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Fluorescent Pseudomonads in the Rhizosphere of Plants and Their Relation to Root Exudates

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ABSTRACT. Fluorescent pseudomonads were present in chernozem soil not influenced by plant roots (10^3-10^4 per g dry soil), in the rhizosphere soil of various plants (10^4-10^5 per g soil) and on roots (10^3 to 10^7 per g fresh roots), depending on the species and age of the plant. Relative species representation of fluorescent pseudomonads changed on the roots and in the plant rhizosphere as compared with free soil. *Pseudomonas fluorescens*, representing 60–93% of the population of fluorescent pseudomonads predominated on the roots of all plants investigated. Somewhat different results were obtained in rhizosphere soil. Relatively higher numbers of *P. fluorescens* were detected in the rhizosphere soil of cucumber and maize, numbers in the rhizosphere soil of wheat were practically the same as in free soil and higher numbers of *P. putida* were found in the rhizosphere soil of barley. Almost all components contained in the root exudates of the plants studied, including β-pyrazolylalanine from the root exudates of cucumbers were utilized as carbon and energy sources. Root sxudates of wheat and maize were utilized by the strain *P. putida* K_2 with an efficiency of 73–91%, depending on species and age of the plant.

At present, fluorescent pseudomonads are considered as an important group, particularly with respect to their relation with plants and their healthy condition. Data concerning production of growth compounds by fluorescent pseudomonads were published (Sequeria and Williams 1964; Hussain and Vančura 1970; Swanson *et al.* 1979). The antagonistic relation of *P. fluorescens* to the fungus *Geaumannomyces graminis* bringing about blackening of wheat roots (Rovira and Campbell 1975; Sivasithamparan and Parker 1979) and synergistic relations between *P. putida* and certain phytopathogenic fungi (Vančura and Staněk 1977) were described. Some authors assume that fluorescent pseudomonads are responsible for "soil sickness" in apple-tree orchards (Bunt and Mulder 1973). *P. aeruginosa* can cause diseases of lower plant organs (Cother *et al.* 1976). Root surface and the rhizosphere soil can be reservoirs of pathogenic bacteria, attacking the upper plant parts, as described in the case of xanthomonads by Staněk and Lasík (1965). Due to this and also for other reasons it is necessary to obtain sufficient information concerning distribution of fluorescent pseudomonads in the soil and rhizosphere of different plants, as well as the ability of bateria of this group to utilize compounds contained in root exudates as nutrition and energy sources.

MATERIALS AND METHODS

Plants. The following plants were used to seed the soil: wheat (*Triticum vulgare* Vill.) cultivar "Iljičovka", barley (*Hordeum vulgare* L.) cultivar "Favorit", cucumber (*Cucumis sativus* L.) cultivar "Bilská", maize (*Zea mays* L.) cultivar "CE-250 Dc", and sugar beet (*Beta vulgaris* subsp. *altissima* var. *saccharifera*), monogerm seed of the cultivar "Dobrovická A". The plants were cultivated in a glass-house in vessels containing 1 kg of the soil. Soil moisture was maintained at 60 % of the maximal water holding capacity.

Soil. Chernozem soil taken from various sites at Líbeznice near Prague was used. Characteristics of the soil: C_t 1.92—2.09 %, N_t 0.22 %, P 0.09 %, exchange pH 7.10—7.20.

Microorganisms. Fluorescent pseudomonads were determined, using the dilution plate method in a nutrient medium according to Barclay and Crosse (1974). Species classification of the isolated strains was performed according to Misaghi and Grogan (1969), utilization of different carbon sources by the isolated strains using a procedure according to Lysenko (1961). The utilization of the following amino acids, and amides was tested: alanine, γ-aminobutyric acid, arginine, asparagine, aspartic acid, citrulline, cystine, phenylalanine, glycine, glutamine, glutamic acid, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, pipecolic acid, proline, serine, threonine, tyrosine and valine. The following sugars and sugar alcohols were tested: arabinose, fructose, galactose, glucose, glycerol, lactose, maltose, mannitol, mannose, sucrose and xylose. The utilization of the following organic acids was examined: benzoic, citric, malic, succinic, malonic, lactic, formic, acetic, oxalic and tartaric.

Utilization of root exudates. The amount of utilized root exudates in a simultaneous culture of plant and *P. putida* was calculated on the basis of microbial mass produced in a medium with root exudates as the only carbon source and residual non-consumed carbon. A yield coefficient was used to recalculate the biomass per quantity of root exudates. Details of the method have already been described (Vančura *et al.* 1978).

RESULTS

Occurrence of fluorescent pseudomonads in the soil, rhizosphere and on plant roots

Table I summarizes the data concerning the amount of fluorescent pseudomonads in the free soil, rhizosphere soil and on the surface of plant roots. In the soil not influenced by plant roots (free soil) the number of fluorescent pseufomonads varied within 10^3—10^4 per g dry soil. Only one sample contained less than 10^3 cells. In the rhizosphere soil the number of cells usually varied within 10^4—10^5. One-fourth of the samples contained 10^3 fluorescent pseudomonads, one sample contained 10^6 cells. On the surface of plant roots usually 10^5 cells per g fresh roots were detected; two samples contained 10^6 and one sample 10^7 cells, three samples contained 10^4 cells and two samples comprised 10^3 cells. Thus, the surface of the roots is characterized by a high variability in the representation of fluorescent pseudomonads, depending also on the species and growth phase of the plant. Most cells were detected on maize roots, medium numbers of pseudomonads were found on wheat, whereas the numbers of cells in barley, sugar beet and cucumbers usually varied within 10^4—10^5.

Table II presents relative counts of individual species of fluorescent pseudomonads: *P. fluorescens*, *P. putida* and other species in the free soil and rhizosphere of different plants. The other pseudomonads include *P. aeruginosa* and phytopathogenic pseudomonads. In soil not influenced by plant roots *P. fluorescent* and *P. putida* predominate. Only lower numbers of *P. aeruginosa* and phytopathogenic pseudomonads are present. In the rhizosphere soil of wheat the relative representation of individual species practically does not change, in spite of the fact that the count of cells increases

TABLE I. The rhizosphere effect of fluorescent pseudomonads in different plants cultivated in chernozem soil

Plant	Time of sampling d	Number of pseudomonads in 1 g dry soil or fresh roots $\times 10^{-3}$		
		free soil	rhizosphere soil	root surface
	22	3.1	11.3	250
Wheat	29	7.0	17.8	450
	50	12.4	596.0	1.400
	57	3.2	17.5	400
Barley	8	0.2	4.2	9.3
	15	5.1	11.4	2.8
	22	1.8	5.9	144
	30	1.2	6.1	144
Maize	8	29.7	480	60,000
	17	46.0	2,170	5,500
Sugar beet	19	6.4	328	350
	26	6.2	52.3	75
	47	20.6	25.3	110
Cucumbers I	15	5.0	7.6	—
	22	1.8	2.9	80
	30	1.2	5.6	76
Cucumbers II	19	6.4	18.7	145
	26	6.2	47.7	175
	47	20.6	107	115

considerably (Table I). In the rhizosphere soil of barley the relative representation of *P. fluorescens* decreased by about one-fourth and the relative representation of *P. putida* increased by one-third. In the rhizosphere soil of maize the relative counts of *P. fluorescens* and *P. putida* increased by one-half and decreased by one-third, respectively, and other species were almost absent. In the rhizosphere of cucumbers the relative number of *P. fluorescens* increased by 60 %, the number of *P. putida* decreased to one-fourth and also the relative occurrence of other species decreased by about 30 %.

P. fluorescens predominated on the roots of all plant species studied (Table II); it represented 60—93 % of all fluorescent pseudomonads. *P. putida* occurred in relatively high quantities only on roots of wheat and barley and on roots of corn and cucumber it represented less than 5 % of the root population of fluorescent pseudomonads. Other species of fluorescent pseudomonads represented only a minor portion of the population, their numbers being higher than 5 % only in cucumbers

Utilization of different carbon sources and root exudates by pseudomonads

A collection of 15 strains of saprophytic fluorescent pseudomonads (10 strains of *P. putida* and 5 strains of *P. fluorescens*) isolated from the surface of roots of wheat

TABLE II. The relative occurrence of fluorescent pseudomonads in the rhizosphere of plants in %

Pseudomonas	Free soil	Rhizosphere soil			
		wheat	barley	maize	cucumber
P. fluorescens	50.1	51.3	38.1	75.0	80.0
P. putida	30.2	25.6	45.2	22.5	8.0
Others	19.7	23.1	16.8	2.5	12.0
		Root surface			
P. fluorescens		60.0	72.5	92.7	88.5
P. putida		37.5	25.0	4.9	4.8
Others		2.5	2.5	2.4	6.7

bean and cucumber was tested with respect to utilization of different compounds usually present in seed and root exudates of plants. All strains tested utilized all the eleven sugars and sugar alcohols as carbon sources, of ten organic acids only oxalic acid was not utilized; strains of *P. fluorescens* did not utilize benzoic acid and two of them also tartaric acid. Out of 24 amino acids, none of the strains of *P. putida* utilized methionine and seven did not utilize serine. All strains tested utilized pipecolic acid and β-pyrazolylalanine.

It was found during a monoxenic cultivation of plants with fluorescent pseudomonads in nutrient solutions, in which root exudates served as the only carbon sources, that root exudates are consumed to a considerable extent (Table III). *P. putida* utilized as the only source of energy and nutrients 80—91 % of root exudates of wheat during the initial growth phases and 73—76 % of root exudates of maize. Mainly proteins and peptides remained unconsumed as *P. putida*, as compared with *P. fluorescens*, does not contain proteolytic enzymes. The utilization of root exudates by bacteria of this species would probably be more complete.

DISCUSSION

Pseudomonads represent the most numerous group of bacteria in the rhizosphere of plants. According to Vágnerová *et al.* (1960) pseudomonads represent 23 % of the bacterial population of the root surface of wheat during the initial growth phases

TABLE III. Utilization of root exudates by *P. putida* K_2 during a simultaneous cultivation with plants

Plant	Cultivation time d	Dry weight of cells of *P. putida*, μg medium	Dry weight of cells of *P. putida*, μg roots	Total carbon found μg	Total carbon calculated μg	Utilization of root exudates, %
Maize	3–8	172	12	49.0	203	76
	9–14	207	38	79.2	293	73
Wheat	3–8	449	103	52.5	570	91
	9–14	103	20	26.3	134	80

and 14 % of the population of the rhizosphere soil. The fluorescent pseudomonads are unevenly distributed on particles of organic matter during the initial stage of decomposition. They are highly dependent on readily available organic compounds. Their amount rapidly decreases with decreasing sources of nutrition (Rovira and Sands 1971; Rovira and Ridge 1973). This basic characteristic of the occurrence of fluorescent pseudomonads was fully confirmed in the experiments referred to here. Due to continuous supply of organic compounds by root exudation and occurrence of dead cells and root filaments on root surface they find a suitable milieu for their development. Also the dynamics of their occurrence is in agreement with the kinetics of exudation of organic compounds by plant roots (Vančura and Hovadík 1965; Vančura and Staněk 1975). Saprophytic fluorescent pseudomonads, such as *P. fluorescens* and *P. putida*, have a series of physiological properties required for the ability to colonize the surface of roots and influence their growth and their healthy condition. They are typical epiphytes of the root surface and fulfill all the requirements of colonization of the rhizosphere environment (Macura 1967). They can utilize a broad spectrum of compounds forming a substantial portion of root exudates as carbon and nitrogen sources. The spectrum includes even pipecolic acid released by germinating seeds and seedlings of bean and β-pyrazolylalanine exuded from seeds and roots of cucumbers (Vančura and Hovadík 1965; Vančura and Staněk 1976). The tests determining the ability of fluorescent pseudomonads to utilize different carbon sources present in root exudates are in agreement with the results of Mishagi and Groggan (1969). Fluorescent pseudomonads grow rapidly and, thus, their competitive ability to utilize nutritional sources increases. They produce a series of vitamins, auxins, gibberellins, kinetins, ethylene, a series of antibiotic and lytic compounds that can influence metabolism and growth of plants and other microorganisms of the root surface. However, the number of pseudomonads on plant roots is subject to considerable variation. A similar finding was published by Rovira and Sands (1970).

The complex role of bacteria of this type in the rhizosphere of plants has not yet been sufficiently studied. They can influence both the growth and nutrition and the healthy condition of plants. In the presence of the fungus *G. graminis* causing blackening of wheat roots the fluorescent pseudomonads colonized the rhizosphere and the soil more efficiently than in the uninfected soil (Bednářová *et al.* 1979). It was demonstrated in monoxenic experiments that during utilization of root exudates as the only carbon source the bacterium *P. putida* produced such an amount of metabolites that plant growth was influenced (Vančura *et al.* 1977). A possible toxic effect of exudates on growth of plants (Rempe and Grjunberg 1970) was eliminated in the experiments referred to here by frequently exchanging the nutrient solution.

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REFERENCES

BARCLAY C.A., CROSSE J.E.: Population of aerobic bacteria associated with the roots of apple and cherry plant. *J.Appl.Bact.* **37**, 475 (1974).

BEDNÁŘOVÁ M., STANĚK M., VANČURA V., VESELÝ D.: Microorganisms in the rhizosphere of wheat colonised by the fungus *Gaeumannomyces graminis* var. *tritici*. *Folia Microbiol.* **24**, 253 (1979).

BUNT J.A., MULDER D.: The possible role of bacteria in relation to the apple replant disease. *Medelingen Fakulteit Landbouwwetenschappen (Gent)* **38**, 1381 (1973).

COTHER E.J., DARBYSHIRE B., BREWER J.: *Pseudomonas aeruginosa:* Cause of Internal Brown Rot of Onion. *Phytopathology* **66**, 828 (1966).

HUSSAIN A., VANČURA V.: Formation of biologically active substances by rhizosphere bacteria and their effect on plant growth. *Folia Microbiol.* **19**, 468 (1970).

LYSENKO O.: Pseudomonas. An attempt at a general classification. *J. Gen.Microbiol.* **25**, 379 (1961).

MACURA J.: Physiological studies of rhizosphere bacteria, p. 379 in *The Ecology of Soil Bacteria* (T.R.G. Gray, D. Parkinson, Eds.). Liverpol Univ. Press, Liverpool 1967.

MISHAGHI I., GROGAN R.G.: Nutritional and biochemical comparisons of plant-pathogenic and saprophytic fluorescent pseudomonas. *Phytopathology* **59**, 1436 (1969).

REMPE E.CH., GRJUNBERG T.M.: Qualitative and quantitative composition of root exudates in steril cultures of maize. (In Russian) *Sel'skochozjajstvennaja biologia* **5**, 703 (1970).

ROVIRA A.D., CAMPBELL R.: A scanning electron microscope study of interactions between microorganisms and *Gaeumannomyces graminis* (Sn. *Ophiololus graminis*) of wheat roots. *Microbiol.Ecology* **2**, 177 (1975).

ROVIRA A.D., RIDGE E.H.: The use of a selective medium to study the ecology of *Pseudomonas* spp. in soil. *Bull.Ecol.Res.Comm.* (Stockholm) **17**, 329 (1973).

ROVIRA A.D., SANDS D.C.: Fluorescent pseudomonads — a residual component in the soil microflora. *J.Appl.Bact.* **34**, 253 (1971).

SEQUERIA L., WILLIAMS P.H.: Synthesis of indole acetic acid by *Pseudomonas solanacearum*. *Phytopathology* **54**, 1240 (1964).

SIVASITHAMPARAM K., PARKER C.A.: Bacterial antagonists to the take-all fungus and fluorescent pseudomonas in the rhizosphere of wheat. *Soil Biol.Biochem.* **11**, 161 (1979).

STANĚK M., LASÍK J.: The occurrence of microorganisms parasitizing on the overground parts of plants in the rhizosphere, p. 300 in *Plant Microbes Relationships* (J.Macura, V. Vančura, Eds.). Publ. House Czech.Acad.Sci., Prague 1965.

SWANSON B.T., WILKINS H.F., KENNEDY B.W.: Factors affecting ethylene production by some plant pathogenic bacteria. *Plant & Soil* **51**, 19 (1979).

VÁGNEROVÁ K., MACURA J., ČATSKÁ V.: Rhizosphere microflora of wheat I. Composition and properties of bacterial flora during the first stages of growth. *Folia Microbiol.* **5**, 298 (1960).

VANČURA V., HOVADÍK A.: Composition of root exudates in the course of plant development, p. 21 in *Plant Microbes Relationships* (J. Macura, V. Vančura, Eds.). Publ. House Czech. Acad. Sci., Prague 1965.

VANČURA V., PŘIKRYL Z., KALACHOVÁ M., WURST M.: Some quantitative aspects of root exudation. *Ecol. Bull.* (Stockholm)) **25**, 381 (1977).

VANČURA V., STANĚK M.: Root exudates of plants. V. Kinetics of exudates from bean roots as related to the presence of reserve compounds in cotyledones. *Plant & Soil* **43**, 547 (1975).

VANČURA V., STANĚK M.: Synergistic relationships of *Pseudomonas putida* and some phytopathogenic fungi in plant rhizosphere (Meeting Abstract). *Folia Microbiol.* **21**, 213 (1976).