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Chemotactic Response of *Azospirillum* Toward Root Exudates of C_3 and C_4 Plants

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Abstract

Chemotactic responses of fifteen *Azospirillum* strains originating from different C_3 and C_4 crop plant roots and belonging to *A. lipoferum* and *A. brasilense* were compared for their chemotactic response to wheat and maize sterile exudates. This chemotaxis towards the root exudates of maize and wheat was determined quantitatively by a channeled-chamber technique. The chemotactic reaction of the strains differed qualitatively and quantitatively toward C_3 and C_4 root exudates. The results suggest general chemotactic behaviour by azospirilla, rather than a specific host dependent response. The different responses to root exudates does not seem to be correlated with the taxonomic position of the strains.

Keywords: *Azospirillum*, chemotaxis, root exudates, C_3, C_4, plants

1. Introduction

Little information is available about the mechanisms of interactions and factors determining host-plant specificity. Chemotaxis may play a role in the initial stage of the establishment of associations between *Azospirillum* spp. and grass roots (Mandimba et al., 1986). It is known that root exudates of various plants can differ in their composition (Vancura, 1964; Vancura et al.,

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1965). It can be postulated that the pattern of chemotactic response to root exudate compounds might be one of the factors determining host-specificity in colonization (Döbereiner et al., 1988). This specificity seems to depend on the origin of the strains and on their taxonomic position (Reinhold et al., 1985). In this work, chemotactic responses of fifteen strains of *Azospirillum* isolated from different C_3 and C_4 crop plant roots toward root exudates of maize and wheat were compared by using the channel chamber method (Barak, 1983).

2. Materials and Methods

Bacterial suspensions of *Azospirillum* strains (Table 1) for chemotaxis experiments were carried out according to Reinhold et al. (1985). For preparation of root exudates, sterilized seeds of maize and wheat were incubated in sterile distilled water (SDW) on a rotary shaker for 8 days. The liquid containing the root exudates was lyophilized and resuspended in a fifth of the initial volume of SDW. The quantitative estimation of chemotaxis was carried out by the channeled chamber method according to the dimensions given by Palleroni (Palleroni, 1976). Simultaneously 20 μl of bacterial suspension and root exudates were injected in each well, in the control chamber the root exudates were replaced by chemotaxis medium.

The chemotactic ratio (R_{che}) was defined as the ratio of cell counts in the

Table 1. *Azospirillum* strains used

Strain	Source	Species affiliation
Sp[1]7	Digitaria	*A. brasilense*
SpRG6xx[2]	wheat	*A. lipoferum*
MRA3c	maize	*A. brasilense*
MRB2	maize	*Azospirillum* sp.
MRC1	maize	*A. brasilense*
MCD1	maize	*A. brasilense*
MCE1	maize	*A. lipoferum*
MCF2	maize	*A. lipoferum*
M6	maize	*A. brasilense*
M1	maize	*A. brasilense*
TA	tobacco	*A. brasilense*
TB	tobacco	*A. lipoferum*
PA	pepper	*A. lipoferum*
PC	pepper	*A. brasilense*
SA	sorghum	*A. lipoferum*

[1] DSM 1690
[2] ATCC 29731
All the other strains are isolates from our laboratory

target well of the sample and control chambers. For chemotaxis assays 6 replicates were used for each strain, confidence level ($p < 0.05$) was calculated by Student's t-test.

3. Results

Thirteen out of fifteen strains of *Azospirillum* have shown chemotactic responses toward root exudates (Table 2). Some chemotactic reactions of the strains differed qualitatively and quantitatively. Figures 1 and 2 show that the strains were strongly attracted by one root exudate preferentially. Only 4 strains gave common chemotactic responses toward both exudates (Fig. 3).

4. Conclusions

Bacteria of the genus *Azospirillum* exhibited chemotactism toward root exudates of C_3 and C_4 plants. The R_{che}s obtained for *Azospirillum* strains isolated from the C_3 and C_4 plants showed clear differences in chemotactic responses toward root exudates of maize and wheat. The results suggest general chemotactic behaviour by azospirilla, rather than a specific host-dependent response. The different responses to C_3 and C_4 root exudates cannot be correlated with the origin of the strains. The chemotactic responses of bacteria to different exudates does not seem to be influenced by the fact that the strains belong to

Table 2. Chemotaxis by *Azospirillum* strains measured in channeled chamber

Strain	Source	Maize-R_{che}*	Wheat-R_{che}*
Sp7[1]	Digitaria	–	8.36
SprG6xx[2]	wheat	13.82	3.95
MRA3c	maize	26.65	14.05
MRB2	maize	0.53	8.82
MRC1	maize	10.00	16.03
MCD1	maize	3.94	19.48
MCE1	maize	14.40	7.92
MCF2	maize	7.32	19.00
M6	maize	0.26	2.14
M1	maize	4.67	0.74
TA	tobacco	0.34	0.97
TB	tobacco	17.31	1.75
PA	pepper	7.91	4.80
PC	pepper	47.26	6.03
SA	sorghum	0.48	1.70

[1] DSM 1690
[2] ATCC 29731
* $p < 0.05$ calculated by Student's t-test

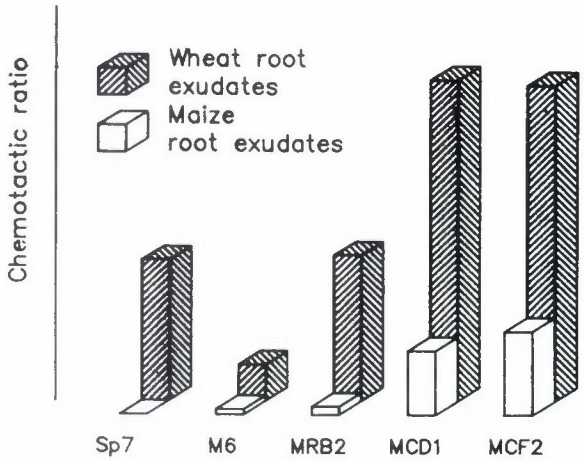

Figure 1. Strains attracted by wheat-root-exudates preferentially

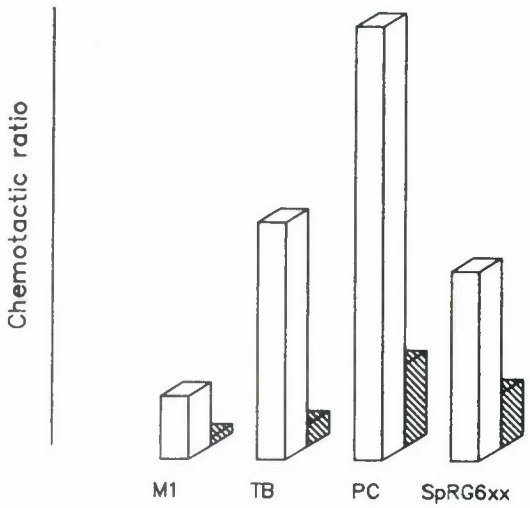

Figure 2. Strains attracted by maize-root-exudates preferentially

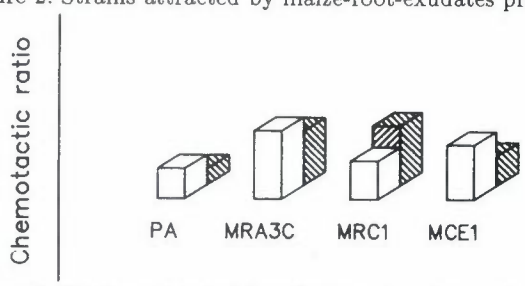

Figure 3. Strains attracted by wheat and maize-root-exudates

A. lipoferum and *A. brasilense*. This research suggests the use of root exudates from different C_3 and C_4 plants instead of only single compounds (amino acids, organic acids and sugars) for a clearer approach to chemotaxis behaviour by azospirilla.

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