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EXPERIMENTAL ARTICLES

The Effect of Tryptophan Present in Plant Root Exudates on the Phytostimulating Activity of Rhizobacteria

L. V. Kravchenko, T. S. Azarova, N. M. Makarova, and I. A. Tikhonovich

All-Russia Research Institute of Agricultural Microbiology, sh. Podbel'skogo 3, St. Petersburg–Pushkin, Russia

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Abstract—Aseptic tomato and radish roots were found to exude, respectively, 2.8–5.3 and 290–390 ng tryptophan per seedling per day. The inoculation of radish plants with rhizosphere pseudomonads increased the root biomass by 1.4 times. The inoculation of tomato plants with the same pseudomonads was ineffective. The beneficial effect of bacterial inoculation on the radish plants can be explained by the fact that the introduced rhizobacteria produce the plant growth–stimulating hormone indole-3-acetic acid. In pot experiments, the addition of this phytohormone to the soil increased the mass of radish roots by 36%. The phytohormonal action of the rhizosphere microflora was found to be efficient provided that the concentration of tryptophan in the rhizosphere is sufficiently high.

Key words: tryptophan, root exudates, seed bacterization, pseudomonads, IAA.

The root system of higher plants is associated with rhizosphere bacteria, some of which can synthesize plant growth–promoting substances, known as phytohormones [1]. The synthesis of phytohormones by rhizosphere bacteria often depends on the presence of phytohormone precursors in the root exudates of plants. Symbiotic rhizobacteria depend extremely on the root exometabolites of host plants. For this reason, the application efficiency of bacterial preparations in agriculture considerably depends on the properties of both symbionts, plant and microorganism.

The presence of L-tryptophan in the medium is a necessary condition of the biosynthesis of indole-3-acetic acid (IAA). When the production of IAA by soil microorganisms is studied under laboratory conditions, tryptophan is deliberately added to the medium [2]. Tryptophan is a metabolic precursor of IAA in almost all soil bacteria [3, 4]. In the plant rhizosphere, the main source of tryptophan is the root exudates of plants [5]. The effect of this amino acid on the phytostimulating activity of rhizobacteria is as yet unknown.

The aim of this work was to study the phytostimulating activity of rhizobacteria on plants whose root exometabolites contain dissimilar amounts of tryptophan.

MATERIALS AND METHODS

Experiments were carried out with the rhizobacteria *Pseudomonas chlororaphis* SPB1217, *Pseudomonas fluorescens* SPB2137, *Pseudomonas corrugata* SPB2142 and SPB2184, *Pseudomonas* sp. SPB4087, and *Curtobacterium* sp. SPB3062 [6]. The rhizobacteria were isolated from soil by the method of active selection for their high response to the root exudates of plants. The plant objects for study were the tomato *Lycopersicon esculentum* Mill cultivars Karmello and Aromato and the common radish *Raphanus sativus* L. cultivars Saksa Nova and Teplichnyi.

Experiments on root exometabolites were carried out as follows: Tomato and radish seeds were sterilized with a 5% solution of sodium hypochlorite and washed many times with sterile water. The seedlings were cultivated in petri dishes with wet filter paper at 27°C for 4 days. Root exudates were extracted from the filter paper with distilled water. The extracts were evaporated in a vacuum at 45°C, and the dry residues were dissolved in 0.5 ml of water. The concentrations of tryptophan in the resulting solutions and of IAA in the culture liquids of rhizobacteria were determined by HPLC with a LiChrosorb RP-18 reversed-phase column, which was eluted with a solution containing 13.7% acetonitrile and 0.22% acetic acid in water [5].

The effect of rhizobacteria on plant growth was studied in a gnotobiotic system described by Simons *et al.* [7]. Sterile quartz sand in a glass flask was moistened with a plant nutrient solution (PNS) containing 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 1 mM KH_2PO_4, and microelements. The solution was added in a relative amount of 10% (v/w). A cell suspension for seed bacterization was prepared by using 12-h-old cells, which were grown in LC medium [8] on a shaker and washed with PNS. Seeds were soaked for 15 min in a cell suspension with an optical density $OD_{620} = 0.1$ (this corresponded to 10^8 cells/ml). Then the bacterized seeds were aseptically placed in the quartz sand to a

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depth of 5 mm. The seedlings were cultivated in a phytotron with 16-h illumination periods at 10000 lx. Cultivation lasted either 7 or 11 days.

Micropot experiments were carried out by using plastic pots with 100 g of a soil–quartz sand mixture (3 : 1). Plants were cultivated for 14 days in a greenhouse with an air humidity of 70% and 16-h illumination periods at 15000 lx. Seeds were bacterized by soaking in a mixture (1 : 1) of a cell suspension and 2% methylcellulose. The suspension of 12-h-old cells had an optical density OD_{620} = 0.7 (this corresponded to 10^9 cells/ml). The bacterized seeds were dried in a flow of sterile air [9]. The dry weight of plant biomass was determined by weighing the biomass dried at 105°C.

The effect of exogenous IAA on radish plants was studied by adding IAA to the pot soil to a final concentration of 10^{-7} M (the control pots did not contain IAA). In these experiments, radish plants were grown from nonbacterized seeds. The wet biomass of radish plants was assessed after 10 days of growth.

RESULTS AND DISCUSSION

To choose plants with contrasting levels of tryptophan in their root exudates, we studied several tomato and radish cultivars (these studies were carried out in the gnotobiotic system). The concentration of tryptophan in the root exudates of different plants differed by 55 to 140 times when calculated per seedling and by 20 to 37 times when calculated per milligram of seeds (Table 1). Radish roots exuded several times more tryptophan than did tomato roots. It can be suggested that the high concentration of tryptophan in the radish rhizosphere may increase the synthesis of IAA by rhizosphere bacteria and stimulate the development of the root system (including edible parts) of radish plants.

Further experiments were carried out with the radish Saksa Nova and the tomato Karmello cultivars. The data presented in Table 2 show that the introduced rhizobacteria *P. chlororaphis* SPB1217, *P. fluorescens* SPB2137, *P. corrugata* SPB2184, and *Curtobacterium* sp. SPB3062 increased the mass of radish roots by approximately 1.4 times, whereas the increase in the mass of the overground parts of radish plants was statistically insignificant. The different plant growth–stimulating effects of the rhizobacteria under study can be explained by their different root-colonizing abilities, as is evident from the observed correlation between the root-colonizing activities of *P. chlororaphis* SPB1217, *P. fluorescens* SPB2137, and *P. corrugata* SPB2184 [6] and their promoting effects on the mass of radish roots.

As for tomato plants, their inoculation with the rhizobacteria produced no growth-stimulating effect (the small increase in the mass of tomato roots and overground parts in response to inoculation with *P. fluorescens* SPB2137 and *Curtobacterium* sp. SPB3062 was statistically insignificant) (Table 2), although the efficiencies of the colonization of radish and tomato plants by the rhizobacteria under study are approximately the same [6].

Table 1. The amount of tryptophan in the root exudates of tomato and radish seedlings

Plant	Mass of one seed, mg	Amount of tryptophan exuded per day	
		ng/seedling	ng/mg seeds
Tomato cultivars:			
Karmello	3.3	5.3 ± 0.7	1.6 ± 0.3
Aromato	3.2	2.8 ± 0.4	0.87 ± 0.10
Radish cultivars:			
Saksa Nova	9.1	293 ± 35	32.2 ± 4.8
Teplichnyi	14.1	390 ± 42	27.7 ± 3.9

Note: The data are the means ± standard deviations of five replicated experiments.

Table 2. The effect of introduced rhizobacteria on the growth parameters of 6-day-old radish plants and 11-day-old tomato plants grown in sand under gnotobiotic conditions

Rhizobacterial strain	Root mass, mg dry wt/10 plants		Mass of overground parts, mg dry wt/10 plants	
	Radish cultivars	Tomato cultivars	Radish cultivars	Tomato cultivars
Control (without bacterization)	28.8	12.0	102.0	24.5
SPB1217	40.7	11.2	110.5	23.5
SPB2137	39.1	12.9	100.1	22.4
SPB2142	33.5	11.6	100.8	24.5
SPB2184	42.1	12.1	103.0	25.7
SPB3062	40.5	12.9	94.1	23.9
SPB4087	27.8	8.8	98.2	20.3
$LSD_{0.95}$	2.9	1.4	6.1	2.6

Note: The data are the means of three replicated experiments. LSD is the least significant difference.

Further pot experiments (Table 3) showed that inoculation with the rhizobacteria caused a statistically significant increase not only in the root biomass but, as opposed to the gnotobiotic experiments described above, in the biomass of the overground parts of 14-day-old radish plants as well. For instance, the inoculation with *P. corrugata* SPB2184 and *Curtobacterium* sp. SPB3062 increased the mass of radish roots and their edible parts by 1.4–1.5 times.

The observed stimulation of the growth of the radish plants can be explained by the production of the plant growth–stimulating phytohormone IAA by the introduced rhizobacteria. Indeed, the addition of L-tryptophan to the medium at a concentration of 50 μM

Table 3. The effect of introduced rhizobacteria on the growth parameters of radish plants grown in pots for 14 days

Rhizobacterial strain	Dry biomass, mg/plant			
	Roots	Roots, % of the control	Overground parts	Overground parts, % of the control
Control (without bacterization)	4.58 ± 0.43	100.0	48.26 ± 5.43	100.0
SPB2184	6.28 ± 0.70	137.1	64.79 ± 8.54	134.2
SPB3062	7.03 ± 0.82	153.5	69.81 ± 6.02	144.6

Note: The data are the means of three replicated experiments.

Table 4. The effect of IAA added to the soil on the growth parameters of radish cultivar Saksa Nova plants

Experimental variant	Wet biomass, mg/plant			
	Green mass	Green mass, % of the control	Overground parts	Overground parts, % of the control
Control (without bacterization)	390 ± 36	100.0	38.2 ± 4.1	100.0
IAA	368 ± 29	94.3	52.5 ± 4.8	136.7

Note: The data are the means of three replicated experiments.

induced the synthesis of IAA by the rhizobacteria at a level of 1.3–5.1 μM, which considerably exceeds the biologically active dose of IAA (0.1 μM) [2].

This hypothesis was substantiated by the results of the experiment with the addition of IAA to the pot soil (Table 4). As is evident from this table, IAA increased the root mass by 36%, leaving the green biomass unchanged.

To conclude, the root exometabolite tryptophan can regulate the synthesis of auxins in soil microflora, including introduced rhizobacteria. The rhizosphere microflora possesses plant growth–promoting activity when the concentration of tryptophan in the rhizosphere is sufficiently high.

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