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Contribution of Chemotaxis and Aerotaxis to the Establishment of *Azospirillum* in the Rhizosphere*

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Abstract

Azospirillum lipoferum ER 15 has a competitive advantage over its chemotactic negative mutant KM 105 on roots of Kallar grass (*Leptochloa fusca* (L.) Kunth) and rice (*Oryzia sativa* L.) both in semisolid medium and sand. Wild type-mutant ratios were up to 40 for rice and up to 150 for Kallar grass.

Introduction

Bacteria capable of motility and chemotaxis have a competitive advantage in nutrient gradients. Chemotactic response of *Azospirillum* towards root exudates of Kallar grass was found to be strain specific and it was postulated that chemotaxis may play a role in the adaptation of *Azospirillum* to their host plants (Reinhold et al., 1985). The aim of this study was to investigate whether *Azospirillum lipoferum* has an advantage over a chemotactic and aerotactic negative but motile mutant in colonizing the rhizoplane of Kallar grass and rice plants.

Materials and Methods

A motile chemotactic negative mutant (KM 105) of *Azospirillum lipoferum* ER 15 (Reinhold et al., 1985) was selected from spontaneous mutations as described by Armstrong (1967) in SSM swarm plates with 1 mM L-malate at pH 6.8. Synthetic malate (SM) medium, chemotaxis wash (CM) medium adjusted to pH 8.0, and chemotaxis semisolid malate (SSM) medium were used according to Reinhold (1985). Plants were grown in Hoagland nutrient solution (HNS) with 29 μg NH_4NO_3 (10 ppm N) and 15 mM phosphate buffer (pH 6.8) added. Surface sterilization and

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germination of the Kallar grass and rice seeds were carried out according to Kloss (1984). Sand was rinsed three times with tap water and then steam-autoclaved. 1 ml inoculum of 5×10^5 cfu/ml was added to 60 g sand in sterile glass tubes (19.5 × 3.5 cm) already supplemented with 12 ml of HNS. Soil moisture was kept at ca. 18%. Another set of tubes was prepared using 40 ml HNS-medium with 0.3% agarose. For competition studies the wild type and mutant were mixed 1:1. Three 2-3-day-old seedlings were transplanted into the prepared tubes and kept in a growth chamber at 30/28° C, 18/6 hr day/night cycle and 20 klx for about 20–25 days. Plants were removed from the tubes, washed thoroughly and the roots were homogenized (blender homogenizer) 5 min at 50 000 rpm in CM medium. 500 µl bacterial dilutions were dropped on plates, uniformly distributed in warm SSM medium (45°C) and incubated at 35°C for 36–48 h. Wild type and mutant were distinguished by chemotactic swarm behavior, e.g. colonies forming chemotactic swarm rings were identified as wild type colonies.

Results and Discussion

The motile mutant KM 105 failed to show either chemotaxis in swarm plates towards several organic acids and amino acids (Reinhold et al., 1985) or aerotaxis as determined in capillary tubes according to Barak (1982) even though it showed the same swimming behavior as the wild type. The specific growth rate of KM 105 ($\mu = 0.65$) was similar to that of the wild type ($\mu = 0.63$) in a batch fermenter culture with NH_4. Without other soil bacteria, absence of chemotaxis played a minor role in

Table 1. Numbers of bacteria recovered from roots inoculated with a 1:1 mixture of *Azospirillum lipoferum* ER 15 and its chemotactic negative mutant KM 105. Numbers of bacteria are given in 10^5 cfu/g roots with standard deviation derived from three samples. Four parallel inoculations were investigated.

Experiments in sand culture					
Kallar grass			*Rice*		
ER 15	KM 105	W/M[a] ratio	ER 15	KM 105	W/M ratio
17±2.8	≤0.4	≥42	20±2.3	7.3±1.5	2.7
31±3.2	0.2±0.4	129	12±2.5	2.6±2.8	4.6
26±1.1	≤0.18	≥147	24±6.0	2.0±0.0	12.0
n.d	n.d		13±0.0	0.3±0.5	39.3
Experiments in semisolid Hoaglandmedium					
10±0.2	0.2±0.0	42.6	42±1.4	2.1±0.8	20.0
23±0.5	0.2±0.2	97.0	50±1.5	6.0±1.0	8.3
41±1.0	4.0±1.2	10.3	15±4.1	1.0±0.0	15.0
9.1±1.8	0.1±0.09	74.2	80±10	4.6±2.5	17.4

[a] Wild type-mutant ratios, n.d. not determined

bacterial colonization of roots, when plant roots were exposed to either the wild type or the mutant KM 105 both in semisolid medium or sand. In the competition study (1:1 mixture), the wild type significantly outcompeted the mutant on the roots of Kallar grass and rice determined at the 1% level according to Wilcoxon matched paires signed rank test. Wild type-mutant ratios (W/M ratio) were between 2.7 and 39 in rice and between 10 and 147 in Kallar grass (Table 1).

The present study has shown a competitive advantage of *Azospirillum lipoferum* ER 15 to colonize the roots of Kallar grass with up to 150 fold increases over the mutant KM 105 and it is proposed that plant-specific chemotaxis reflects an adaptation of *Azospirillum* to the host plant Kallar grass (Reinhold et al., 1985, Barak et al., 1983).

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