

Review article

The Role of Taxis in the Ecology of *Azospirillum*

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Abstract

The possible mechanisms involved in the migration of azospirilla towards plant roots are discussed. The chemotactic response of *Azospirillum* appears to depend on its current metabolism, which is determined by available plant root metabolites.

Keywords: *Azospirillum brasilense*, azospirilla, plant colonisation, rhizosphere, symbiosis, bacterial chemotaxis, bacterial motility

1. Introduction

Bacteria of the genus *Azospirillum* are typical rhizosphere microorganisms. The term "associative symbiosis" was coined as a result of the close association this organism was found to have with plant roots, and this led to the development of a new branch of soil microbiology.

The colonization of plant roots, with their constant release of nutrients, appears to be important for the growth and survival of azospirilla. Directed movement in response to natural gradients of different stimuli, i.e. taxis, is a mechanism by which motile microorganisms may reach their appropriate ecological niche. In natural, rather than laboratory conditions, it is possible that

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the growth limiting factors provide the main behavioural stimuli. In the case of the heterotrophic microaerophilic *Azospirillum*, organic compounds and oxygen may be growth limiting, and respiring plant roots produce gradients of both of these factors within the rhizosphere. Taxis in response to the gradient of nutrients excreted by the roots and the microaerophilic conditions created in the rhizosphere may provide a survival advantage for motile organisms competing for nutrients.

This article summarizes recently published and unpublished data which may help elucidate the mechanisms by which azospirilla direct their movement towards plant roots, and the specificity of the process. Although all the representatives of the genus *Azospirillum* are motile, only two species *A. brasilense* and *A. lipoferum* have been the subjects of systematic studies into their motility and taxis.

2. Results and Discussion

Motility

In the first paper to describe the genus *Azospirillum* it was mentioned that the bacteria were motile by means of flagella (Tarrand et al., 1978). The bacteria show mixed flagellation, with a single polar flagellum when grown in liquid culture but numerous lateral flagella if grown on solid media. The lateral flagella are thinner and have a shorter waveform than the polar flagella and the two types can be distinguished immunologically, suggesting that they are made of different proteins (Hall and Krieg, 1984). It has been suggested that the different flagella may perform different functions: the polar flagellum being responsible for swimming motility and the lateral flagella being responsible for swarming across solid surfaces (Hall and Krieg, 1983). In this study we have only investigated swimming motility, the function of the polar flagellum.

Although azospirilla are motile, motility alone does not provide a survival advantage for a bacterium. Simple logical reasoning (Glagolev, 1984) and mathematical modelling (Poole et al., 1991) show that motile bacteria become trapped in unfavourable environments (anaerobiosis, absence of substrate) if they are unable to bias their direction of swimming up a favourable gradient. Active movement must therefore be "directed" i.e. there must be chemotactic control over bacterial motile behaviour.

At present, bacterial chemotaxis is one of the best understood behavioral systems in Nature (for a recent review, see Armitage, 1992). The basis of the chemotactic response is an increase or decrease in the frequency with which bacteria change their swimming direction. This direction changing is usually the result of the flagella switching the direction of rotation from

counterclockwise (CCW) to clockwise (CW). However, in three species, the monoflagellate *Rhodobacter sphaeroides* (Armitage and Macnab, 1987), the peritricous *Rhizobium meliloti* (Gotz and Schmitt, 1987) and *Agrobacterium tumefaciens* (Loake et al., 1988), direction changing is brought about by brief stops in the rotation of flagella that only rotate in one direction. As these are all rhizosphere organisms, it was important to characterize the motile behaviour of *Azospirillum* before studying chemotaxis.

Reiner and Okon (1986), using cells tethered to glass slides by anti-flagella antibody, reported that *A. brasilense* rotated its flagella in both CW and CCW directions. These results were confirmed by following the behaviour of free-swimming cells using computerized motion analysis (I.B. Zhulin and J.P. Armitage, unpublished) i.e. *A. brasilense* changes direction by switching the direction of flagellar rotation.

Chemotaxis in vitro

Early studies of chemotaxis in *Azospirillum* showed that the bacteria were attracted by oxygen, some chemicals and root exudates (Okon et al., 1980; Alvarez-Morales and Lemos-Pastrana, 1980). During the next few years the list of attractants for *A. brasilense* and *A. lipoferum* was extended to include some 40 organic compounds: organic acids, amino acids and sugars (Barak et al., 1983; Reinhold et al., 1985; Zhulin et al., 1988b). Apparently the best growth substrates, organic acids, were the best attractants and poor substrates, e.g. amino acids, were weak attractants. No repellents were identified with the exception of cysteine which caused a weak response at high concentrations, which may be the result of its reducing properties (Zhulin et al., 1988a).

Aerotaxis

In modern studies of bacterial behaviour the ability of azospirilla to find an optimum oxygen concentration in natural gradients is so precise that the formation of an aerotactic band in an oxygen gradient was described for *A. brasilense* (Barak et al., 1982) before it was described for the "model" chemotaxis organism *Escherichia coli* (Shioi et al., 1987). The tactic response of *Azospirillum* toward oxygen is so strong that it tends to mask responses toward chemical substrates when both oxygen and chemical gradients occur in one direction (Barak et al., 1982). The functioning redox chain, the final component of bacterial catabolism, has been reported to be necessary for aerotaxis in *A. brasilense* (Reiner and Okon, 1986). The critical role of the redox chain was confirmed by the observation that *A. brasilense* cells were attracted to and banded at an

optimal redox potential, even in the absence of an oxygen gradient (Grishanin et al., 1991). Compounds which serve as electron acceptors (nitrate, nitrite, fumarate) and/or compounds which can alter the rates of electron flow through the redox chain and thus the electrochemical proton gradient (pmf) (lipophilic acids and bases, ions and substrates transported via the pmf) may be attractants for *Azospirillum*. It is possible therefore to consider plant roots as the source of a three-dimensional redox and concentration gradient to which rhizosphere bacteria may respond.

Taxis in vivo

Although chemotaxis and aerotaxis can be demonstrated under laboratory conditions using quantitative and semi-quantitative methods, it was not clear whether the phenomenon occurred in natural environments, particularly the soil. Bashan and coworkers answered this ecological question (Bashan, 1986; Bashan and Levanony, 1987). A series of experiments under both field and controlled conditions showed that azospirilla were capable of moving tens of centimeters vertically and horizontally. The movement of the bacteria towards plant roots was found to be limited by soil moisture, suggesting that the bacteria swim through the water space, rather than swarm when responding to a tactic gradient. The bacteria were shown to travel as a massive band toward plant roots from the point of inoculation (Bashan, 1986). This suggests that azospirilla respond to a complex gradient generated by plant roots and chemotactic responses direct them to the optimal conditions for their metabolism, which at any one time would be located in a narrow zone causing band formation. The local consumption of nutrients and secretion of by-products by the local concentration of bacteria would cause the cells to move further along the gradient, in essence imposing a new gradient upon the plant generated gradient. A similar situation occurs during bacterial chemotaxis *in vitro*. Azospirilla have been shown to travel in self-generated spatial gradients of chemicals (Barak et al., 1983), oxygen (Barak et al., 1982) and even in artificial redox gradients (Grishanin et al., 1991).

The experiments carried out under field conditions provide direct evidence that azospirilla are able to migrate a reasonable distance in the soil and this movement is directed towards plant roots. The movement pattern observed is similar to that seen for individual attractants under laboratory conditions.

In search of specificity

Is the chemotactic response of azospirilla towards a host plant a specific process? Different species of azospirilla have been shown to respond to different

ranges of attractants. For example *A. brasilense* showed a strong response towards malate (Barak et al., 1983) whereas *A. lipoferum* responded more strongly towards sucrose (Heinrich and Hess, 1985). Fendrik and coworkers found more distinct differences at both the species and strain level (Reinhold et al., 1985). Bacteria isolated from the roots of C₄-pathway plants were all attracted by a similar spectrum of compounds but this spectrum was different for azospirilla from C₃-pathway plants.

The different specificity could be the result of the evolution of different chemoreceptors in response to the specific effectors exuded by the different host plants, alternatively it may reflect the different induced metabolic pathways in different environments. Specific membrane bound receptors and sensory transducers, methyl accepting chemotaxis proteins (MCPs) are known to be an important part of the chemosensory pathway in *E. coli* and many other bacterial species, including *Bacillus*, *Spirochaetes* and *Halobacteria* (for review see Armitage, 1992, and Eisenbach, 1991). These receptors have a high affinity for a limited range of effectors (the range may vary from species to species). Binding of a chemoeffector to the receptor controls the direction-changing frequency of the flagellum and provides a high level of sensitivity for chemotaxis towards that range of effectors. However, not all chemoeffectors are sensed via MCPs, many sugars are sensed as part of the phosphotransferase transport pathway (see Taylor and Lengeler, 1990 for review) and the photosynthetic bacterium *Rhodobacter sphaeroides* controls chemotaxis via a change in intermediary metabolism (Armitage et al., 1990). A recent study indicates that *A. brasilense* may not use MCPs for chemosensing (I.B. Zhulin and J.P. Armitage, unpublished). Table 1 summarizes some of the features of *A. brasilense* behaviour in comparison with those of *R. sphaeroides* and *E. coli*. As can be seen the behaviour is similar to that characterised in *R. sphaeroides* rather than *E. coli* and is probably independent of specific chemoreceptors and dependent on at least limited metabolism.

The attempts to identify specific azospirilla chemoeffectors in plant roots were made after the report that the legume *Lotus corniculatus* produces a glycoprotein which attracted only a homologous strain of *Rhizobium*, and the threshold for this response was high (Currier and Strobel, 1977 and 1981). A heat labile, high molecular weight attractant was subsequently found in the roots of *Leptochloa fusca* which attracted the homologous strain of *A. lipoferum* ER15 (Reinhold et al., 1985). Similarly a protein fraction isolated from membranes and cell walls of wheat roots was a weak attractant for *A. brasilense* Sp245, a strain originating from wheat roots (Galkin et al., 1988). None of these responses to any of the compounds is inconsistent with a "metabolism" based chemosensing system. The semi-quantitative analysis

Table 1. Comparison of specific features of chemotactic response in *A. brasilense*, *R. sphaeroides* and *E. coli*

Chemotaxis features	<i>A. brasilense</i> and <i>R. sphaeroides</i>	<i>E. coli</i>
1. The best attractants	effective electron donors and acceptors (organic acids and oxygen)	Amino acids and sugars
2. Threshold for response towards main attracts	~ 1 mM	~ 1 μ M
3. Repellents	None identified	Some amino acids, organic acid, heavy metals
4. Specific chemoreceptors	No receptors identified: <ul style="list-style-type: none"> - methionine auxotroph showed the same behavior as wild type; - methylation <i>in vivo</i> revealed no MCPs; - antibodies raised against <i>E. coli</i> MCP did not react with cell extracts 	Dedicated chemoreceptors, MCPs: <ul style="list-style-type: none"> - methionine auxotroph showed abnormal pattern of chemotaxis; - methylation <i>in vivo</i> revealed MCPs
5. Chemokinesis in response to main attractants	present	not identified

and the use of buffer systems subsequently shown to interfere with receptor-independent chemotactic responses (Poole et al., 1991) mean that the conclusions should be analysed with care.

3. Conclusions

Experimental evidence suggests that *Azospirillum* does not have specific chemotactic receptors. The chemotactic response appears to be dependent on the metabolic pathways induced under current growth conditions. The mechanism can provide a more flexible although less sensitive response when compared to specific receptors.

When bacteria are faced with a complex gradient of metabolites produced by a plant, they would be expected to induce the metabolic pathways essential for the utilization of those compounds. It is possible that the early stages of recognition and induction are also involved in directing the bacteria up the chemical gradient. This is supported by the finding that chemotaxis towards many different attractants is inducible in *A. brasilense* (Zhulin et al., 1988a), an observation inconsistent with MCP activity, and that there is a lag-period before azospirilla shows movement towards plant roots (Bashan et al.,

1986). This delay may reflect the time taken for the bacteria to "adjust" their metabolic pathways and subsequently become capable of chemotaxis towards the individual plant producing that metabolite.

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