Folia Microbiol. 33, 277–280 (1988)

Chemotaxis of *Azospirillum brasilense* towards Compounds Typical of Plant Root Exudates

I.B. ZHULIN, S.E. TRETYAKOVA and V.V. IGNATOV

Institute of Biochemistry and Physiology of Plants and Microorganisms, USSR Academy of Sciences, Saratov, 410 015 USSR

Received July 7, 1987

ABSTRACT. Nitrogen-fixing *Azospirillum brasilense* exhibits positive chemotaxis towards a large number of organic compounds (amino acids, saccharides, organic acids) typical for plant root exudates. The most effective attractants were malate, succinate, fructose and other compounds which serve as substrates for *A. brasilense*. Chemotactic response towards the majority of compounds was inducible, but in case of malate and succinate it was constitutive. Tactic response during the nitrogen-fixing process was essentially the same as in the presence of fixed nitrogen in the medium. Among organic compounds tested no repellents were found.

A number of papers have been published recently to review the investigation of chemotaxis in the genus *Azospirillum*. This genus is attracted by some organic compounds (Barak *et al.* 1983; Reinhold *et al.* 1985), wheat seedlings exudates (Zhulin 1986), and cereal root exudates (Gafny *et al.* 1986; Heinrich and Hess 1985; Mandimba *et al.* 1986). A certain specificity of chemotactic response in *Azospirillum* spp. was shown towards plant root exudates (Mandimba *et al.* 1986; Reinhold *et al.* 1985). In experiments *in vivo A. brasilense* migrated rather rapidly towards wheat roots in wet soil (Bashan 1986). All these data indicate that bacterial chemotaxis occurs towards the substances exuded by plant roots in the initial stage of establishing *Azospirillum* spp. association with plants.

The purpose of this work is to investigate chemotaxis towards a large number of organic substrates typical of plant root exudates.

MATERIALS AND METHODS

Azospirillum brasilense strain 7 (ATCC 29 145) was grown in a liquid synthetic medium (Nelson and Knowles 1978) in 300-mL Erlenmeyer flasks filled with 100 mL of the medium each during 16 h at 30 °C on a rotary shaker.

Chemotaxis was determined by the method of Adler (1966) that allows to estimate metabolized attractants. In the centre of the Petri dish containing buffer and the tested chemoeffector with an addition of 0.3 % Bacto agar, 20 μL of cell suspension (cell concentration 108/mL) was inoculated and incubated during 36 h at 30 °C. Tactic response was observed in the form of a concentric expanding band (chemotactic ring) of bacterial population.

The most effective attractants determined by Adler's method were tested additionally by the method of Tso and Adler (1974). Semi-solid agar blocks cut from solid agar and containing the chemoeffector were introduced into a bacterial suspension. Chemotaxis was determined by chemotactic ring formation around a solid agar block in 15—20 min after the start of incubation. All reagents used as chemoeffectors were from *Fluka*, *Serva*, *Reanal* and *Chemapol*.

TABLE I. *A. brasilense* attractants in a metabolism-dependent variant of chemotactic response[a]

Attractant	Relative efficiency[b]	Attractant	Relative efficiency[b]
Amino acids			
L-Alanine	+++	L-Serine	+
L-Proline	+++	L-Methionine	+
L-Asparagine	+++	L-Leucine	+
β-Alanine	+++	L-Lysine	+
L-Histidine	+++	L-Threonine	+
L-Phenylalanine	+++	L-Cysteine	+
L-Glutamine	+++	D-Alanine	+
L-Aspartate	+++	D-Asparagine	+
L-Tryptophan	++	L-Isoleucine	NT
L-Arginine	++	D-Arginine	NT
L-Tyrosine	++	D-Aspartate	—
L-Glutamate	++	D-Valine	—
Saccharides			
D-Fructose	+++	N-Acetyl-D-galactosamine	+
D-Mannose	+++	Glycerol	+
D-Galactose	+++	D-Mannitol	NT
L-Rhamnose	+++	Galactitol	NT
D-Arabinose	+++	D-Glucitol	NT
D-Xylose	+++	Sucrose	NT
D-Gluconate	+++	Lactose	NT
L-Fucose	++	Melezitose	NT
L-Arabinose	++	Cellobiose	NT
D-Glucose	++	Raffinose	NT
D-Ribose	+	D-Glucuronate	NT
2-Deoxy-D-glucose	+	D-Galacturonate	—
Maltose	+	D-Glucosamine	—
N-Acetyl-D-glucosamine	+	L-Ribose	—
N-Acetyl-D-mannosamine	+	D-Fucose	—
Organic acids			
Malate	+++	2-Oxoglutarate	++
Succinate	+++	Pyruvate	++
Oxalate	+++	*cis*-Aconitate	++
Lactate	+++	Oxaloacetate	++
Citrate	++	Formate	+

[a] Results from four experiments in two replicates. All substances were tested at a concentration of 1 mmol/L.

[b] +++, strong attractants; ++, medium attractants; +, weak attractants; NT, not tested; —, no chemotaxis.

RESULTS AND DISCUSSION

It is well known that plant root exudates contain a large number of organic compounds including the whole pool of amino acids (Berestetsky and Kravtchenko 1980), saccharides and organic acids (Ivanov 1973). All these substances were tested by Adler's method as chemoeffectors for *A. brasilense* (Table I).

TABLE II. Attractants for *A. brasilense* tested by the method of Tso and Adler (1974)[a]

Attractant	Chemotaxis[b]	
	minimal medium	medium with effector
L-Proline	—	+++
L-Alanine	NT	+++
L-Asparagine	NT	+++
L-Histidine	—	++
D-Fructose	—	+++
D-Galactose	—	+++
D-Mannose	—	++
L-Rhamnose	—	+
D-Xylose	—	—
L-Fucose	—	—
Malate[c]	+++	+++
Succinate	++	+++

[a]The experiments were repeated four times. All substances were tested at a concentration of 10 mmol/L.
[b]For symbols see Table I.
[c]Cells were grown in the medium with succinate.

All natural stereoisomers of organic substances typical of root exudates proved to be effective attractants for *A. brasilense*, the most effective being malate, succinate, and D-fructose.

A number of attractants were tested by the method of Tso and Adler (1974). The experiments were conducted in two variants, (1) the cells were grown in a minimal medium with malate, (2) the medium was supplemented with 10 mmol/L chemoeffector tested. The results are represented in Table II.

Chemotaxis of *A. brasilense* towards most chemoeffectors is inducible, *i.e.* it is exhibited when the bacteria have been previously grown in the presence of a chemoeffector. Tactic response towards malate and succinate seems to be constitutive.

In all experimental variants chemotaxis was not dependent on nitrogen nutrition and was practically the same under nitrogen-fixing conditions as well as in the presence of 0.5 % NH_4Cl in the medium.

Among the organic substances tested there was no repellent.

The results obtained indicate a close relationship between metabolism and chemotaxis in *A. brasilense*. All the substrates taken up and assimilated by the bacteria induced the chemotactic response. Thus, utilizing the root exudates in the rhizosphere azospirilla would inevitably move in the direction of increasing substrate concentration, *i.e.* towards plant roots.

Chemotactic response specificity at the "strain—variety" level can hardly be explained by trophic taxis towards substances with a low molar mass. Taxis towards a large number of organic compounds provides competitiveness for *A. brasilense* under rhizosphere conditions. Specificity is probably provided by the attractants of higher molar mass exuded by roots (Reinhold *et al.* 1985).

REFERENCES

Adler J.: Chemotaxis in bacteria. *Science* **153**, 708—716 (1966).

Barak R., Nur I., Okon Y.: Detection of chemotaxis in *Azospirillum brasilense*. *J.Appl.Bacteriol.* **53**, 399—405 (1983).

Bashan Y.: Migration of the rhizosphere bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens* towards wheat roots in the soil. *J.Gen.Microbiol.* **132**, 3407—3414 (1986).

Berestetsky O.A., Kravtchenko L.V.: Exudation of amino acids by seedlings of wheat and pea. *Soviet Plant Physiol.* **27**, 419—422 (1980).

Gafny R., Okon Y., Kapulnik Y., Fisher M.: Adsorption of *Azospirillum brasilense* to corn roots. *Soil Biol.Biochem.* **18**, 69—75 (1986).

Heinrich D., Hess D.: Chemotactic attraction of *Azospirillum lipoferum* by wheat roots and characterization of some attractants. *Can.J.Microbiol.* **31**, 26—31 (1985).

Ivanov V.P.: *The Plant Root Exudates and Their Role in the Life of Phytocenosis.* (In Russian) Nauka, Moscow 1973.

Mandimba G., Heulin T., Bally R., Guckert A., Balandreau J.: Chemotaxis of free-living nitrogen-fixing bacteria towards maize mucilage. *Plant & Soil* **90**, 129—139 (1986).

Nelson L.M., Knowles R.: Effect of oxygen and nitrate on nitrogen fixation and denitrification by *Azospirillum brasilense* grown in continuous culture. *Can.J.Microbiol.* **24**, 1395—1403 (1978).

Reinhold B., Hurek T., Fendrik I.: Strain-specific chemotaxis of *Azospirillum* spp. *J.Bacteriol.* **162**, 190—195 (1985).

Tso W.-W., Adler J.: Negative chemotaxis in *Escherichia coli*. *J.Bacteriol.* **118**, 560—576 (1974).

Zhulin I.B.: Wheat seedling exudate attracts diazotropic phytohormone producing bacteria, p. 29 in Abstr. *4th Internat. Youth Symp. Plant Metabolism Regulation*, Varna (Bulgaria) 1986.