospirillum* sp. B510 codes for six chemotaxis pathways and 89 chemotaxis receptors. While most soil bacteria possess a large number of chemotaxis receptors, the average number is around 25–30. Bacteria of the genus *Azospirillum* have thus an extremely large repertoire of chemotaxis receptors, which further complicates the identification of their sensory abilities. Indeed, the sensory specificity of most chemotaxis receptors is not known (Krell et al. 2011). This complexity also suggests that chemotaxis signal transduction provides *Azospirillum* with a competitive advantage in the soil and the rhizosphere. Such a large repertoire of putative chemotaxis receptors suggests that *Azospirillum* spp. can monitor diverse environmental cues. This ability is likely contributing to explaining their ubiquitous distribution. However, the exact advantage that possessing multiple pathways provides cells with is not yet clear.

Of the multiple chemotaxis pathways encoded within the *Azospirillum* spp. genomes, most are predicted to control flagellar-based motility and thus probably direct swimming and/or swarming. One of the chemotaxis operons found in all *Azospirillum* spp. is not predicted to control flagellar motility but rather another cellular function (belonging to a larger group of ACF (Alternative Cellular Function)-like pathways) (Wuichet and Zhulin 2010). In *A. brasilense* Sp7, chemo- and aerotaxis are characterized by a transient change in swimming speed as well as a transient change in the swimming motility bias. Each of these behaviors is controlled by at least two different chemotaxis pathways (Stephens et al. 2006; Bible et al. 2008, 2012); one pathway controls transient changes in swimming speed during chemo- and aerotaxis (named Che1 (Bible et al. 2012)) and a second one (or perhaps more?), not yet identified, is implicated in controlling transient changes in swimming directions (Russell et al. 2013). In addition, signaling cross talk between Che1 and another chemotaxis pathway has been identified but is yet to be characterized at the molecular level (Stephens et al. 2006).

6.5 Conclusions and Outlook

Several genome sequences and a suite of quantitative and qualitative assays are available to study taxis behaviors in *Azospirillum* spp. The extended tool kit to assess taxis responses in this species provides an opportunity to use *Azospirillum* chemotaxis signal transduction as a model system for understanding how sensing and signaling are coordinated and integrated by multiple chemotaxis pathways. The complexity of chemotaxis signal transduction and the large number of chemotaxis receptors challenges the notion that chemotaxis could be manipulated to enhance the ability of bacteria to colonize the roots of plants and thus benefit plant health. However, deciphering the role of taxis responses in maintenance of *Azospirillum* spp. in the rhizosphere as well as the soil and in competing with other organisms could suggest avenues in which chemotaxis could be used in agricultural applications.

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