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Investigations on Root Exudates of Korean Rice

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Abstract

Root exudates of Korean wild rice and a cultivated rice cultivar, both Japonica types, have been investigated under sterile and monoaxenic conditions in order to study the role of root exudation in the establishment of stable bacteria-plant root associations. For inoculation, the isolates W03 (a variety of *Azospirillum lipoferum*). KO18 and F (similar *Azospirillum lipoferum* ATCC 29708 (SpRg20a)) were used. Main root exudates were the sugars glucose and ribose, the organic acids malate, acetate and pyruvate, and the amino acids serine, glycine, alanine, and aspartic acid. As compared to the cultivar, the wild rice exuded more sugars, organic acids and amino acids. The inoculated bacteria consumed mainly glucose, malate and succinate, but contributed acetate, pyruvate, and ribose to the nutrient solution. In contrast to KO18, W03 exuded considerable amounts of amino acids. Positive effects on plant development were observed in all inoculation experiments.

Keywords: root exudates, plant growth promoting effects, nitrogen fixation, rhizosphere

1. Introduction

Rice and other grain crops form the staple food for the majority of people in the world. Production is in most cases limited by nitrogen supply. Therefore high amounts of nitrogen fertilizers are necessary, forming economical as well as

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ecological problems because of high costs and leaching of nitrogen compounds into the ground water. Under these aspects, biological nitrogen fixation by root associated bacteria could contribute considerably to the nitrogen supply of plants and thus reduce fertilizer application. Associations between plants and nitrogen fixing bacteria have been described (Patriquin et al., 1983; Bashan et al., 1989) also with respect to rice plants. For practical application, well founded knowledge about nature and function of these associations are necessary. Interesting results were obtained in experiments with *Azospirillum* spp. (Kapulnik et al., 1981b; Watanabe and Lin, 1984). Inoculation with these bacteria led among other effects to increased plant growth and changes in root morphology (Baldani and Döbereiner, 1980; Kapulnik et al., 1981a; Bashan, 1986). The mechanisms involved in these plant growth promoting effects have not yet been clarified. Apart from the improvement of plant nitrogen supply, stimulation of plant development by bacterial phytohormones has been discussed (Tien et al., 1979; Bothe et al., 1988). Okon et al. (1986) demonstrated an increase of nutrient absorption capacity by inoculation.

One important aspect of plant-bacterium interaction in these associations is the supply of the bacteria with energy and carbon compounds by plant exudates like sugars, organic acids, amino acids, vitamins and high molecular compounds (Lynch, 1982). In this paper, rice root exudation is studied in model experiments under sterile and monoaxenic conditions in order to gain information about the influence of plant type and bacterial inoculation on the exudation pattern. In particular, sugars, organic acids and amino acids were analysed by HPLC-techniques.

2. Materials and Methods

Plants

Two Korean rice varieties of *Oryza sativa* L. were used, one unnamed wild rice variety, and the Hwajinbyo cultivar, both Japonica types. Rice seeds were harvested in Suweon (Korea), propagation of rice plants was also performed in Hannover, using the original rice soil.

Microorganisms

Nitrogen fixing bacteria were isolated from fresh Korean rice soils (wild and cultivated). The most efficient diazotrophs were WO3 (from wild rice soil), an *Azospirillum lipoferum* variety (Bazzicalupo, pers. comm.) and the not yet identified KO18 from cultivated rice soil. A further efficient nitrogen fixing bacterium (F) from the root surface of wild rice was identified by DNA restriction

fingerprinting as similar to *Azospirillum lipoferum* ATCC 29708 (SpRg20a) (Bazzicalupo, pers. comm.). Strains were maintained on nutrient agar plates (Merck No. 5450), at 30°C in nitrogen-free semisolid medium (Döbereiner, 1980), and with 10% DMSO in liquid nitrogen. For inoculation, 24 hrs old cultures were adjusted to an optical density of 1.2 at 578 nm.

Plant experiments

All plant experiments were carried out in triplicate in a special growth chamber, constructed for long-term plant experiments under sterile and monoaxenic conditions (Waschütza, 1990). After surface sterilisation (Nilsson, 1957, modified), rice seeds were germinated on nutrient agar plates, and after 3–4 days transferred to flasks (4 per flask). During the tests, incubation conditions were: 31°C/26°C and 10/14 hrs day/night cycle. Each flash contained 400 ml Hoagland solution (Hoagland, 1950, modified), either plus or minus 80 ppm of ammonium chloride. Inoculations were carried out after transfer of the seedlings into the flasks.

The experimental arrangements were: Parallels with combined nitrogen without microorganisms **(+N/−MO)** for positive plant growth control; parallels without nitrogen and without microorganisms **(−N/−MO)** for deficiency control; parallels without combined nitrogen, but with microorganisms **(−N/+MO)** for the investigation of bacterial influence on plant growth and exudation; parallels with nitrogen and microorganisms **(+N/+MO)** for the investigation of possible additional effects on the plants, and parallels without combined nitrogen and without plants, but the medium supplemented with 1 g glucose/l **(−N/−P/+MO)** for the registration of bacterial metabolism products and for bacterial growth control. Moreover, parallels were included without combined nitrogen, but with dead bacteria (121°C/3 bar/15 min) **($-N/+MO_{dead}$)**, investigating the influence of bacterial surface properties and decomposition products on plant exudation. Samples were taken every second day during a 30 day test, and were used for HPLC-analyses as well as for the specification of bacterial and plant parameters, and for pH-control.

HPLC analyses

Root exudates were quantified by three different HPLC-systems:

Sugars

Column CarboPac TM PA 1 (Dionex); Detector PAD/Gold (Dionex)/RT; Eluant 0.1 M NaOH; flow rate 1 ml/min; sample size 20 μl.

Organic acids

Column Aminex HPX-87H (Biorad)/65°C; detector UV 210 nm (Milton-Roy); eluant 5 mM H_2SO_4; flow rate 0.6 ml/min; sample size 20 μl.

Amino acids

Column Spherisorb ODS2 (Grom)/RT; detector fluorescence Ex 330 nm; Em 450 nm (Pharmacia); derivatisation OPA; eluant A: sodium phosphate buffer (5% methanole/1% THF); eluant B: sodium phosphate buffer (14.5% methanole/4.5% THF/35% acetonitrile); flow rate 0.8–1.2–0.8 ml/min; sample size 20 μl.

3. Results and Discussion

The experiments performed provide information about quality and quantity of rice root exudates as being influenced by plant type and bacterial inoculation, and about the influence of inoculation on plant development.

In flasks with inoculated plants **(−N/+MO, +N/+MO)** the isolate WO3 showed a growth promoting effect on wild rice plants, as well as the isolate KO18 on the cultivar Hwajinbyo. Isolate F similar to (*A. lipoferum* ATCC 29708 (SpRg20a)) promoted the growth of both wild and cultivated rice. Dead bacteria **(−N/+MO_{dead})** had no growth promoting effect on either plant type. Nitrogen deficiency marks could clearly be seen in the **−N/−MO** experiment. Table 1 summarizes the shoot size of the plants influenced by the microorganisms. Increasing yield caused by bacterial growth promoting factors was discussed by various authors (Tien et al., 1979; Bothe et al., 1988), also under suboptimal nitrogen fertilizer conditions (Kapulnik et al., 1981a). Growth promoting effects under inoculated conditions were also shown by Rao et al. (1983), Watanabe and Lin (1984), and Okon et al. (1987).

Table 1. Shoot size (cm) after 28 days

	Wild rice/WO3	Wild rice/F	Hwajinbyo/KO18	Hwajinbyo/F
+N/−MO	21.25	47.5	19	26.19
−N/−MO	12.25	11.2	12.3	11.4
−N/+MO	15.8	15	18.3	16
−N/+MO_{dead}	12.5	11.3	13.1	12.9
+N/+MO	n.d.	55	26.2	51.7

All data were obtained from triplicates.

In this work it could be clearly demonstrated that bacterial inoculation in the −N experiments led to plant development similar to the +N/−MO variants, while in the +N/+MO flasks further increase of growth could be observed. Whether these effects are only due to bacterial nitrogen supply, or whether additional growth promoting effects were involved, could not be decided in our setup. Tables 2 and 3 contain detailed data about the root exudates of wild and cultivated rice under sterile and monoaxenic conditions after a 28 day

Table 2. Wild rice root exudates (μmol/l) after 28 d

	+N/−MO	−N/−MO	−N/+MO	$-N/+MO_{dead}$	+N/+MO	WO3
Exudates (μmol/l)						
Oxalate	0.120	0.0768	0.230	0.100	0.200	0.092
Citrate	0.600	0.4660	0.600	0.460	0.700	0.276
Malate	1.600	0.0000	0.300	0.400	1.100	0.700
Succinate	0.390	0.0000	0.000	0.200	0.390	0.000
Acetate	5.800	4.9800	3.320	5.100	6.640	1.328
Pyruvate	1.100	1.1600	0.440	1.000	1.500	0.800
Total organic acids	9.610	6.6828	4.890	7.260	10.530	3.196
Sorbit	0.170	0.1690	0.170	0.090	0.180	0.072
Arabinose	0.160	0.0000	0.000	0.000	0.070	1.710
Glucose	5.900	1.4800	0.000	0.780	1.160	
Fructose	0.450	0.3370	0.000	0.400	0.290	0.320
Ribose	1.240	0.7400	0.700	1.200	1.300	0.640
Isomaltose	0.300	0.0000	0.000	0.000	0.000	0.068
Total sugars	8.220	2.7260	0.870	2.470	3.000	2.810
Aspartic acid	0.300	0.1700	0.260	0.133	0.400	0.200
Serine	0.720	0.3400	0.600	0.210	1.350	0.360
Glycine	0.770	0.2400	0.500	0.190	1.140	0.320
Threonine	0.240	0.1100	0.180	0.110	0.280	0.120
Alanine	0.380	0.1700	0.290	0.090	0.550	0.200
Tyrosine	0.210	0.1900	0.170	0.150	0.180	0.160
Valine	0.120	0.0560	0.150	0.030	0.240	0.080
Histidine	0.090	0.0500	0.080	0.040	0.100	0.040
Arginine	0.070	0.1660	0.180	0.069	0.150	0.024
Glutamine	0.010	0.0000	0.100	0.000	0.100	
Glutaminic acid	0.130	0.0700	0.090	0.047	0.120	0.040
Thryptophane	0.001	0.0000	0.001	0.000	0.001	0.000
Isoleucine	0.080	0.0450	0.090	0.030	0.120	0.040
Leucine	0.120	0.0510	0.100	0.035	0.140	0.040
Phenylalanine	0.070	0.0355	0.070	0.025	0.130	0.048
Lysine	0.070	0.0270	0.060	0.012	0.120	0.040
Total amino acids	3.380	1.7210	2.920	1.171	4.700	1.712

Exudates (μmol/l) estimated per plant from triplicates with a standard variation of 20%

Table 3. Hwajinbyo root exudates (μmol/l) after 28 d

	+N/−MO	−N/−MO	−N/+MO	−N/+MO_{dead}	+N/MO	KO18
Exudates (υmol/l)						
Oxalate	0.115	0.310	0.120	0.260	0.150	0.612
Citrate	0.230	0.000	0.120	0.100	0.230	0.840
Malate	1.920	0.260	0.260	0.000	0.530	2.480
Succinate	0.390	0.100	0.000	0.000	0.000	0.476
Acetate	3.300	3.300	3.300	4.000	4.980	1.480
Pyruvate	0.730	0.940	0.430	1.100	1.160	1.400
Total organic acids	6.685	4.910	4.230	5.460	7.100	7.288
Sorbite	0.640	0.456	0.800	0.640	0.900	0.028
Arabinose	0.200	0.000	0.000	0.050	0.260	2.256
Glucose	2.700	2.680	0.600	0.300	2.000	
Fructose	0.410	0.200	0.136	0.300	0.600	0.680
Ribose	1.300	1.200	2.250	1.700	3.800	0.240
Isomaltose	0.330	0.130	0.300	0.200	0.260	0.148
Total sugars	5.580	4.680	4.100	3.190	7.820	3.352
Aspartic acid	0.198	0.155	0.090	0.127	0.094	0.088
Serine	0.380	0.279	0.164	0.160	0.140	0.072
Glycine	0.244	0.193	0.147	0.200	0.140	0.164
Threonine	0.152	0.145	0.230	0.130	0.160	0.040
Alanine	0.200	0.169	0.097	0.198	0.090	0.184
Tyrosine	0.264	0.220	0.230	0.163	0.220	0.148
Valine	0.090	0.117	0.085	0.170	0.070	0.040
Histidine	0.090	0.080	0.040	0.080	0.020	0.012
Arginine	0.060	0.120	0.050	0.100	0.080	0.036
Glutamine	0.010	0.000	0.010	0.000	0.010	
Glutamic acid	0.080	0.064	0.040	0.030	0.030	0.036
Thryptophane	0.001	0.000	0.001	0.000	0.001	0.000
Isoleucine	0.070	0.070	0.040	0.070	0.030	0.012
Leucine	0.080	0.080	0.018	0.090	0.045	0.016
Phenylalanine	0.080	0.080	0.045	0.100	0.035	0.032
Lysine	0.024	0.030	0.050	0.040	0.020	0.012
Total amino acids	3.441	1.782	1.320	1.636	1.190	0.872

Exudates (μmol/l) estimated per plant from
Triplicates with a standard variation of 20%

cultivation period. In the **+MO** experiments, wild rice seedlings were inoculated with the strain WO3, cultivated rice with KO18, both isolates from the respective soils.

Total exudation under non-inoculated conditions **(+N/−MO)** was clearly higher in wild rice for organic acids and sugars, while no difference existed for amino acids. In both cases, main exudates were the organic acids acetate and malate and the sugars glucose and ribose. In the **-N/+MO** tests, WO3 consumed all sugars except the sugar alcohol sorbit, and used also malate, succinate, acetate, and pyruvate. In contrast, KO18 did not make use of acetate and ribose and the concentrations of sorbite and ribose even increased. No significant amounts of amino acids were consumed by the microorganisms in both cases. When nitrogen was present in the inoculated culture **(+N/+MO)**, sugars and malate were used as energy source by WO3; acetate, pyruvate, and ribose were present even in higher concentrations than under non-inoculated conditions. KO18 consumed mainly malate, succinate and glucose; again concentrations of acetate, pyruvate, and ribose increased, but in this case also sorbite and fructose. Strong differences were found in the amino acid exudation. While in the **+N/+MO** case for KO18 the concentrations were the same or slightly lower, WO3 inoculation led to a strong increase of most amino acids. Apparently the bacteria contribute considerably to the exudate mixture, as also can be seen in the **−N/−P/+MO** results. In the negative controls **(−N/−MO)** and **$-N/+MO_{dead}$**, due to nitrogen deficiency, the plant root exudation was strongly reduced.

With regard to quality, our results correspond well with the investigations of Boureau (1977) and Lin et al. (1989), though we included more sugars and organic acids in our analyses. Quantitative differences between the results presented in this paper and the earlier publications can be explained by different experimental setups. It still has to be proved, whether the observed plant growth promoting effects result from nitrogen fixation activity of the bacteria, their production of phytohormones, or of a combination of both. Therefore experiments are now being performed with *A. lipoferum* ATCC 29708 (SpRg20a), our isolate F, and a nitrogenase negative mutant strain of the same organism.

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