JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 41, No. 3 2001

BACTERIAL AND FUNGAL POPULATIONS IN THE RHIZOSPHERE OF VARIOUS PLANTS AS RELATED TO ROOT EXUDATES

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Accepted: July 9, 2001

Abstract: The purpose of the studies conducted in the years 1996–1998 was to determine the quantitative composition of bacteria and fungi populations in the rhizosphere of spring wheat, winter wheat, potato and soybean, and in non-rhizosphere soil. Besides, the effect of root exudates of these plants on the formation of antagonistic microorganisms is presented. A microbiological analysis found out that 1 g of rhizosphere soil dry weight of the examined plants and non-rhizosphere soil contained from 4.24×10^6 to 5.97×10^6 bacteria colonies on average. The lowest number of bacteria was found in non-rhizosphere soil (4.24×10^6 on average), and the highest in rhizosphere of potato (5.97×10^6 on average). The fewest fungi colonies (28.59×10^3 on average) were isolated from 1 g of dry weight of winter wheat rhizosphere, and the most (93.41×10^3) from soybean rhizosphere. Antagonistic bacteria of genera *Bacillus* and *Pseudomonas,* and fungi of *Gliocladium, Penicillium* and *Trichoderma* genera dominated in winter wheat rhizosphere. Soybean roots exuded the greatest number of aminoacids (1.088 mg/ml of the solution), while spring wheat roots exuded the smallest amount (0.148 mg/ml of the solution). The percentage of aromatic and alkaline aminoacids was the lowest in potato root exudates, while the highest was found out in the exudates of winter wheat.

Key words: soybean, potato, spring and winter wheat, antagonistic microorganisms, root exudates

I. INTRODUCTION

The greatest biological activity within soil environment is characteristic for rhizosphere soil of cultivated plants. Quantitative and qualitative composition of bacteria and fungi populations undergoes changes under the effect of various biotic and abiotic factors (Parke 1990; Schroth and Weinhold 1986). Plants are the main factors causing these changes which by their root exudates and post-harvest residue, form the population of microorganisms (Funck-Jensen and Hockenhull 1984; Schoruvitz and Zeigler 1989; Sytnik et al. 1977). The root exudates are a rich source of aminoacids, sugars, organic acids, vitamins, metal ions, phenolic acids and other metabolites (Funck-Jensen and Hockenhull 1984; Pięta 1988; Sytnik et al. 1977). These substances as well as organic residue may have a stimulating or inhibiting effect on the growth and development of the populations of bacteria and fungi, including antagonistic microorganisms (Funck-Jensen and Hockenhull 1984; Martyniuk et al. 1991; Schoruvitz and Zeigler 1989; Sundin et al. 1990). Among the enumerated compounds of root exudates, sugars soluble in water and acid aminoacids have a stimulating effect, while phenolic compounds, aromatic and alkaline aminoacids have an

inhibiting effect on the development of pathogenic fungi (Milczak and Piotrowski 1980; Pięta 1988; Piotrowski and Milczak 1982).

Special attention should be paid to the stimulating effect of root exudates on the development of antagonistic bacteria (*Bacillus* spp., *Pseudomonas* spp.) and soil-borne fungi (*Gliocladium* spp., *Penicillium* spp., *Trichoderma* spp.) (Pięta and Patkowska 2000). These antagonistic bacteria and fungi have an ability to inhibit the development of soil-borne pathogenic fungi by anti-biosis, competition and parasitism (Dowling and O'Gara 1994; Kloepper et al. 1999; Lin et al. 1994; Mukherjee et al. 1995; Pietr and Sobiczewski 1993).

The purpose of the studies was to determine quantitatively populations of bacteria and fungi in the rhizosphere of spring wheat, potato, soybean, and in non-rhizosphere soil. Moreover, numbers of antagonistic bacteria (*Bacillus* spp. and *Pseudomonas* spp.) and fungi (*Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp.) were estimated. Besides, the studies aimed at explaining the effect of root exudates of those plants on the formation of antagonistic microorganisms.

II. MATERIAL AND METHODS

The studies were performed in the years 1996–1998 on an experimental field belonging to the Department of Plant and Soil Cultivation of the University of Agriculture in Lublin. The plot was situated at the Experimental Station at Czesławice near Nałęczów. The object of the studies was rhizosphere of spring wheat cv. Sigma, winter wheat cv. Kobra, potato cv. Bronka and soybean cv. Poland, as well as non-rhizosphere soil. The plants were cultivated in crop-rotation with 25% of soybean – potato, spring wheat, soybean, winter wheat. Non-rhizosphere soil was sampled from belts lying between the plots mechanically treated in black fallow.

The experiment was set in complete randomised blocks with three replications, on grey brown podsolic soil formed from loess soils belonging to the second complex of agricultural suitability of the soil (good wheat complex).

1. Microbiological analysis of rhizosphere of plants and non-rhizosphere soil

Each year the soil was sampled at anthesis of the analysed plant species. Soil samples and microbiological analyses were performed according to the method described by Martyniuk et al. (1991). The rhizosphere and non-rhizosphere soil (from the depth of 5–10 cm) was put to sterile Petri dishes. Afterwards, in sterile laboratory conditions a soil solution was prepared in the dilution ranging from 10^{-1} to 10^{-7}. The total number of bacteria in 1g of soil dry weight was determined on "Nutrient agar" from the soil solutions with the dilutions of 10^{-5}, 10^{-6}, 10^{-7}. "Triptic soy agar" and dilutions 10^{-4}, 10^{-5}, 10^{-6} were used for the bacteria from *Bacillus* spp., while "Pseudomonas agar F" and dilutions of 10^{-2}, 10^{-3}, 10^{-4} were used for *Pseudomonas* spp. The total number of fungi in 1 g of soil dry weight was determined on Martin nutrient medium (1950) with the dilutions of 10^{-2}, 10^{-3}, 10^{-4}.

The results of quantitative analysis, concerning the numbers of bacteria and fungi, were statistically analysed, and the significance of differences was determined on the basis of Tukey's confidence intervals (Oktaba 1987).

2. Effect of bacteria and saprophytic fungi on pathogenic fungi

Each year of the studies, the isolates of bacteria (200 isolates of *Bacillus* spp., and 200 isolates of *Pseudomonas* spp.) as well as all the isolates of saprophytic fungi (the genera of *Gliocladium, Penicillium, Trichoderma*) were used to determine their antagonistic effect towards such pathogenic fungi as *Botrytis cinerea, Fusarium culmorum, Fusarium oxysporum, Fusarium solani, Phoma exigua, Pythium irregulare, Rhizoctonia solani* and *Sclerotinia sclerotiorum.* To establish the antagonistic effect of the examined bacteria on pathogenic fungi the five-degree scale described by Martyniuk et al. (1991) and the degrees of inhibition of phytopathogen growth provided by Pięta (1999) were used. The estimation of the effect of saprophytic fungi on the studied pathogenic fungi was performed by means of the method of biotic rows (Mańka 1974; Mańka and Mańka 1992), and the individual effect of antagonistic effect was determined on the basis of the scale provided by Mańka and Kowalski (1968).

3. Chemical analysis of the plant root exudates

Quantity of the soluble sugars and the composition of aminoacids in root exudates of experimental plants were analyzed in order to explain the effect of root exudates on the formation of antagonistic fungi and bacteria populations. The way of preparing water solution of root exudates of the examined plant species, and the chemical analyses were conducted according to the methods described by Pięta (1988). Chemical compounds were determined in the Department of Chemistry of Medical Academy in Lublin.

III. RESULTS

1. Microbiological analysis of rhizosphere of plants and non-rhizosphere soil

On the basis of the microbiological analysis of rhizosphere of spring wheat, winter wheat, potato and soybean, and non-rhizosphere soil, it was found out (3 years of studies) that on average 1 g of soil dry weight contained from 4.24×10^6 to 5.97×10^6 bacteria strains (Tab. 1). The lowest number was found out in non-rhizosphere soil (4.24×10^6 strains on average), while the highest in potato rhizosphere (5.97×10^6 on average) (Tab. 1).

The number of bacteria of *Bacillus* genus in 1 g of soil dry weight ranged from 1.19×10^6 to 3.0×10^6 colonies. Most numerous strains of *Bacillus* spp. were in 1 g of dry weight of spring wheat rhizosphere (2.10×10^6 on average). They were less numerous in soybean rhizosphere (1.93×10^6 on average), non-rhizosphere soil (1.91×10^6 colonies) (Tab. 1). Strains of *Bacillus* spp. were in minority isolated from 1 g of dry weight of winter wheat rhizosphere (1.43×10^6 colonies on average). A reverse relation was observed for bacteria

Table 1

The number of bacteria and fungi in the plant rhizosphere and non- rhizosphere soil

Type of soil	Total number of bacteria (mln/1g d. m. of soil)				Number of bacteria of *Bacillus* genus (mln/1g d. m. of soil)				Number of bacteria of *Pseudomonas* genus (mln/1g d. m. of soil)				Total number of fungi (thous./1g d. m. of soil)			
	1996	1997	1998	mean	1996	1997	1998	mean	1996	1997	1998	mean	1996	1997	1998	mean
Rhizosphere of spring wheat	4.30^{a}	6.57^{c}	3.96^{ab}	4.94^{ab}	1.73^{a}	2.40^{c}	2.19^{b}	2.10^{c}	2.18^{cd}	1.67^{c}	1.25^{b}	1.70^{b}	22.30^{a}	47.10^{c}	65.85^{c}	45.08^{b}
Rhizosphere of winter wheat	5.40^{b}	4.87^{b}	4.96^{c}	5.07^{b}	1.26^{a}	1.78^{b}	1.25^{a}	1.43^{a}	2.75^{e}	1.45^{c}	2.74^{c}	2.31^{c}	33.90^{b}	17.42^{a}	34.47^{b}	28.59^{a}
Rhizosphere of potato	6.69^{c}	3.96^{ab}	7.26^{d}	5.97^{c}	3.0^{b}	1.23^{a}	1.19^{a}	1.80^{b}	1.91^{bc}	0.96^{b}	2.03^{b}	1.63^{b}	59.90^{c}	27.37^{b}	69.96^{c}	52.41^{b}
Rhizosphere of soybean	8.70^{d}	3.21^{a}	4.60^{bc}	5.50^{bc}	1.54^{a}	2.26^{c}	2.0^{b}	1.93^{c}	2.48^{de}	0.58^{ab}	1.24^{b}	1.43^{b}	86.0^{d}	70.51^{d}	123.74^{d}	93.41^{c}
Non- rhizosphere soil	4.75^{ab}	4.47^{b}	3.50^{a}	4.24^{a}	1.17^{a}	2.25^{c}	2.33^{b}	1.91^{c}	1.03^{a}	0.44^{a}	0.19^{a}	0.55^{a}	34.10^{b}	51.38^{c}	16.16^{a}	33.88^{a}

Means in columns differ significantly (P = 0.05), if they are not marked with the same letter

of *Pseudomonas* genus. The lowest number of these bacteria was found in spring wheat rhizosphere (1.70×10^6 on average), while the highest in winter wheat rhizosphere (on average, 2.31×10^6 colonies in 1 g of soil dry weight). The lowest number of *Pseudomonas* spp. was observed in non-rhizosphere soil.

In respective years of studies, the total number of fungi in 1 g of soil dry weight ranged from 16.16×10^3 to 123.74×10^3 colonies (Tab. 1). The lowest quantities of fungi (28.59×10^3 on average) were isolated from 1 g of dry weight of winter wheat rhizosphere, and the most high quantity (93.41×10^3 colonies) from soybean rhizosphere. Non-rhizosphere soil was also characterised by small quantities of fungi. Besides, almost twice as many fungi colonies were found in spring wheat in comparison to winter wheat rhizosphere (Tab. 1).

2. Effect of bacteria and saprophytic fungi on pathogenic fungi

Laboratory tests showed that within populations of microorganisms from both the rhizosphere of the studied plants and non-rhizosphere soil, there were different numbers of bacteria of *Bacillus* spp. and *Pseudomonas* spp., and fungi of *Gliocladium* spp., *Penicillium* spp., *Trichoderma* spp. antagonistic to the pathogenic fungi tested. The largest number of antagonistic bacteria (283 strains) and fungi (428 isolates) was revealed in winter wheat rhizosphere, while the lowest number (48 and 56 isolates, respectively) was found in soybean rhizosphere (Tab. 2). Spring wheat rhizosphere comprised of almost four time more antagonistic bacteria and 1.5 time more antagonistic fungi than the non-rhizosphere soil (Tab. 2).

3. Chemical analysis of the plant root exudates

The chemical analysis of root exudates of soybean, spring wheat, winter wheat and potato revealed differences in the quantitative and qualitative composition of free aminoacids

Table 2

Antagonistic microorganisms isolated from the plant rhizosphere and non-rhizosphere soil (mean of isolates from the years 1996–1998)

Bacteria and fungi	Number of isolates				
	rhizosphere of spring wheat	rhizosphere of winter wheat	rhizosphere of potato	rhizosphere of soybean	non-rhizosphere soil
Bacillus spp.	78	100	71	19	18
Pseudomonas spp.	116	183	99	29	34
Total bacteria	194	283	170	48	52
Gliocladium spp.	18	26	7	3	4
Penicillium spp.	168	229	166	42	119
Trichoderma spp.	71	173	51	11	47
Total fungi	257	428	224	56	170

Table 3

The contents of free amino acids and sugars (in mg/ml) in the exudates of plant roots

Plants	Sour amino acids		Aromatic amino acids		Alkaline amino acids			treo-nine	serine	glycine	alanine	valine	metio-nine	isoleu-cine	leucine	Total amino acids	water souble sugars
	aspartic acid	glutam. acid	tyro-sine	phenyl-alannine	lysine	histi-dine	argi-nine										
Spring wheat	0.029	0.030	0.009	0.010	0.004	0.006	0.004	0.011	0.001	0.011	0.006	0.010	0.007	0.003	0.007	0.148	trace
Winter wheat	0.091	0.099	0.086	0.017	0.037	0.046	0.026	0.017	0.014	0.009	0.010	0.013	0.003	0.012	0.004	0.484	0.117
Potato	0.052	0.054	0.007	0.004	0.006	0.004	0.003	0.004	–	0.001	0.004	0.010	0.003	–	0.001	0.153	0.363
Soybean	0.312	0.277	0.136	0.031	0.036	0.038	0.035	0.113	0.010	0.006	0.004	0.017	0.048	0.015	0.005	1.088	0.121

presented in 1 ml of water solution (Tab. 3). The highest amount of aminoacids (1.088 mg/ml of the solution) was exuded by soybean roots, and the lowest (0.148 mg/ml and 0.153 mg/ml, respectively) by the roots of spring wheat and potato. For winter wheat the amount of free aminoacids was 0.484 mg/ml of the solution (Tab. 3).

Out of 15 free aminoacids found in root exudates of the analysed plants two amino-acids: aspartic acid and glutamic acid dominated. Their value in root exudates of soybean and potato roots was respectively 54% and 69% of the total amount of free aminoacids. The lowest percentage of these compounds was found in spring wheat (39.8%) and winter wheat (39% of the total amount of aminoacids).

Roots of the examined plants also exuded alkaline aminoacids like lysine, histidine and arginine. The lowest amount of alkaline aminoacids was exuded by potato roots (8.5%). Larger amount of these aminoacids (22.5%) was found in root exudates of winter wheat than in spring wheat (9.4% of the total quantity of the compounds discussed here).

Moreover, free aromatic aminoacids, like tyrosine and phenylalanine were found in root exudates. The largest amount of these compounds was exuded by winter wheat roots (21%), and the smallest by potato roots (7% of the total amount of free aminoacids).

The other aminoacids: treonine, serine, glycine, alanine, valine, metinine, isoleucine and leucine present in winter wheat root exudates constituted 0.082 mg/ml of the solution. For spring wheat it was 0.056 mg/ml of the solution of potato 0.023 mg/ml of the solution, and of soybean 0.218 mg/ml of the solution (Tab. 3).

Apart from aminoacids, the roots of spring wheat exuded trace quantities of sugars soluble in water. The roots of winter wheat and soybean exuded slightly more of these compounds, i.e. 0.117 mg/ml and 0.121 mg/ml of the water solution, respectively. On the other hand, the highest amounts

of sugars soluble in water were found in potato root exudates (0.363 mg/ml of the water solution) (Tab. 3).

IV. DISCUSSION

Results of the studies showed different composition of bacteria and fungi populations in rhizosphere of several plant species, and in non-rhizosphere soil. Winter wheat rhizosphere had the most favourable composition of microorganisms, since it contained the lowest total quantity of fungi. Besides, it created good conditions for the development of antagonistic bacteria (*Bacillus* spp., *Pseudomonas* spp.) and fungi (*Gliocladium* spp., Penicillium *spp., Trichoderma* spp.). According to Keel (1992) and Weller (1988), bacteria of *Pseudomonas* spp. are capable of active settlement of plant roots, owing to which they efficiently compete with pathogens for food elements available in root exudates becoming this way a factor of biological control of plants. The studies performed by Księżniak and Kobus (1993) also report that bacteria of *Pseudomonas* genus, being able to produce siderophores, inhibit the development of phytopathogens. In spring wheat rhizosphere the species of antagonistic fungi and bacteria were less numerous than in winter wheat rhizosphere. Among the plants rhizosphere studied the lowest quantity of antagonists analysed, occurred in soybean rhizosphere. On the other hand, potato rhizosphere contained nearly four times more antagonistic bacteria and fungi than soybean rhizosphere.

The presented results confirmed the findings that a plant species has the foremost effect on the formation of microorganisms populations (including antagonists) (Funck-Jensen and Hockenhull 1984). It seems that by acidic, aromatic and alkaline aminoacids and by the products of post-harvest residue decomposition, winter wheat, spring wheat and potato created especially favourable nutritive conditions for the development of antagonistic microorganisms.

It is known that considerable nutritive requirements and great biochemical activity distinguish antagonistic microorganisms from non-rhizosphere microorganisms (Curl 1982; Funck-Jensen and Hockenhull 1984; Papavizas 1985; Sundin et al. 1990). In the presented studies the lowest quantity of antagonists was observed in soybean rhizosphere. It might be explained by the fact that root exudates of *Papilionaceae*, including soybean and products of decomposition of its tissues, especially inhibit the development of *Trichoderma* spp. (Darcy 1982; Papavizas 1985). According to Darcy (1982), Funck-Jensen and Hockenhull (1984) and Pięta (1988), the substances exuded by soybean roots are rich in aminoacids, and many post-harvest residues contain their proteins. Therefore cultivation of these plants have a stimulating effect on the growth of pathogenic fungi, this way reducing the number of antagonistic microorganisms.

V. CONCLUSIONS

1. The mean number of bacteria and fungi in rhizosphere of the studied plant species was usually higher than in non-rhizosphere soil.
2. Antagonistic *Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp., *Penicillium* spp., and *Trichoderma* spp. dominated in winter wheat rhizophere.
3. Soybean roots exuded the largest total number of aminoacids and spring wheat roots the smallest. The lowest proportion of aromatic and alkaline aminoacids was found in potato roots exudates, while the highest in winter wheat exudates.
4. It seems that root exudates of winter wheat showed the most stimulating effect on the growth of propagation units of antagonistic bacteria and fungi. On the other hand, soybean rhizosphere contained the lowest quantity of antagonists, which may suggest an inhibiting effect of compounds exudated by the roots of this plant.
5. Root exudates of spring wheat had more positive effect on the number of antagonistic bacteria and fungi than potato root exudates.

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ZBIOROWISKA BAKTERII I GRZYBÓW W RYZOSFERZE RÓŻNYCH ROŚLIN W ZALEŻNOŚCI OD ICH WYDZIELIN KORZENIOWYCH

STRESZCZENIE

Celem badań przeprowadzonych w latach 1996–1998 było określenie składu ilościowego zbiorowisk bakterii i grzybów w ryzosferze pszenicy jarej, pszenicy ozimej, ziemniaka, soi oraz w glebie pozaryzosferowej. Ponadto starano się wyjaśnić, jaki wpływ na kształtowanie się mikroorganizmów antagonistycznych dla grzybów patogenicznych mogły mieć wydzieliny korzeniowe tych roślin. W wyniku przeprowadzonej analizy mikrobiologicznej stwierdzono, że w 1g s. m. gleby średnia liczebność bakterii i grzybów w glebie ryzosferowej badanych gatunków roślin była z reguły większa, aniżeli w glebie pozaryzosferowej. Antagonistyczne *Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp., *Penicillium* spp. i *Trichoderma* spp. dominowały w glebie ryzosferowej pszenicy ozimej. Najwięcej ogółem aminokwasów wydzielały korzenie soi, a najmniej korzenie pszenicy jarej. Wydaje się, że

wydzieliny korzeniowe pszenicy ozimej wykazywały najbardziej stymulujący wpływ na wzrost jednostek propagacyjnych bakterii i grzybów antagonistycznych. Natomiast w ryzosferze soi wystąpiło najmniej antagonistów, co może sugerować hamujące oddziaływanie związków wydzielanych przez korzenie tej rośliny. Wydzieliny korzeniowe pszenicy jarej wpłynęły korzystniej na liczebność antagonistycznych bakterii i grzybów aniżeli wydzieliny korzeniowe ziemniaka.