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Aerotaxis and Chemotaxis of *Azospirillum brasilense*: A Note

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Abstract. *Azospirillum brasilense* was attracted to capillaries containing either phosphate buffer, distilled water, or saline. The number of bacteria in these capillaries was 3–4 $\times$ 10^4, after 1 h of incubation. In the presence of phosphate buffer + attractants, the number of cells accumulated in the capillary increased only to 5 $\times$ 10^4–1.1 $\times$ 10^5 cells. It was not possible, therefore, to measure chemotaxis in *A. brasilense* as distinct from aerotaxis by the capillary method. Chemotaxis was observed in semi-solid agar plates and was determined by a growth band oriented towards the attractant. Positive chemotactic response was obtained with peptone, tryptone, yeast extract, amino acids, organic acids, arabinose and galactose.

Note

The nitrogen-fixing bacterium *Azospirillum brasilense* (11) has been isolated from the rhizosphere of tropical grasses (6). Cells of *A. brasilense* are gram negative, highly motile vibroid cells with a polar flagellum (11). The bacterium requires microaerobic conditions (0.005 PO_2) for optimal growth when fixing N_2 (8). Generally, organic acids are preferred as carbon and energy sources, but certain sugars can be utilized during N_2 fixation or for growth in the presence of a source of combined nitrogen (6, 7, 11). These properties make *A. brasilense* a typical rhizosphere organism, more abundant around roots than in the soil. It was not known, however, whether *Azospirillum* is capable of being actively attracted to organic substances such as those excreted by the roots (10), i.e., chemotaxis, or to an oxygen depletion gradient by aerotaxis (3). The present study was designed to examine these possibilities.

Azospirillum brasilense ATCC 29145 (Sp 7), isolated from *Digitaria decumbens* roots, Sp 51e from wheat and Sp 80 from maize (6), and an isolate from *Cynodon*

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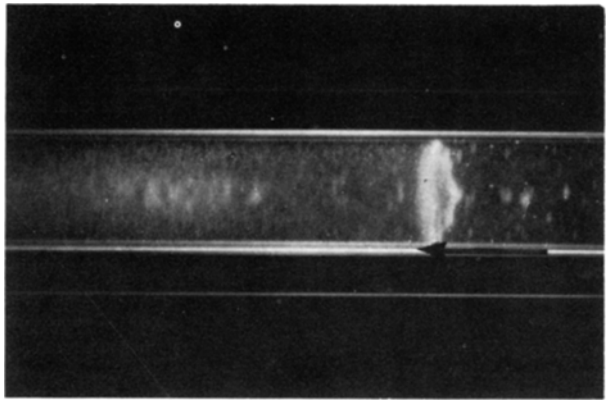

Fig. 1. Typical band of *Azospirillum brasilense* formed in the capillary containing either phosphate buffer 0.1 M, saline, tap water, distilled water, or buffer + tested attractant. Arrow shows direction of moving band.

dactylon ATCC 29729 (11), were grown in a static synthetic malate liquid medium containing 0.05% NH_4Cl at 30°C or in semi-solid agar medium without NH_4Cl (8). A suspension of cells from a 24-h-old culture grown either on semi-solid agar without N_2 or in liquid culture containing NH_4Cl, which contained approximately 5×10^7 colony-forming units (CFU)/ml (8), was used for the aerotaxis and chemotaxis experiments.

Accumulation of bacteria in the capillary was measured by the method of Adler (2) modified by sealing one end of the capillary with Celloseal (Fisher Scientific Co.); capillaries (0.08 mm in diameter, 2 μl in volume), purchased from Modulohm Vasekaer, Denmark, were filled with the test substances dissolved in 0.1 M phosphate buffer (pH 7.0). Control capillaries contained only potassium phosphate buffer. The capillaries were placed with the open tip in 0.2 ml of a bacterial suspension and incubated for 1 h at 30°C. The number of bacteria accumulated in the capillary was determined by diluting its contents in phosphate buffer and plating portions on nutrient agar plates.

In most chemotaxis systems such as those utilizing *Escherichia coli* or *Pseudomonas lachrymans* (1, 4), the bacteria usually entered the control capillary in a random fashion with $1–3 \times 10^3$ bacterial cells/1 μl capillary after 30 min, whereas when the bacteria chemotactically responded to attractants, they formed bands and $10^4–10^5$ bacterial cells/capillary were present. With all tested isolates of *A. brasilense*, a band of bacteria (Fig. 1) started to form within 5 min in the 2 μl capillary with either phosphate buffer, tap water, saline, or distilled water, and bacterial concentration reached $3–4 \times 10^4$ CFU after 1 h.

Similar aerotactic response was described by Caraway and Krieg (3) for *Spirillum volutans*—an obligatory microaerophilic bacterium which responded aerotactically to self-created oxygen gradients in capillary tubes. In this organism band formation required oxidizable substrate, inorganic ions and chelating agents. In the *A. brasilense* system, cell suspensions contained oxidizable substrate (malate) and inorganic ions because the cells used were not washed from the medium. When attractants, such as 0.1% w/v peptone, 10^{-3} M glutamic acid, pyruvic acid, or galactose, were added to the buffer the same band was observed and the capillary contained $5 \times 10^4–1.1 \times 10^5$ CFU. Although in other chemotactic systems the number of bacteria in the buffer + test substance was high compared to that in the buffer alone, with *A. brasilense* maximum ratios detected were only in the range of 3:1. Similarly, low chemotactic ratios were also obtained with *Rhizobium* (5).

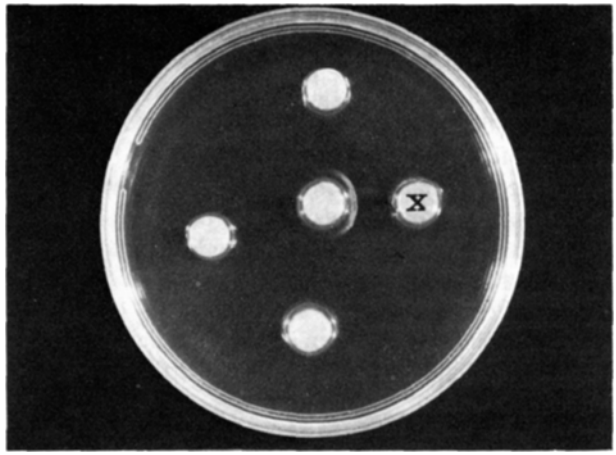

Fig. 2. Chemotaxis assay for *Azospirillum brasilense* in semi-solid agar. *X*, Agar disc with attractant. Attractants were malate, succinate, pyruvate, fumarate, galactose, arabinose, peptone, yeast extract, tryptone, glutamate, alanine, leucine. Glucose and mannitol did not attract *Azospirillum*.

It was not possible, therefore, to measure chemotaxis as distinct from aerotaxis in *A. brasilense* by the capillary method. If an oxidizable substrate is present in the capillary, it seems likely that such an oxygen depletion gradient would be increased; therefore more organisms might be able to respond to it. Thus the various compounds tested might not necessarily be attractants, but merely oxidizable substrates that lead to formation of an oxygen gradient. The organisms might be responding to such gradients, not to the compounds themselves.

A method for testing the chemotactic response of *A. brasilense* towards attractants that could distinguish chemotaxis from aerotaxis was developed. An 8 mm diameter, 2 mm thick agar disc containing the tested attractant (2.5% w/v) was placed on a semi-solid (0.4%) synthetic agar medium which was either free of N or supplemented with NH_4Cl, opposite to a disc covered with a 24 h culture of *Azospirillum*. Three discs of water agar were placed at opposite distances as controls (Fig. 2). After 14 h incubation, a band of growing bacteria oriented towards the attractant was observed (Fig. 2). Twenty-four hours after inoculation, an expanding band similarly oriented as well as bacteria growing around the attractant disc could be observed, whereas no response was observed towards the three water agar discs which served as controls.

A similar test was considered by Adler (1) suitable for testing chemotaxis. In Adler's test the bacterial inoculum was placed in the center of semi-solid agar, the cultures growing in a concentric expanding band following a self-created gradient of attractant contained in the medium.

In general, complex organic substances used for growth media such as peptone, tryptone, yeast extract, organic acids, and amino acids showed positive chemotaxis. The tested isolates of *A. brasilense* neither grew nor fixed N_2 with glucose or mannitol as sole carbon source. These sugars also did not attract the organism. Growth was supported by galactose and arabinose (7, 11), which were attractants for *A. brasilense*.

Nitrogen fixation by *A. brasilense* occurring only under microaerophilic conditions may be due to the poor protection of its nitrogenase system from O_2 destruction (6, 7). The organism grew well under aerobic conditions when combined nitrogen such as NH_4Cl was present in the medium (9). However, when *Azospirillum* (0.1 ml suspension) grown on either N_2 or NH_4Cl was inoculated on the surface or mixed with freshly prepared semi-solid synthetic medium (10 ml in 15 ml, 1.5 cm diameter test tubes)

containing 0.01–0.5% agar either with or without 0.05% NH_4Cl, the cultures developed a growth zone. The depth of this growth zone was proportional to decreasing agar concentration. The bacteria did not grow, however, on the surface of the medium indicating that *A. brasilense* was aerotactically attracted to microaerobic conditions when not fixing N_2.

The mechanism by which *A. brasilense* associates with roots of grasses is not known. Chemotaxis may play a role in this association, since organisms may be attracted to the root surface from the surrounding soil by root exudates, such as amino acids, organic acids, and sugars (10), which are later utilized as substrate for growth. The aerotactic properties of *A. brasilense* towards low O_2 concentrations and its capability to grow either on combined nitrogen or to fix N_2 under these conditions may explain the close association of these bacteria with root surfaces (9).

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