FORMATION OF BACTERIAL AND FUNGAL COMMUNITIES IN THE RHIZOSPHERE OF SOYBEAN (GLYCINE MAX. (L.) MERRILL) AND THEIR ANTAGONISM TOWARDS PHYTOPATHOGENS

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Abstract. The purpose of the studies conducted in the years 1996-1998 was to determine the quantitative and qualitative composition of bacterial and fungal communities in rhizosphere of soybean cultivated in monoculture and non-rhizosphere soil. Besides, the proportion of bacteria and fungi, which were distinguished by their antagonistic effect towards soil-borne pathogens was established. A microbiological analysis of 1g of dry weight of soil from rhizosphere of soybean resulted in 3.21×10^6 to 8.70×10^6 bacterial colonies and from 70.51×10^3 to 123.74×10^3 fungal colonies. In the case of non-rhizosphere soil, 3.50×10^6 to 4.75×10^6 bacterial colonies and 16.16×10^3 to 51.38×10^3 fungal colonies were obtained. Besides, soybean cultivation in monoculture had a negative effect on the number of antagonistic isolates of bacteria (*Bacillus* spp., *Pseudomonas* spp.) and fungi (*Gliocladium* spp., *Penicillium* spp., *Trichoderma* spp.). Smaller numbers of antagonistic bacteria and fungi in rhizosphere soil of soybean cultivated in monoculture as compared to non-rhizosphere soil, can prove little biological activity, which results in a worse phytosanitaty condition of the soil.

Key words: soybean, rhizosphere, phytopathogens, antagonistic bacteria and fungi

I. INTRODUCTION

The quantitative and qualitative composition of bacterial and fungal populations in the soil undergoes changes under the effect of biotic and abiotic factors (Parke 1990; Schroth and Weinhold 1986). The highest biological activity within the soil environment is characteristic of rhizosphere soil of field crops. In the vegetative period, a big influence on the communities of microorganisms is exerted by root exudates, which are a rich source of aminoacids, sugars, organic acids, vitamins, metal ions, phenolic acids and their derivatives (Funck-Jensen and Hockenhull 1984; Pieta 1981; Sytnik et al. 1977). On the other hand, after harvest an important role in the formation of microbial populations is played by remaining organic residues (Batalin 1962; Huber and Watson 1970). Root exudates and organic residues can have a stimulatory or inhibitory effect on the growth and development of populations of bacteria and fungi, including antagonistic ones (Funck-Jensen and Hockenhull 1984; Martyniuk et al. 1991; Pięta et al. 1999; Schoruvitz and Zeigler 1989; Sundin et al. 1990). Antagonistic bacteria (Bacillus spp., Pseudomonas spp.) (Defago and Haas 1990; Keel 1992) and fungi (Gliocladium spp., Penicillium spp.) (Hwang and Chakravarty 1993; Lorito et al. 1993; Papavizas 1985) have the ability to inhibit growth of pathogenic soil-borne fungi through antibiosis, 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 the quantitative and qualitative composition of bacterial and fungal communities in rhizosphere of soybean and non-rhizosphere soil. Besides, the studies established the proportion of microorganisms antagonistic towards pathogenic soil-borne fungi.

II. MATERIAL AND METHODS

The studies were carried out in the years 1996-1998 on an experimental plot of the Department of Plant and Soil Cultivation of the University of Agriculture in Lublin. The plot was localised at the Experimental Station in Czesławice near Lublin. The subject of the studies was rhizosphere soil of soybean cv. Poland, gorwn in a 4-year-long monoculture, and non-rhizosphere soil.

Each year rhizosphere soil was sampled at anthesis of soybean. Non-rhizosphere soil samples were taken from belts mechanically treated in bare fallow. Sampling of rhizosphere soil and microbiological analysis were performed according to the method described by Martyniuk et al. (1991). Soil samples were taken from the depth of 5-10 cm, and soil dilutions ranging from 10^{-1} to 10^{-7} were prepared for microbiological analysis.

The total number of bacteria in 1g of soil dry weight the number of bacteria of genera *Bacillus* and *Pseudomonas* as well as the total number of fungi were determined according to the method described by Pięta (1999).

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

Each year the obtained isolates of bacteria (200 isolates of Bacillus spp. and 200 isolates of Pseudomonas spp.), all isolates of saprophytic fungi from the genera of Gliocladium and Trichoderma, and 5 isolates from each species of Penicillium genus were used in order to determine their antagonistic effect towards the following pathogenic fungi Botrytis cinearea, Fusarium culmorum, F. oxysporum f.sp. glycines, F. solani, Phoma exigua var. exigua, Pythium irregulare, Rhizoctonia solani and Sclerotinia sclerotiorum. Antagonistic effect of the examined bacteria and fungi on pathogenic fungi was determined according to methods described by Martyniuk et al. (1991) and Pięta (1999).

III. RESULTS

The microbiological analysis of rhizosphere of soybean and non-rhizosphere soil showed that the number of bacteria in 1 g of dry weight of rhizosphere soil ranged from 3.21×10^6 to 8.70×10^6 bacteria, and in non-rhizosphere soil from 3.50×10^6 to 4.75×10^6 bacteria were found (Tab. 1). The mean number of bacteria of *Bacillus* spp. in 1 g of dry weight of rhizosphere soil was 1.93×10^6 , and in non-rhizosphere soil 1.91×10^6 bacteria.

Almost three times more bacterial colonies from *Pseudomonas* spp. were obtained from rhizsophere soil of soybean as compared to non-rhizosphere soil. The total number of fungi in 1g of dry weight of rhizosphere soil of soybean ranged from 70.51×10^3 to 123.74×10^3 , while in non-rhizsophere soil from 16.16×10^3 to 51.38×10^3 (Tab. 1).

The mycological analysis of rhizosphere soil of soybean revealed the presence of 367 fungal isolates, and in non-rhizosphere soil 296 of fungal isolates in 1 g of dry soil (Tab. 2). Among pathogenic fungi the most frequent were Fusarium culmorum, F. oxysporum, F. solani, Phoma exigua and Pythium irregulare, while the last two were not isolated from non-rhizosphere soil. The other species were much more frequently obtained from rhizosphere soil of soybean as compared to non-rhizosphere soil (Tab. 2). On the other hand, such saprophytic fungi as Penicillium spp., Gliocladium spp. and Trichoderma spp. were more frequently isolated from non-rhizosphere soil than from rhizosphere soil of soybean (Tab. 2).

Among the tested bacteria isolated in the years 1996-1998 from 1 g of dry weight of rhizosphere soil of soybean, 19 isolates of *Bacillus* spp. and 29 isolates of *Pseudomonas* spp. showing antagonistic effect towards the studied phytopathogens were found. They were the most effective in inhibiting the growth and development of *P. irregulare* and *Phoma exigua* var. *exigua*, since the value of the total antagonistic effect for three years of studies was +219 and +161, respectively (Tab. 3). Totally, the antagonistic effect of bacteria *Bacillus* spp. and *Pseudomonas* spp. isolated from soybean rhizosphere towards all tested phytopathogens was +760 (Tab. 3).

1 g of dry weight of non-rhizosphere soil contained 18 colonies of *Bacillus* spp. and 34 colonies of *Pseudomonas* spp. characterised by antagonistic effect towards the studied phytopathogens. These bacteria were the most effective in inhibiting the growth of *B. cinerea* (+66), *F. oxysporum* f. sp. glycines (+68) and *P. exigua* var. exigua (+67). Totally, the antagonistic effect of studied bacteria from non-rhizosphere soil towards soybean phytopathogens was +388 (Tab. 4).

The number of bacteria and fungi in soybean rhizosphere and non-rhizosphere soil

Type of soil	Tota (m	Total number of bacteria (mln/1g d. w. of soil)	r of bact w. of so	teria il)	z º Ē	Number of bacteria of Bacillus genus mln/1g d. w. of soil)	f bacter us genu w. of sc	ia s vil)	Nu Ps	Number of bacteria of Pseudomonas genus (mln/1g d. w. of soil)	bacteria nas gent w. of so	of sı (li	Tot (tho	Total number of fungi (thous./1g d. w. of soil)	er of fur w. of s	ngi oil)
	1996	.996 1997 1998 mean 1996 1997 1998	1998	mean	9661	1997		mean	9661	1996 1997 1998		mean	9661	1996 1997	8661	mean
Rhizosphere of soybean Non- rhizosphere soil	8,70 ^b 4,75 ^a	3,21ª 4,47b	4,60b 3,50a	3,21a 4,60b 5,50b 1,54b 4,47b 3,50a 4,24a 1,17a	1,54 ^b 1,17 ^a	2,26ª 2,25ª	2,0ª 2,33b	1,93ª	2,48 ^b 1,03 ^a	0,58ª	1,24 ^b 0,19 ^a	1,43 ^b 0,55 ^a	86,0 ^b 34,10 ^a	1,93* 2,48* 0,58* 1,24* 1,43* 86,0* 70,51* 123,74* 93,41* 1,91* 1,03* 0,44* 0,19* 0,55* 34,10* 51,38* 16,16* 33,88*	123,74 ^b 16,16 ^a	93,41 ^b

Means in columns differ significantly (P \leq 0.05) if they are not marked with the same letter.

Table 2
Fungi isolated from rhizosphere of soybean and non-rhizosphere soil

			N	umber o	of isolate	s		
Fungus species	rhiz	osphere	of soyb	ean	no	n-rhizo	sphere s	oil
	1996	1997	1998	total	1996	1997	1998	total
Aspergillus fumigatus Fres.					4	13	5	22
Fusarium avenaceum (Corda ex Fr.) Sacc.					1		2	3
Fusarium culmorum (W. G. Sm.) Sacc.		3	6	9		1	1	2
Fusarium equiseti (Corda) Sacc.		3	4	7	3	4	3	10
Fusarium oxysporum Schl.	42	57	66	165	3	2	1	6
Fusarium solani (Mart.) Sacc.	10	9	9	28	2	2	1	5
Gliocladium roseum Bainier		1	2	3	2		2	4
Penicillium chrysogenum Thom					8	12	14	34
Penicillium funiculosum Thom					10	11	12	33
Penicillium verrucosum Dierckx var.								
verrucosum Samson, Stolk, et Hadlok	5	1	1	7	10	12	8	30
Penicillium spp.	21	12	5	38	6	9	7	22
Phoma eupyrena Sacc.					11	20	6	37
Phoma exigua Desm.	15	17	7	39				
Pythium irregulare Buisman	14	15	6	35				
Trichoderma hamatum (Bon.) Bain	1	1		2	1		1	2
Trichoderma harzianum Rifai						2		2
Trichoderma koningii Oud.			2	2	2	5	1	8
Trichoderma pseudokoningii Rifai	3	4		7	2	13	8	23
Trichoderma viride Pers. ex S.F.Gray					4	3	5	12
Other saprophytic fungi	2	5	16	45	14	22	5	41
Total	113	128	126	367	83	131	82	296

The results concerning the effect of saprophytic fungi isolated from rhizosphere soil of soybean on the plant pathogens showed that 56 colonies of various species (42 colonies of *Penicillium* spp., 11 colonies of *Trichoderma* spp. and 3 colonies of *Gliocladium* spp.) were antagonistic. The growth of *Fusarium solani* and *Penicillium exigua* var. *exigua* was most strongly inhibited, since the total antagonistic effect was +219 and +208, respectively (Tab. 5). The total antagonistic effect of saprophytic fungi isolated from rhizosphere soil towards the studied pathogenic fungi was +1160 (Tab. 5).

Non-rhizosphere soil contained 182 fungal isolates characterised by antagonistic effect. They belonged to the genera: Penicillium – 119 isolates, Trichoderma – 47 isolates, Gliocladium roseum – 4 isolates, Torula herbarum – 5 isolates and Verticillium tenerum – 7 isolates. These antagonistic fungi were the most effective towards F. solani (the total antagonistic effect +711) (Tab. 6). The total antagonistic effect of fungi isolated from non-rhizosphere soil towards the studied phytopathogens was +3,827 (Tab. 6).

Activity of bacteria Bacillus spp. and Pseudomonas spp. isolated from rhizosphere of soybean towards pathogenic fungi

Genus of bacteria	Number of anta- gonistic	B. cii	nerea	F. culm	orum		sporum sp. ines	F. sc	olani .	P. ez va exiş	2000	P. irre	gulare	R. se	olani	S. scler	otiorum	Altogether effect of antagonistic
	isolates	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	activity
									19	96								
Bacillus spp.	2	+4	+8	+3	+6	+3	+6	+6	+12	+3	+6	+4	+8	+2	+4	+2	+4	+54
Pseudomonas spp.	4	0	0	0	0	+1	+4	+1	+4	+2	+8	+2	+8	0	0	+1	+4	+28
Total effect of antagonistic activity			+8		+6		+10		+16		+14		+16		+4		+8	+82
									19	97								
Bacillus spp.	7	+1	+7	+1	+7	+1	+7	+1	+7	+2	+14	+4	+28	+1	+7	+1	+7	+84
Pseudomonas spp.	9	+1	+9	0	0	+1	+9	+1	+9	+1	+9	+3	+27	0	0	0	0	+63
Total effect of antagonistic activity			+16		+7		+16		+16		+23		+55		+7		+7	+147
									19	98								
Bacillus spp.	10	+4,5	+45	+1	+10	+2	+20	+3	+30	+6	+60	+6	+60	0	0	+3	+30	+255
Pseudomonas spp.	16	+1	+16	+0,25	+4	+1	+16	+1	+16	+4	+64	+5,5	+88	+0,5	+8	+4	+64	+276
Total effect of antagonistic activity			+61		+14		+36		+46		+124		+148		+8		+94	+531
Altogether effect of antagonistic activity			+85		+27		+62		+78		+161		+219		+19		+109	+760

^{1 -} individual effect of antagonistic activity; 2 - total effect of antagonistic activity

Genus of bacteria	Number of anta- gonistic	B. ci	nerea	F. culr	norum	f.	sporum sp.	F. se	olani		xigua ar. gua	P. irre	gulare	R. s	olani	S. scler	otiorum	Altogether effect of antagonistic
	isolates	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	activity
									19	96								
Bacillus spp.	6	+1	+6	+1	+6	+1	+6	+1	+6	+2	+12	+1	+6	0	0	0	0	+42
Pseudomonas spp.	11	0	0	0	0	+1	+11	+1	+11	+1	+11	0	0	0	0	0	0	+33
Total effect of antagonistic activity			+6		+6		+17		+17		+23		+6		0		0	+75
									19	97								
Bacillus spp.	3	+2	+6	0	0	+2	+6	+2	+6	+1	+3	+1	+3	+1	+3	+1	+3	+30
Pseudomonas spp.	13	+2	+26	0	0	+2	+26	+1	+13	+2	+13	+1	+13	+1	+13	+1	+13	+117
Total effect of antagonistic activity			+32		0		+32		+19		+16		+16		+16		+16	+147
									19	98								
Bacillus spp.	9	+2	+18	+1	+9	+1	+9	0	0	+2	+18	+3	+27	+1	+9	+4	+36	+126
Pseudomonas spp.	10	+1	+10	0	0	+1	+10	0	0	+1	+10	+1	+10	0	0	0	0	+40
Total effect of antagonistic activity			+28		+9		+19		0		+28		+37		+9		+36	+166
Altogether effect of antagonistic activity			+66		+15		+68		+36		+67		+59		+25		+52	+388

^{1 -} individual effect of antagonistic activity; 2 - total effect of antagonistic activity

Activity of selected saprophitic fungi isolated from rhizosphere of soybean towards pathogenic fungi

Fungus species	Mean number of isolates in	В. с	nerea	F. culi	morum		sporum sp. ines	F. s	olani		xigua ar. gua	P. irre	egulare	R. s	olani	S. scler	otiorum
	1996-1998	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Gliocladium roseum	3	+6	+18	+4	+12	+5	+15	+8	+24	+8	+24	+7	+21	+3	+9	+2	+6
Penicillium brevi-compactum	14	+3	+42	+3	+42	+3	+42	+4	+56	+4	+56	+4	+56	-2	-28	-2	-28
Penicillium decumbens	7	+1	+7	+1	+7	+1	+7	+2	+14	+1	+7	+1	+7	+1	+7	0	0
Penicillium janthinellum	9	+1	+9	+1	+9	+1	+9	+2	+18	+1	+9	+1	+9	-4	-36	-4	-36
Penicillium meleagrinum Penicillium verrucosum	5	-2	-10	-1	-5	+1	+5	+1	+5	+2	+10	+1	+5	-2	-10	-2	-10
var. verrucosum	7	+1	+7	+1	+7	+2	+14	+2	+14	+2	+14	+1	+7	+1	+7	+1	+7
Trichoderma hamatum	2	+8	+16	+7	+14	+8	+16	+8	+16	+8	+16	+8	+16	+7	+14	+8	+16
Trichoderma koningii	2	+8	+16	+8	+16	+8	+16	+8	+16	+8	+16	+8	+16	+8	+16	+8	+16
Trichoderma pseudokoningii	7	+7	+49	+7	+49	+8	+56	+8	+56	+8	+56	+8	+56	+7	+49	+8	+56
Total effect																	
of antagonistic activity			+154		+151		+180		+219		+208		+193		+28		+27

 ^{1 -} individual effect of antagonistic activity
 2 - total effect of antagonistic activity

Activity of selected saprophitic fungi isolated from non-rhizosphere soil towards pathogenic fungi

Fungus species	Mean number of isolates in	В. с	inerea	F. culi	morum	f.	sporum sp. ines	F. s	olani	v	xigua ar. gua	P. irre	gulare	R. s	olani	S. scler	otiorui
	1996-1998	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Gliocladium roseum	4	+6	+24	+6	+24	+6	+24	+7	+28	+7	+28	+7	+28	+4	+16	+3	+12
Penicillium chrysogenum	34	+2	+68	+1	+34	+2	+68	+5	+170	+4	+136	+4	+136	-4	-136	-4	-136
Penicillium funiculosum	33	+1	+33	+1	+33	+1	+33	+1	+33	+1	+33	+1	+33	-3	-99	-2	-66
Penicillium nigricans	6	+1	+6	0	0	+1	+6	+2	+12	+1	+6	0	0	-4	-24	-3	-18
Penicillium purpurogenum	12	0	0	0	0	+2	+24	+2	+24	+1	+12	+1	+12	-1	-12	-1	-12
Penicillium roseo-purpureum	4	0	0	0	0	+1	+4	+3	+12	+2	+8	+1	+4	+1	+4	+1	+4
Penicillium verrucosum																	
var. verrucosum	30	+1	+30	+1	+30	+2	+60	+1	+30	+1	+30	+1	+30	0	0	0	0
Torula herbarum	5	0	0	0	0	+1	+5	+1	+5	0	0	0	0	-3	-15	-3	-15
Trichoderma hamatum	2	+8	+16	+6	+12	+8	+16	+8	+16	+8	+16	+8	+16	+6	+12	+7	+14
Trichoderma harzianum	2	+8	+16	+8	+16	+8	+16	+8	+16	+8	+16	+8	+16	+7	+14	+8	+16
Trichoderma koningii	8	+7	+56	+7	+56	+8	+64	+8	+64	+8	+64	+8	+64	+7	+56	+8	+64
Trichoderma pseudokoningii	23	+8	+184	+7	+161	+8	+184	+8	+184	+8	+184	+8	+184	+7	+161	+7	+161
Trichoderma viride	12	+8	+96	+8	+96	+8	+96	+8	+96	+8	+96	+8	+96	+8	+96	+8	+96
Verticillium tenerum	7	+1	+7	+1	+7	+2	+14	+3	+21	+3	+21	+2	+14	+1	+7	+2	+14
Total effect																	
of antagonistic activity			+536		+469		+614		+711		+650		+633		+80		+134

^{1 -} individual effect of antagonistic activity
2 - total effect of antagonistic activity

IV. DISCUSSION

The results pointed out at different composition of bacterial and fungal communities in rhizosphere of soybean and non – rhizosphere soil. Rhizosphere soil generally contained more bacteria and fungi than non-rhizosphere soil. The increase of the number of microorganisms in rhizosphere soil was most probably due to the effect of root exudates of this plant. Extensive information given in literature relates the effect of root exudates and crop residues to the qualitative and quantitative composition of microorganisms (Funck-Jensen and Hockenhull 1984; Huber and Watson 1970; Rovira 1965; 1969; Schoruvitz and Zeigler 1989). According to numerous researchers (Funck-Jensen and Hockenhull 1984; Pięta 1988; Piotrowski and Milczak 1982; Sytnik et al. 1977) soluble sugars and acid aminoacids occurring in root exudates stimulate the growth of pathogenic fungi, whereas phenolic compounds, aromatic and alkaline aminoacids inhibit it.

Results of microbiological analysis showed that soybean rhizosphere contained almost three times more bacterial colonies from *Pseudomonas* spp. than in non-rhizosphere soil. According to Keel (1992) and Weller (1988) bacteria of *Pseudomonas* spp. are capable of active colonisation of plant roots, thanks to what they actively compete with pathogens for nutrients available in root exudates being a biological factor of biological control of diseases. Besides, a greater number of pathogenic fungi in rhizosphere of soybean cultivated in monoculture as compared to non-rhizosphere soil, can be explained by smaller numbers of bacteria and fungi showing antagonistic effect. On the other hand, a smaller proportion of pathogenic fungi in non-rhizosphere soil could have been caused by more numerous occurrence of isolates of antagonistic bacteria (*Bacillus* spp., *Pseudomonas* spp.) and fungi (*Gliocladium* spp., *Penicillium* spp., *Trichoderma* spp.) in that environment. This fact is confirmed by numerous authors (Łacicowa and Pięta 1985a; 1985b; 1989; Papavizas 1985) who indicate that fungi of *Gliocladium* spp. and *Trichoderma* spp. can significantly inhibit soil-borne phytopathogens.

V. CONCLUSIONS

- The mean number of bacteria and fungi in rhizosphere soil of soybean cultivated in monoculture was higher than in non-rhizosphere soil. Almost three times more bacterial colonies of *Pseudomonas* genus and fungal colonies were obtained from rhizosphere of soybean than from non-rhizosphere soil.
- 2. Antagonistic isolates of bacteria (Bacillus spp., Pseudomonas spp.) and fungi (Gliocladium spp., Penicillium spp., Trichoderma spp.) prevailed in non-rhizosphere soil.
- 3. The lower number of antagonistic isolates of bacteria and fungi in rhizosphere of soybean cultivated in monoculture can testify to little biological activity of microorganisms, which contributed to a worse phytosanitary condition of the soil.

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KSZTAŁTOWANIE SIĘ ZBIOROWISK BAKTERII I GRZYBÓW W RYZOSFERZE SOI (*GLYCINE MAX* (L.) MERRILL) I ICH ANTAGONIZM WZGLĘDEM FITOPATOGENÓW

STRESZCZENIE

Przedmiotem badań przeprowadzonych w latach 1996-1998 była gleba ryzosferowa soi uprawianej w monokulturze oraz gleba pozaryzosferowa. Analiza mikrobiologiczna wykazała, że w 1 g s.m. gleby ryzosferowej średnia liczebność bakterii oraz grzybów była większa, aniżeli w glebie pozaryzosferowej. W próbach badanych gleb liczebność bakterii z rodzaju *Bacillus* była zbliżona. W przypadku bakterii z rodzaju *Pseudomonas* ogólna ich liczebność była prawie trzykrotnie większa w ryzosferze soi, aniżeli w glebie pozaryzosferowej.

Badania laboratoryjne wykazały, że znacznie więcej antagonistycznych Bacillus spp., Pseudomonas spp., Gliocladium spp., Penicillium spp. i Trichoderma spp. wystapiło w glebie pozaryzosferowej, aniżeli w ryzosferze soi. Mniejsza liczebność antagonistycznych bakterii i grzybów w ryzosferze soi uprawianej w monokulturze może świadczyć o małej aktywności biologicznej mikroorganizmów, co przyczynia się do pogorszenia fitosanitarnego stanu gleby.