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EXPERIMENTAL ARTICLES

Root Exudates of Tomato Plants and Their Effect on the Growth and Antifungal Activity of *Pseudomonas* Strains

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Abstract—The study of the effect of the root exometabolites of tomato plants on the growth and antifungal activity of plant growth–promoting *Pseudomonas* strains showed that the antifungal activity of plant growth–promoting rhizobacteria in the plant rhizosphere may depend on the sugar and organic acid composition of root exudates.

Key words: root exudates, tomato, pseudomonads, antifungal activity.

The root exudates of plants considerably influence their symbiosis with bacterial strains introduced into the plant rhizosphere. Plant growth–promoting rhizobacteria (PGPR) are an efficient and ecologically safe alternative to pesticides [1]. The PGPR that are able to rapidly colonize the rhizosphere econiche suppress the growth of phytopathogens [2]. It is known that the expression of the rhizobacterial *auf* genes, controlling the biosynthesis of antibiotics, can be determined by the composition of the root exometabolites exuded into the rhizosphere [3, 4].

The aim of this work was to study the effect of the root exometabolites of tomato plants on the growth and antifungal activity of plant growth–promoting pseudomonads.

MATERIALS AND METHODS

The plant growth–promoting rhizobacteria used in this work, *Pseudomonas chlororaphis* SPB1217 and *Pseudomonas fluorescens* SPB2137, were isolated from soil by the method of active selection, accounting for their affinity to root exudates [5]. The strains are able to inhibit spore germination and the growth of a wide range of phytopathogenic fungi, i.e., possess antifungal activity. In this work, the antifungal activity of the strains was tested in vegetative experiments using the phytopathogenic fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici.* The effect of root exudates was studied using the tomato *Lycopersicon esculentum* cultivar Karmello.

The growth dynamics of the pseudomonads was studied by cultivating them at 28°C in a mineral medium (10 ml) in Bunsen flasks. The medium was supplemented with 1 g/l organic acids and sugars (individual or in some combinations) contained in the root exudates of tomato plants. Growth was monitored by measuring the optical density of cultures at 660 nm (OD_{660}).

The effect of carbon sources on the antifungal activity of the *Pseudomonas* strains was studied by the agar well method. The strains were grown in liquid cultures under stationary conditions for 4 days in the presence of particular carbon sources at a concentration of 10 g/l. Then aliquots of bacterial suspensions (100 μl) were poured into wells 8 mm in diameter made in agar plates. The wells were preliminarily inoculated with *F. culmorum* spores. The plates were incubated at 28°C for 2–4 days, after which the antifungal activity of the pseudomonads was assessed by the diameter of the zone of inhibited fungal growth around the wells.

Biocontrol experiments were performed by the method of Chin-A-Woeng *et al.* [6].

In experiments with root exudates and extracts of tomato seeds and seedlings, tomato seeds were sterilized with 5% sodium hypochlorite for 3 min, washed with sterile water, and incubated on wet filter paper placed in petri dishes. After 2 and 4 days of incubation, the tomato seedlings were extracted with water, and the extracts were concentrated in a vacuum rotary evaporator. Root exudates were obtained from 14-day-old tomato plants grown aseptically in special flasks [7].

The sugar and organic acid composition of root exudates was determined using a Jasco LC-900 system (Japan) equipped with a Supelcogel C-610H ion-exchange column (7.8 mm × 30 cm). The mobile phase was 10 mM H_3PO_4 at a flow rate of 0.7 ml/min. The col-

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Table 1. The content of organic acids in root exudates and extracts of tomato seeds and seedlings, ng/seed (seedling)

Organic acid	Cultivation time, days					
	2	%	4	%	14	%
Oxalic	296.2 ± 45.3	48.9	635.8 ± 76.7	16.6	1410 ± 250	5.7
Citric	ND	–	2060 ± 320	53.7	13630 ± 790	55.0
Ketoglutaric	104.6 ± 36.5	17.3	22.3 ± 14.5	0.5	ND	–
Pyruvic	112.5 ± 27.2	18.6	295.2 ± 17.1	7.6	1040 ± 90	4.2
Malic	ND	–	180.0 ± 20.4	4.7	3780 ± 470	15.3
Aconitic	ND	–	0.85 ± 0.30	0.1	20.1 ± 4.7	0.1
Succinic	traces	–	102.1 ± 36.4	2.6	1870 ± 180	7.6
Lactic	77.0 ± 12.3	12.7	472.5 ± 62.3	12.3	2480 ± 316	10.0
Acetic	2.1 ± 0.5	0.3	1.4 ± 0.9	0.1	ND	–
Fumaric	2.8 ± 0.7	0.5	8.1 ± 1.1	0.2	110.1 ± 20.2	0.4
Propionic	ND	–	ND	–	69.9 ± 18.2	0.3
Pyroglutamic	10.1 ± 2.7	1.7	61.2 ± 1.3	1.6	349.9 ± 31.7	1.4
Total	605.3	100.0	3839.5	100.0	24760	100.0

Note: Data presented in Tables 1 and 2 are the means of five replicated experiments. ND stands for "not detected."

umn was kept at 30°C. Eluted products were monitored at 210 nm.

Reducing sugars in the eluate were assayed automatically with a reagent containing 2.0 g 2,3,5-triphenyltetrazolium chloride in 1 l of 0.18 M NaOH. Sugars were analyzed on a Supelcosil LC-NH_2-5 μm column (4.6 mm × 25 cm). The mobile phase was an acetonitrile–water (85 : 15, v/v) mixture at a flow rate of 0.8 ml/min. The column was kept at 30°C. The reagent was supplied at a flow rate of 0.2 ml/min. The chromogenic complex was detected at 487 nm using a Jasco UV-975 monitor.

RESULTS AND DISCUSSION

The dynamics of organic acids (12 in number) and sugars (seven in number) revealed in extracts and root exudates of tomato seeds and seedlings are shown in Tables 1 and 2, respectively.

Let us consider first the dynamics of organic acids (Table 1). The extracts of swollen seeds incubated on wet filter paper at 4°C for 2 days were dominated by oxalic, pyruvic, and ketoglutaric acids. The extracts of 4-day-old tomato seedlings were dominated by citric and oxalic acids, and the root exudates of 14-day-old seedlings by citric and malic acids. In this case, the content of dominant organic acids decreased from 97.5% of the total quantity of organic acids (swollen seeds) to 82.6 (4-day-old seedlings) and 80.3% (14-day-old seedlings). The total amount of organic acids calculated per seed or seedling increased from about 0.6 μg (swollen seeds) to 3.8 μg (4-day-old seedlings) and 24.7 μg (14-day-old seedlings), i.e., by more than 40 times. The amount of organic acids in swollen seeds and in the exudates of 4- and 14-day-old seedlings calculated per mg dry weight was equal to 183.7 ng, 1163 ng, and 44.2 μg per mg dry wt, respectively.

The sugars of swollen seeds were dominated by fructose and glucose (more than 94% of the total sugar content of the seeds) (Table 2). These sugars, as well as maltose, were also dominant in the root exudates of 4-and 14-day-old seedlings (90.9 and 87.7% of the total sugar content, respectively). The total amount of sugars in the exudates of 14-day-old seedlings was 9 times greater than it was in swollen seeds. The amount of sugars in swollen seeds and in the exudates of 4- and 14-day-old seedlings calculated per mg dry weight was equal to 223 ng, 375 ng, and 11.9 μg per mg dry wt, respectively.

As can be seen from these data, swollen seeds contain more sugars than organic acids, whereas the exudates of 4- and 14-day-old seedlings contain more organic acids than sugars. Accordingly, organic acids may play an important role in the energy metabolism of rhizobacteria present in the tomato rhizosphere. The study of the effect of individual root exometabolites on the growth of rhizobacteria in the mineral liquid medium showed that both rhizobacteria studied utilized organic acids more easily than they utilized sugars (Figs. 1, 2). When grown on citric acid, *P. chlororaphis*

Table 2. The content of sugars in root exudates and extracts of tomato seeds and seedlings, ng/seed (seedling)

Sugar	Cultivation time, days					
	2	%	4	%	14	%
Ribose	ND	–	16.8 ± 5.1	1.4	86.9 ± 9.7	1.3
Xylose	256.4 ± 46.7	34.8	95.8 ± 9.8	7.7	2011 ± 360	30.3
Fructose	4.3 ± 0.6	0.6	535.0 ± 88.0	43.2	408.3 ± 60.1	6.2
Glucose	441.2 ± 79.5	59.8	412.8 ± 51.6	33.3	1807 ± 216	27.3
Sucrose	ND	–	ND	–	309.4 ± 59.1	4.7
Maltose	35.3 ± 6.9	4.8	178.5 ± 31.2	14.4	1962 ± 269	30.1
Melibiose or gentobiose	ND	–	ND	–	55.9 ± 9.4	0.1
Total	737.2	100.0	1238.9	100.0	6640.7	100.0

Table 3. The effect of individual root exometabolites on the antifungal activity of plant growth–promoting pseudomonads

Exometabolite	*P. chlororaphis* SPB1217		*P. fluorescens* SPB2137	
	OD_{660}	growth inhibition zone, mm	OD_{660}	growth inhibition zone, mm
Sugars				
Xylose	0.5	0	0.8	0
Fructose	0.9	14	0.9	20
Glucose	1.0	13	0.8	0
Cellobiose	0.20	12	0.25	30
Sucrose	1.2	12	1.0	10
Maltose	0.27	13	0.29	20
Ribose	0.8	15	0.8	0
Organic acids				
Citric	0.8	16	0.6	23
Pyruvic	1.2	18	1.4	11
Malic	0.8	18	0.7	20
Succinic	1.0	18	1.2	24
Ketoglutaric	0.9	18	1.0	18
Oxalic	0.45	18	0.45	30
$LSD_{0.95}$		2		3

Note: LSD stands for “least significant difference.”

SPB1217 and *P. fluorescens* SPB2137 accumulated a maximum biomass (i.e., reached the stationary growth phase) after only 14 h of cultivation. The growth of these rhizobacteria on pyruvic, succinic, and malic acids was poorer and slower than it was on citric acid, so that the stationary growth phase was reached only after 24 h of cultivation. It should be noted that neither rhizobacterium can grow on oxalic acid, which is one of the dominant organic acids of root exudates.

Both rhizobacteria studied grew on sugars more poorly than on organic acids: the lag phase was extended and the stationary growth phase was not reached (Fig. 2). The optical density of bacterial cultures reached the values that are typical of their growth on organic acids after 48–60 h of cultivation. In this case, the consumption of xylose, ribose, and maltose was very low.

The rhizobacteria showed good growth on a mixture of organic acids and sugars taken in a proportion of 3 : 1, which is typical of the proportion of these substances in root exudates. The stationary growth phase was reached after only 12 h of cultivation (Fig. 3). These data suggest that the rhizobacteria studied can efficiently utilize

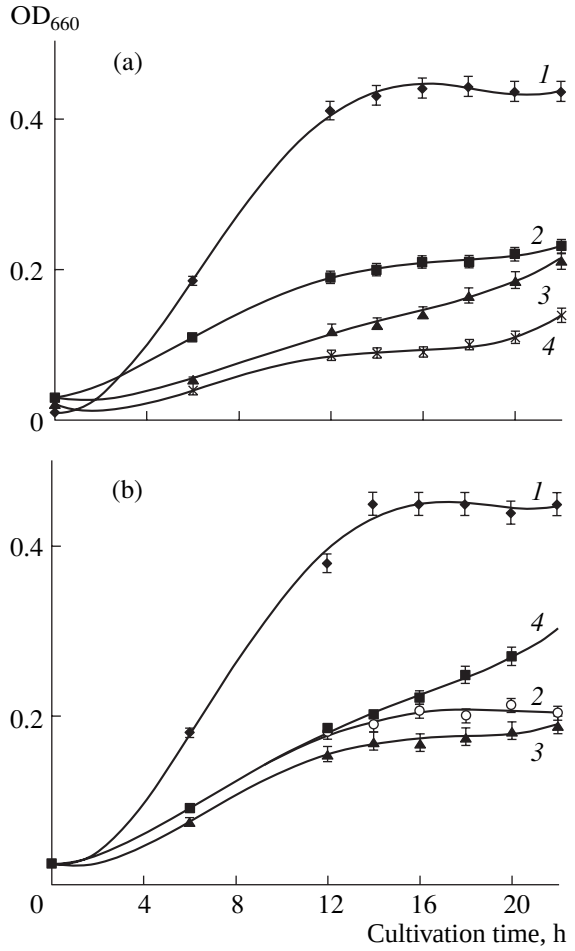

Fig. 1. Growth of (a) *P. chlororaphis* SPB1217 and (b) *P. fluorescens* SPB2137 on (*1*) citric, (*2*) malic, (*3*) succinic, and (*4*) pyruvic acids. The data points are the means of five replicated measurements. The error bars represent standard errors.

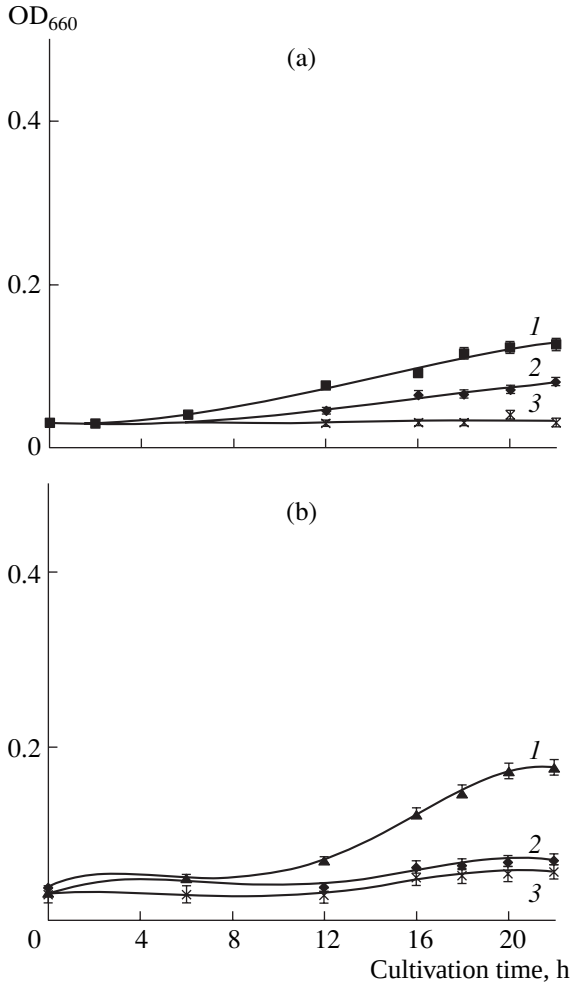

Fig. 2. Growth of (a) *P. chlororaphis* SPB1217 and (b) *P. fluorescens* SPB2137 on (*1*) glucose; (*2*) fructose; and (*3*) xylose, ribose, or maltose. The data points are the means of five replicated measurements. The error bars represent standard errors.

nutrients present in tomato root exudates and hence can be successfully introduced into the tomato rhizosphere.

Actually, low-molecular-weight carbohydrates and organic acids present in the rhizosphere may play an important role in the bacterial colonization of plant roots. Earlier, we showed that a mutant of *P. fluorescens* WSC 365 that was not able to grow on organic acids poorly colonized tomato roots, whereas a mutant that was not able to grow on sugars colonized the roots with the same efficiency as the wild-type strain WSC 365 [8, 9]. Those observations show that the ability of rhizobacteria to colonize plant roots may be related to their ability to utilize root exometabolites.

The study of the effect of individual root exometabolites on the antifungal activity of the rhizobacteria showed that the exometabolites influence this activity, although no correlation was observed between the ability of particular exometabolites to support rhizobacterial growth and the degree of their influence on antifungal activity (Table 3). As a rule, the level of antifungal activity was higher when the rhizobacteria were grown on organic acids than when they were grown on sugars. Some sugars (xylose in the case of both pseudomonads and glucose and ribose in the case of *P. fluorescens* SPB2137) did not promote the synthesis of antifungal metabolites, although the growth of the rhizobacteria on these sugars was comparable with their growth on the other sugars studied.

Reportedly, carbon sources may affect the synthesis of antibiotics by bacteria. For instance, James and Gutterson showed that the synthesis of oomycin A by *P. fluorescens* Hv37a is induced by glucose and is suppressed by some amino acids used as carbon sources [10]. Likewise, oomycin A synthesis was substantially suppressed at high aeration of the medium [3]. These data indicate that the antagonistic activity of rhizobacteria introduced into a rhizosphere may considerably depend on the ecochemical properties of the rhizosphere.

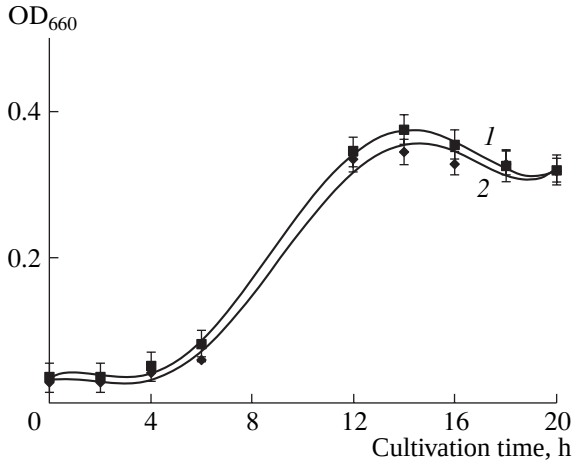

Fig. 3. Growth of (*1*) *P. fluorescens* SPB2137 and (*2*) *P. chlororaphis* SPB1217 on a mixture of organic acids and sugars taken in a proportion of 3 : 1. The data points are the means of three replicated measurements. The error bars represent standard errors.

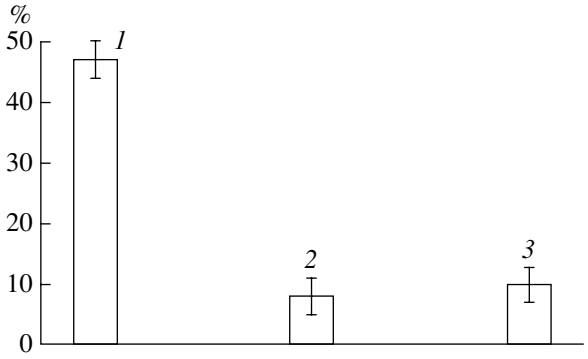

Fig. 4. The percent of tomato plants infected by *F. oxysporum* in (*1*) the control (without introduction of the plant growth–promoting pseudomonads) and in the presence of the introduced (*2*) *P. chlororaphis* SPB1217 and (*3*) *P. fluorescens* SPB2137 strains. The open bars are the means of 15 replicated measurements. The error bars represent standard errors.

Experiments on the antiphytopathogenic activity of the rhizobacteria introduced into the tomato rhizosphere showed that the introduced rhizobacteria diminished the number of tomato plants infected by the phytopathogen *F. oxysporum* by more than 5 times (Fig. 4).

Thus, the utilization of root exometabolites by plant growth–promoting rhizobacteria may influence their antifungal activity. There are grounds to believe that the antifungal activity of rhizobacteria introduced into the plant rhizosphere depends on the sugar and organic acid composition of the root exudates of these plants.

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