Polish Journal of Microbiology
2004, Vol. 53, No 3, 175–182

An Attempt to Protect Winter Wheat Against *Fusarium culmorum* by the Use of Rhizobacteria *Pseudomonas fluorescens* and *Bacillus mycoides*

JANUSZ CZABAN*, ANDRZEJ KSIĘŻNIAK and ANDRZEJ PERZYŃSKI

Department of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation
Puławy, Poland

Received in revised form 20 May 2004

A b s t r a c t

Inoculation of wheat seeds with two strains of *Pseudomonas fluorescens* (III107 and II21) and two strains of *Bacillus mycoides* (JC192 and K184) isolated from winter wheat roots, as well as with one strain of *P. fluorescens* (ID13) isolated from oat roots, reduced the negative influence of *Fusarium culmorum* on winter wheat in a 28 day pot experiment. The bacterial strains (especially III107 and chitinolytic JC192) markedly increased the plant seedlings emergence and the plant biomass (the shoots weight up to 252%, and the roots weight up to 229%) in comparison to the experimental series with *F. culmorum* alone. Also in a microplot experiment the yield of grain and straw of winter wheat, inoculated with the bacterial strains (especially JC192 and III107) and growing in soil contaminated with *F. culmorum*, was higher (the grain yield up to 120%, and the straw yield up to 139%) than in a series with *F. culmorum* alone (100%). In both experiments the highly cyanogenic strain II21 was least effective. A linear correlation ($r = 0.926$) and a rank Spearman's correlation ($r_{Sp} = 0.991$), both significant at $p < 0.01$, between the weight of plant biomass in the pot experiment and the yield of whole shoots in the microplot experiment were found. It suggests that the same mechanisms worked in both experiments, although with different intensity.

K e y w o r d s: winter wheat protection *F. culmorum*, rizobacteria

Introduction

The fungi belonging to genus *Fusarium* are ubiquitous in soils on which wheat is grown. They are particulary numerous and active in the rhizosphere region of wheat, especially on its roots (Čatska *et al*., 1960; Peterson, 1958; Pląskowska, 1997). The genus *Fusarium* includes many phytopathogenic varieties. Among those *F. culmorum*, which causes foot and root rot diseases, is particularly harmful to the plants (Bandurska *et al.*, 1994; Cook, 1992; Jenkins *et al*., 1988).

The aim to reduce the use of pesticides, because of their harmful side-effect to the environment and man, has resulted in increasing the interest in the use of biological specimens of plant protection (Singh and Prithiviraj, 1997; Uoti, 1995). Microorganisms that can grow in the rhizosphere are ideal to be used as biocontrol agents, since the rhizosphere provides the front-line defense for roots against the attack by pathogens and they can directly stimulate crop growth (Bowen and Rovira, 1999; Weller, 1988).

Fluorescent *Pseudomonas* and *Bacillus* are prime candidates for biological control because of their ecological and physiological characteristics. There is good evidence from soil experiments confirming the involvement of siderophores, different antibiotics and antifungal volatiles produced by both bacterial genera in the biocontrol of plant root diseases (Fiddamann and Rosall, 1993; O'Sullivan and O'Gara, 1992; Święcicka and Hauschild, 1996; Weller, 1988). There is a number of reports from many countries around the world on the potential of these groups of bacteria as biological control agents against fungal pathogens (Bowen and Rovira, 1999; Jaroszuk-Ściseł and Kurek, 2001; Kim *et al*., 1997; Mariano *et al.*, 1997; Singh and Prithiviraj, 1997; Tsuchiya, 1997; Weller, 1988; Wenhua and Hetong, 1997). Fluorescent pseudomonads were also involved in natural suppressiveness of soils to fusarium wilts and take-all (Wong and Baker, 1984).

* Corresponding author: Janusz Czaban, Department of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation, 8 Czartoryskich St., 24-100 Puławy, Poland; e-mail: czaban@iung.pulawy.pl

In the previous paper (Czaban *et. al.*, 2004) we studied the abilities of four plant growth promoting rhizobacterial (PGPR) strains of *Pseudomonas fluorescens* and *Bacillus mycoides* isolated from rhizosphere of winter wheat to protect winter wheat against *Gaeumannomyces graminis* var. *tritici*.

The purpose of the present work is a preliminary evaluation of the potential of these strains of *P. fluorescens* and *B. mycoides* to control *Fusarium culmorum* (W.G. Smith) Sacc. on winter wheat.

Experimental

Materials and Methods

Inoculum of the pathogen. The pathogenic fungus, *Fusarium culmorum* was obtained from Department of Plant Pathology, Agricultural University of Lublin. Inoculum of *F. culmorum* was prepared by growing the fungus on autoclaved oat grains. In the pot experiment the inoculum was mixed with the whole soil in the amount of 0.5% (w/w). In the microplot experiment the inoculum was mixed with the upper (20 cm) layer of the soil (40 g of the inoculum per a 0.07 m^2 microplot).

The source of the rhizobacterial strains. The rhizobacterial strains (*P. fluorescens* strains III107 and II21, and *B. mycoides* – JC192 and K184) were isolated from the winter wheat roots according to the method described by Kobus *et al.* (1993). *P. fluorescens* – ID13 was isolated from oat roots (Paszkowski, 2001).

Metabolic activities of the rhizobacteria:

Antagonistic activity of the isolated strains against *F. culmorum* was determined on Petri dishes (9 cm diameter) with Difco potato-dextrose agar (PDA). The plugs of agar with *F. culmorum* (4 mm diameter) were placed in the middle of the plates, and the tested bacteria were inoculated at the distance of 3.5 cm from the middle of the plates. The zones of *F. culmorum* inhibition were measured after 10 days of the incubation at 28^0C. Chitinolytic activity was determined in tubes with agar medium (Strzelczyk *et al.*, 1990) containing 0.5% (w/v) of colloidal chitin. The bacteria were inoculated at the top of the agar medium. The depth of clear zone indicating the rate of chitin hydrolysis was measured after 28 days of incubation at 28°C. Ability to synthesize Fe^{+3} complexing compounds was studied in mineral nutrient medium containing 2.5% (v/v) of glycerol (Księżniak and Kobus, 1993). After 21 days of incubation at 28°C the cultures were centrifugated and 0.3 ml of 6% (w/v) $FeCl_3.6H_2O$ in 0.1 N HCl were added to 1 ml of culture supernatants. After 1 h, OD_{520} of the mixture was measured and the concentration of Fe^{+3} complexing compounds was estimated from the calibration curve, prepared for a synthetic iron chelator-deferoxamine mesylate USP (Desferal – CIBA-GEIGY) after its reaction with $FeCl_3$ (Jaroszuk-Ściseł and Kurek, 2001). The capacity of the bacterial strains for HCN production was assayed in sealed test tubes with slants of solid King's medium B containing 4.4 g glycine l^{-1} (Bakker and Schippers, 1987) and 20 μM $FeCl_3$ (Voisard *et al.*, 1991) and a piece of filter paper impregnated with 0.5% picric acid and 2% sodium carbonate (Bakker and Schippers, 1987). The microbial production of HCN was indicated by color change (from yellow to orange-brown) of the filter paper after 4 days of incubation at 28°C.

Preparation of the rhizobacterial inocula. The inocula of the strains were prepared by rinsing off the bacterial cells after 48 h growth on solid King's medium B, with 1% (w/v) CM-cellulose (15 ml per 1 Petri dish). The seeds of wheat were soaked in the bacterial suspensions (10^9 CFU/ml) for 30 min before sowing. The seeds from the series non-inoculated with the bacteria were soaked for 30 min in 1% solution of CM-cellulose after rinsing off the plates containing the non-inoculated nutrient medium.

Characteristics of the soils. In the pot experiment a brown soil developed from sandy slight loam (pH_{KCl} 6.9; organic C content 1.18%; total N 0.09%; CEC 15.82 meq $100g^{-1}$; clay 9%; silt 19% and sand 72%), taken from a garden at IUNG in Puławy was used. The soil, sieved through a 2 mm screen, was placed in the pots (1 kg per pot). For microplot experiments 30 cm diameter polyvinyl cylinders were sunk into a brown soil developed from sandy loam (pH_{KCl} 6.0; organic C content 1.13%; total N 0.11%; CEC, 16.35 meq $100g^{-1}$; clay 17%; silt 33% and sand 50%).

Plant tests. The pot experiment was conducted in April in a glasshouse with additional electric light. In this experiment 20 seeds of winter wheat cv. Gama per one pot (containing 1 kg of the soil) were sown. The number of emerged plants was determined on 5^{th}, 6^{th}, 7^{th} and 19^{th} day after sowing. The number of plants was reduced to 8 per pot on the 19^{th} day after sowing. The plants were harvested after 28 days of growth. Then the dry weights of the plant shoots and roots, and the tiller numbers were determined. These measurements were done with 4 replicates.

In the microplot experiment the soil was enriched with mineral fertilizers corresponding to 70 kg N, 20 kg P, 45 kg K per ha. Winter wheat cv. Almari was sown in the amount of 30 seeds per one microplot (0.07 m^2 area). The plants were grown from 8.10 till 6.08. After the harvest, the yield of the grain, the straw and the whole shoots were determined. All determinations were done with 4 replicates.

Statistical evaluations. All the data (in 4 replicates) were subjected to analysis of variance and separated with Student's t-test ($p = 0.05$ for the glasshouse experiment and $p = 0.1$ for the microplot experiments). For the estimation of examined relationships linear and rank (Spearman's) analyses were used. Together with correlation coefficients (r and r_{Sp}), probability (p) and the pair numbers (n) are presented.

Results

Table I presents characteristics of the rhizobacteria used in the studies. All tested strains had capabilities for *F. culmorum* growth inhibition on PDA nutrient medium. *B. mycoides* strain JC192 was able to degradation of chitin, and *P. fluorescens* strain II21 was highly cyanogenic. All strains of *P. fluorescens* (especially strain ID13) produced high amounts of Fe^{3+} complexing compounds.

Table I
Characteristics of bacterial strains chosen to vegetation experiments with *Fusarium culmorum*

Bacterial species	Strain symbol	Inhibition of *F. culmorum* growth (inhibition zone in mm)	Fe^{3+} chelating compounds (μM Desferal l^{-1})	Chitinase synthesis (clearing zone in mm)	HCN synthesis (Scale 0–5)
Pseudomonas fluorescens	III107	9	125	0	0
	II21	4	190	0	5
	ID13[P]	8.5	442	0	0
Bacillus mycoides	JC192	0*	12	25	0
	K184	0*	18	0	0

* – the lack of the inhibition zone. The growth of *F. culmorum* was very strongly inhibited by intensively expanding bacteria.
[P] – the results concerning strain ID13 were taken from Paszkowski W.L. Microflora of oat rhizosphere and their effect on development and healthiness of plants. (in Polish), PhD thesis, IUNG Puławy, Poland (1993) and Paszkowski (1997).

In the pot experiment the infection of the soil with *F. culmorum* strongly inhibited the emergence of the wheat seedlings (Fig. 1) and decreased the weight of wheat shoots and roots (Table II). Inoculation of the wheat seeds with the bacterial strains reduced the negative effect of *F.c.* on the emergence of wheat seedlings (Fig. 1) and on the plant weight (Table II), although the protection of the emerging seedlings was incomplete. The best protection against *F. culmorum* was obtained in the cases of *B. mycoides* strain JC192 and *P. fluorescens* strains III107 and ID13, whereas the worst effect was obtained in the case of *P. fluorescens* strain II21 (Fig.1 and Table II). Except for cyanogenic *P. fluorescens* strain II21, dual inoculation of soil with *F.c.* and seeds with one of the bacterial strains gave higher wheat biomass (especially shoots) than in the non-inoculated control (Table II).

The infection of the soil with *F. culmorum* increased the shoot weight to root weight ratio (S:R). The inoculation of the wheat seeds with the rhizobacteria intensified (especially with *P. fluorescens* strains) this increase (Table II). The highest value of S:R was obtained in the case of cyanogenic strain II21. Dual inoculation with *F. culmorum* and the rhizobacteria increased the number of shoots in comparison to the non-inoculated control (Table II), but this increase was statistically significant (at $p = 0.05$) only in the cases of the experimental series with strains JC192 and II21. In the microplot experiment the infection of the soil

Table II
Protection of winter wheat against deleterious effect of *F. culmorum* by *P. fluorescens* strains III107B4 and II21y4, and *B. mycoides* strains JC192 and K184 in the pot experiment

Treatment	Dry weights (in mg per pot) of:				Shoot weight to root weight ratio (S/R)		Tiller numbers per pot	
	shoots		roots					
	(% of C)	(% of *F. culmorum*)	(% of C)	(% of *F. culmorum*)	(% of C)	(% of *F. culmorum*)	(% of C)	(% of *F. culmorum*)
Control (C)	**939 b*** (100)		**873 b** (100)		**1.08 a** (100)		**8.0 a** (100)	
Fusarium culmorum	**528 a** (56)	(100)	**439 a** (50)	(100)	**1.20 b** (112)	(100)	**8.3 a** (103)	(100)
F. culmorum + III107B4	**1239 cd** (132)	(234)	**881 bc** (101)	(201)	**1.41 c** (131)	(117)	**8.5 ab** (106)	(103)
F. culmorum + II21y2	**800 a** (85)	(151)	**549 a** (63)	(125)	**1.46 c** (136)	(121)	**9.3 bc** (116)	(112)
F. culmorum + ID13	**1215 c** (129)	(230)	**874 b** (100)	(199)	**1.40 c** (130)	(116)	**8.8 a** (109)	(106)
F. culmorum + JC192	**1331 c** (142)	(252)	**1004 c** (115)	(229)	**1.33 c** (123)	(110)	**10.8 c** (134)	(130)
F. culmorum + K184	**1080 bd** (115)	(204)	**896 bc** (103)	(204)	**1.21 b** (112)	(100)	**8.8 ab** (109)	(106)

* – the means in each column with different letters are significantly different at $p = 0.05$.

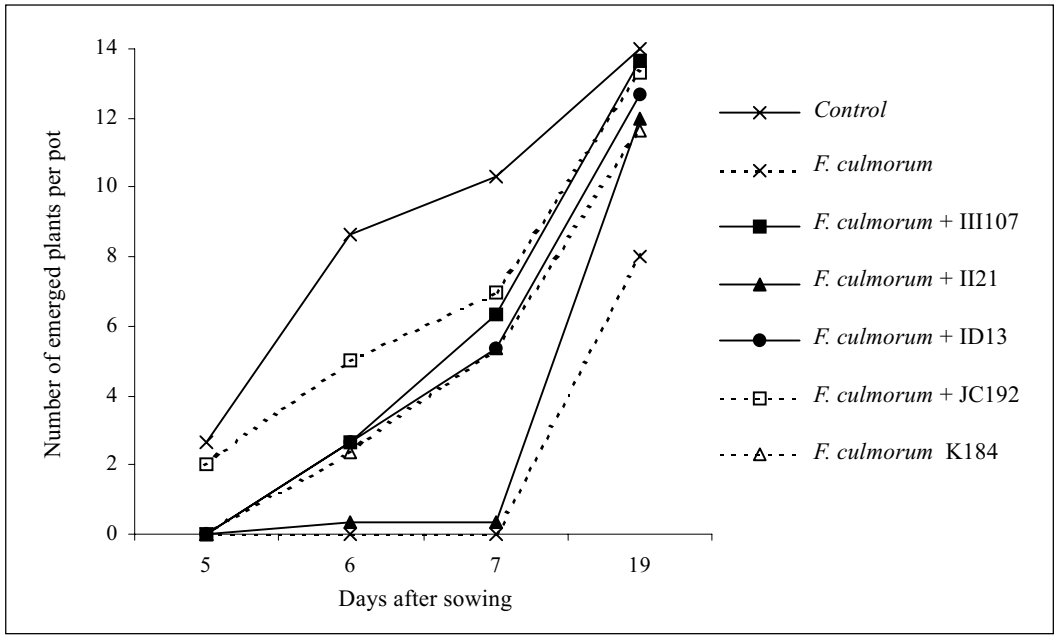

Fig. 1. The influence of *Fusarium culmorum* and the rhizobacteria (*Pseudomonas fluorescens* strains III107, II21, ID13 and *Bacillus mycoides* strains JC192, K184) on the emergence of winter wheat seedlings in the pot experiment

with *F. culmorum* (in series with seeds not inoculated with bacteria) slightly reduced the yield of winter wheat grain and practically did not change the yield of straw.

The combined inoculation (of soil with *F. culmorum* and seeds with the bacterial strains) increased (except strain II21) the yield of straw of the winter wheat in comparison to the non-inoculated control series and the series with *F. culmorum* alone, but the statistically significant (at p = 0.1) effect was obtained only in the cases of both strains of *B. mycoides* (Table III). The positive influence of the bacterial strains (especially III107, JC192 and ID13) on the yield of wheat grain in the presence of *F.c.* was also observed, but all the differences between the means were statistically (at p = 0.1) insignificant (Table III).

Table III

Influence of *F. culmorum* and the rhizobacteria (*P. fluorescens* strains III107B4, II21y4, ID13 and *B. mycoides* strains JC192, K184) on the yield of winter wheat grain, straw and whole shoots in the microplot experiment

Experimental series	Yield of grain (in g)		Yield of straw without ears (in g)		Yield of whole shoots (in g)	
	(% of C)	(% of *F. culmorum*)	(% of C)	(% of *F. culmorum*)	(% of C)	(% of *F. culmorum*)
Control (C)	**47.8 a*** (100)		**31.8 a** (100)		**90.0 a** (100)	
Fusarium culmorum	**43.0 a** (90)	(100)	**30.5 ab** (96)	(100)	**83.7 a** (93)	(100)
F. culmorum + III107	**51.4 a** (107)	(120)	**34.8 abd** (109)	(114)	**96.8 a** (108)	(116)
F. culmorum + II21	**45.6 a** (95)	(106)	**29.3 ac** (92)	(96)	**84.5 a** (94)	(101)
F. culmorum + ID13	**48.9 a** (102)	(114)	**35.0 abd** (110)	(115)	**94.5 a** (105)	(113)
F. culmorum + JC192	**50.4 a** (105)	(117)	**42.3 cd** (133)	(139)	**105.3 a** (117)	(126)
F. culmorum + K184	**46.6 a** (97)	(108)	**37.8 bd** (119)	(124)	**94.5 a** (105)	(113)

* – the means in each column with different letters are significantly different at p = 0.1

Table IV
A linear correlation between the weights of winter wheat in the pot experiment and the yield of winter wheat in the microplot experiment

The pot experiment			The microplot experiment		
Weight of shoots	Weight of roots	Weight of whole plants	Yield of grain	Yield of straw	Yield of whole shoots
1			0.931**	0.795*	0.918**
	1		0.854*	0.826*	0.907**
		1	0.912**	0.820*	0.926**

**, * – significant at $p \leq 0.01$ and $p \leq 0.05$, respectively (n = 7)

Table V
The weight of whole plants in the pot experiment and the yield of whole shoots in the microplot experiment presented as percentage of the value of series with *F. culmorum* alone and as ranks

Experimental series	Weight of whole plants in the pot experiment		Yield of whole shoots in the microplot experiment	
	in % of *F. culmorum*	ranks	in % of *F. culmorum*	ranks
F. culmorum + JC192	241	7	126	7
F. culmorum + III107	219	6	116	6
F. culmorum + ID13	216	5	113	4.5
F. culmorum + K184	204	4	113	4.5
Control	184	3	108	3
F. culmorum + II21	146	2	101	2
F. culmorum	967 mg = 100%	1	83.7 g = 100%	1

A very strong linear correlation was found between the weight of plant biomass in the pot experiment and the yield of winter wheat grain, straw and whole shoots in the microplot experiment (Table IV). Table V shows that arrangement of experimental series from the highest to the lowest value of winter wheat biomass is almost identical in both the pot and the plot experiments, so the Spearman's rank correlation coefficient between the weight of whole plants in the pot experiment and the yield of whole shoots in the microplot experiment is very high ($r_{Sp} = 0.991$).

Discussion

The four PGPR strains (*P. fluorescens* – III107 and II21, and *B. mycoides* – JC192 and K184), isolated from winter wheat roots and chosen to the present studies, were previously tested as biocontrol agents to protect winter wheat against *Gaeumannomyces graminis* var. *tritici* with promising results (Czaban *et al.*, 2004). The best protection of winter wheat against take-all was obtained in the cases of strains III107 and JC192 (Czaban *et al.*, 2004). Chitinolytic ability of *B. mycoides* strain JC192 was probably one of the main bacterial features controlling take-all. Chitinolytic ability of this rhizobacterial strain may also have played a role in control of *F. culmorum* in our pot and microplot experiments. Paszkowski (1998b) found a significant negative correlation between the incidence of *Fusarium* on oat roots and the number of chitinolytic microorganisms. Also, many chitinolytic microorganisms became evidently effective biocontrol agents against fungal pathogens inclusive of various *Fusarium* strains (Aziz, 2002; Fridlender *et al.*, 1993; Toyota *et al.*, 1994; Tsuchiya, 1997; van Loon, 1998).

The capability for Fe^{3+} chelators production could be one of the main features of *P. fluorescens* strains in wheat protection against *F. culmorum* This bacterial feature was very important in biocontrol of various fusarioses (Jaroszuk and Kurek, 1998 and Lemanceau *et al.*, 1992). *P. fluorescens* strain ID13 was an especially strong producer of Fe^{3+} chelators (Paszkowski, 1997). This strain, isolated from oat roots by Paszkowski, was used by us as a reference for evaluation of the effectiveness of our bacterial strains,

isolated from winter wheat roots, for protection of the plants against *F. culmorum.* Strain ID13 was very efficient in protection of various cereals against *F. culmorum* (Paszkowski, 2001). Paszkowski *et al.* (2001) also found that ID13 introduced on oat seeds significantly decreased the propagule number of *F. culmorum* in the oat rhizosphere.

P. fluorescens strain II21 produced high amounts of HCN. Although this feature of bacteria helped to suppress diseases caused by various fungal pathogens *e.g. Thielaviopsis basicola* on tobacco, *Pythium ultimum* on cucumber and cress, *Rhizoctonia solani* on cotton, *Gaeumannomyces graminis, Septoria tritici* and *Puccinia graminis* on wheat, *Colletotrichum orbiculare* on cucumber (Défago *et al.*, 1990; Hornby, 1998; Van Loon, 1998; Paszkowski, 1998a; Voisard *et al.*, 1989), it may not be useful in control of diseases caused by *F. culmorum.* Paszkowski (1998a) found that *F. culmorum* (contrary to other pathogens *e.g. Gaeumannomyces graminis* var. *tritici*) was highly resistant to HCN, and this resistance might have been connected with the use of cyanide as a source of nitrogen by this strain. Nevertheless, we decided to check II21y4 as a biocontrol agent of *F. culmorum* in our studies. As expected, this rhizobacterial strain proved the worst (among the studied strains) biocontrol agent against *F. culmorum*, so probably its cyanogenic ability did not play a positive role in winter wheat protection against the fusariosis. In the pot experiment strain II21 increased shoot weight to root weight ratio by the highest degree. It is probably caused by strong cyanogenic activity of these bacteria. Czaban *et al.* (2001) found that cyanogenic strains of fluorescent pseudomonads significantly decreased, in comparison with non-cyanogenic strains, the weight of wheat roots.

In the pot experiment both the negative influence of the pathogen on wheat growth and the protective power of the bacterial strains were very distinctly visible, contrary to the results of the microplot experiment. Perhaps the changeable natural conditions of the microplot experiment were not as favourable to the growth of both *F. culmorum* and the rhizobacteria as the conditions of the pot experiment. The pot experiment was conducted in a heated glasshouse with mean temperature about 20°C. Additionally, strong insolation on some days raised the ambient temperature to about 25°C. Such conditions were probably very favourable to *F. culmorum* and the rhizobacteria and they might have also caused the winter wheat plants to be more sensitive to the microbial action. Jaroszuk and Kurek (1998) found that negative influence of different *Fusarium* species (including *F. culmorum*) on rye was stronger in higher temperatures (they tested 10, 15 and 20°C) and that the stronger effect at higher temperatures was connected with the higher propagule number of the pathogens. They also found that ability of pseudomonad strain 26 to protect rye against various fusaria (including *F. culmorum*) in soil developed from sand, was also higher in higher temperatures. In higher temperatures the inoculation of rye seedlings with pseudomonad strain 26 decreased the number of *F. culmorum* and *F. oxysporum* in the rye rhizosphere to a higher extent. Another explanation is also possible. Winter wheat cv. Almari could be less sensitive to the microbial action than cv. Gama, used in the pot experiment.

The changeable conditions of the field studies caused the results on biocontrol to be a little reproducible (Wenhua and Hetong, 1997). Two-growing season study of Jaroszuk-Ściseł and Kurek (2001) on biocontrol of *F. culmorum* on rye by various strains of *P. fluorescens* are a good example of such case. In the first growing season *F. culmorum* decreased the yield of rye grain and straw by 26% and 21%, respectively, but in the second growing season only by 14% and 4% (the degree of the latter rye yield decrease is similar to that obtained in our microplot experiment). Moreover, the effectiveness of the bacterial strains used were very changeable in these two growing seasons.

Although in the microplot experiment the effect of the combined inoculation (of soil with *F. culmorum* and wheat seeds with the bacterial strains) on the yield of straw and grain of the winter wheat was statistically insignificant at $p = 0.05$ (and at $p = 0.1$, except for the influence of both *B. mycoides* strains on the straw yield) due to a great dispersion of the results, very strong linear and rank correlation between the weight of plant biomass in the pot experiment and the yield of winter wheat biomass in the microplot experiment suggest that in both experiments the same mechanisms worked however with different intensity, and that the results obtained in the microplot experiment are not fortuitous.

In both experiments dual inoculation of soil with *F. culmorum* and wheat seeds with the bacterial strains (except strain II21) increased the plant weight in comparison to the values of the noninoculated control. Perhaps, this phenomenon may be explained by the enrichment of the soil with some nutrients available to plants from soil substances and autoclaved oat grain, used as a carrier and a nutrient medium for *F. culmorum*, transformed by the microorganisms. Another explanation is also possible. Synergistic influence of various biologically active substances produced by the pathogen and the rhizobacteria is very probable. Significant changes of tiller numbers and shoot to root ratio in the pot experiment suggest the production of some biologically active substances by the microorganisms. Similar regularities concerning the common influ-

ence of *F. culmorum* and *P. fluorescens* strains are visible on the basis of the results presented by Jaroszuk-Ściseł and Kurek (2001). The yield of rye grain and straw as well as the number of ears were higher in the case of dual inoculation with *P. fluorescens* strain 26 (alone or in mixture with other bacterial strains) and *F. culmorum* in comparison to series with the only bacterial inoculation or with *F. culmorum* alone (and higher than the values of noninoculated control in one of two growing seasons). Moreover, Jaroszuk in her PhD thesis (Jaroszuk J., 1997. Rhizosphere microflora active in protection of rye against fusariosis (in Polish). UMCS Lublin, Poland) found that combined inoculation of rye seedlings with *F. culmorum* and some *Trichoderma* strains (or their culture filtrates) gave higher biomass of rye shoots and roots than the noninoculated control. In the previous studies (Czaban *et al.*, 2004) we observed that winter wheat seeds inoculation with *P. fluorescens* strains III107 and II21 as well as with *B. mycoides* strains JC192 and K184 intensified the negative effect of *Gaeumannomyces graminis* var. *tritici* on the winter wheat seedlings emergence until the 10th day of the incubation, although during this period, the same rhizobacterial strains stimulated very strongly the emergence of the seedlings in the soil not infested with the pathogen.

Among the studied rhizobacterial strains, bacterization of winter wheat seeds with *P. fluorescent* strain III107 and *B. mycoides* strain JC192 increased the weight of the winter wheat biomass in both experiments at the highest rate in comparison to the series with *F. culmorum* alone. On the basis of the results of the pot and the microplot experiments we may conclude that these two bacterial strains seem to be eligible candidates for biological *F. culmorum* control agents (with similar ability to *P. fluorescens* strain ID13), but their *F. culmorum* biocontrol ability should be confirmed in further experiments with various strains of *F. culmorum*, especially in the field conditions.

Literature

Aziz N.H. 2002. The role of chitinase of *Serratia marcescens* in controlling the production of zearalenone by *Fusarium graminearum*. *Acta Microbiol. Pol.* **51**: 131–137.

Bakker A.W. and B. Schippers. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp-mediated plant growth-stimulation. *Soil Biol. Biochem.* **19**: 451–457.

Bandurska H., J. Chełkowski and H. Wiśniewska. 1994. Free proline accumulation in wheat seedlings influenced by *Fusarium culmorum* infection and the pathogen metabolite deoxynivalenol. *Acta Physiol. Plant.* **16**: 111–116.

Bowen G.D. and A.D. Rovira. 1999. The rhizosphere and its management to improve plant growth. *Adv. Agron.* **66**: 1–102.

Cook R.J. 1992. Wheat root health management and environmental concern. *Can. J. Plant Pathol.* **14**: 76–85.

Czaban J., W. Paszkowski and J. Kobus. 2001. Ability of *Pseudomonas* rhizobacteria to produce HCN and their influence on winter wheat growth. *Allelopathy J.* **8**: 260.

Czaban J., A. Księżniak, B. Wróblewska and W.L. Paszkowski. 2004. An attempt to protect winter wheat against *Gaeumannomyces graminis* var. *tritici* by using rhizobacteria *Pseudomonas fluorescens* and *Bacillus mycoides*. *Pol. J. Microbiol.* **53**: 101–110.

Čatska V., J. Macura and K. Vágnerová. 1960. Rhizosphere microflora of wheat. III. Fungal flora of wheat rhizosphere. *Folia Microbiol.* **5**: 320–330.

Défago G., C. Keel, M. Maurhofer, P. Sacherer, B. Wüthrich, V. Gaia, J. Laville and D. Haas. 1990. HCN as a signal in plant-microbe interactions. p. 199–200. In: C. Keel, B. Koller, G. Défago (eds), Plant Growth-promoting Rhizobacteria. Progress and Prospects. Interlaken, Switzerland.

Fiddaman P.J. and S. Rosall. 1993. The production of antifungal volatiles by *Bacillus subtilis*. *J. Appl. Bacteriol.* **74**: 119–126.

Fridlender M., J. Inbar and I. Chet. 1993. Biological control of soilborne plant pathogens by a β-1,3 glucanase-producing *Pseudomonas cepacia*. *Soil Biol. Biochem.* **25**: 1211–1221.

Hornby D. 1998. Take-all disease of cereals. A regional perspective. CAB INTERNATIONAL Oxon, New York.

Jaroszuk J. and E. Kurek. 1998. Siderophore mediated fusarioses biocontrol by *Pseudomonas* 26 strain under different environmental conditions. p. 25–33. In: Orlikowski and Cz. Skrzypczak (eds), Biological Agents and their Effectiveness in the Control of Plant Pathogens. Section for Biological Control of Plant Diseases of the Polish Phytopathological Society, Research Institute of Pomology and Floriculture, Skierniewice, Poland.

Jaroszuk-Ściseł J. and E. Kurek. 2001. Biocontrol of rye fusariosis by *Pseudomonas fluorescens* under field conditions. *Bull. Pol. Acad. Sci. Biol. Sci.* **49**: 275–285.

Jenkins J.E.E., W.S. Clark and A.E. Buckle. 1988. *Fusarium* diseases of cereals. *HGCA Res. Rev.* **4**: 89.

Kim D.-S., R.J. Cook and D.M. Weller. 1997. *Bacillus* sp. L324-92 for biological control of tree root diseases of wheat grown with reduced tillage. *Phytopathology* **87**: 551–558.

Kobus J., J. Czaban, A. Gajda, D. Masiak and A. Księżniak. 1993. Wheat rhizosphere microflora and its effect on plant nutrition and some pathogenic fungi. part I. Changes of rhizobacterial populations with development of winter wheat. *Roczn. Glebozn.* **44**: 45:53.

Księżniak A. and J. Kobus. 1993. The participation of the rhizosphere microflora of wheat, barley and oat in the siderophore production. (In Polish with English summary) *Pam. Puł.* **102**: 77–90.

Lemanceau P., P.A.H.M. Bakker, W.J. DeKogel, C. Alabouvette and B. Schippers. 1992. Effect of Pseudobactin 358 production by *Pseudomonas putida* WCS358 on suppression of *Fusarium wilt of carnations by non-pathogenic*

Fusarium oxysporum Fo47. *Appl. Environ. Microbiol.* **59**: 74–82.

Mariano R.L.R., S.J. Michereff, E.B. Silveira, S.M.P. Assis and A. Reis. 1997. Plant growth-promoting rhizobacteria in Brazil. p. 22–29. In: A. Ogoshi, K. Kobayashi, Y. Homma, F. Kodama, N. Kondo, S. Akino (eds), Plant Growth-Promoting Rhizobacteria. Present Status and Future Prospects. Sapporo, Japan.

O'Sullivan D. and F. O'Gara. 1992. Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. *Microbiol. Rev.* **56**: 662–676.

Paszkowski W.L. 1997. Characteristics of *Pseudomonas fluorescens* Id13 strain and its effect on cereal yields. p. 201–204. In: A. Lagauskas, D. Bridziuviene, J. Repeckiene (eds), Ecological effects of microorganism action. Vilnius, Lithuania.

Paszkowski W.L. 1998a. The effect of HCN excreted by *Pseudomonas* fluorescens on the growth of some phytopathogenic fungi. (in Polish with English summary). *Zesz. Nauk. Akad. Roln. we Wrocławiu. Konferencje* **332**: 85–92.

Paszkowski W.L. 1998b. Relations between degree of colonization of roots by *Fusarium* and communities and properties of other groups of oat rhizosphere microorganisms. (in Polish with English summary). *Zesz. Nauk. Akad. Roln. we Wrocławiu. Konferencje* **332**: 93–101.

Paszkowski W.L. 2001. Effectiveness of *Pseudomonas fluorescens* in the control of *Gaeumannomyces graminis* var. *tritici* and *Fusarium culmorum* on cereal plants. *Bull. Polish Acad. Sci.* **49**: 381–385.

Paszkowski W.L., A. Księżniak and B. Wróblewska. 2001. The influence of *Pseudomonas fluorescens* on degree of the oat roots settling by endomicorhizal fungi and *Fusarium culmorum*. p. 203–208. In: A. Marszałek (ed.), Drobnoustroje środowiska glebowego. Aspekty fizjologiczne, biochemiczne, genetyczne. Zakład Mikrobiologii, Instytut Biologii Ogólnej i Molekularnej Uniwersytetu Mikołaja Kopernika w Toruniu. Poland (in Polish).

Peterson E.A. 1958. Observation on fungi associated with plant roots. *Can. J. Microbiol.* **4**: 257–265.

Pląskowska E. 1997. The fungi communities of the soil environment of *Triticum eastivum* and its forecrops: *Hordeum vulgare*, *Vicia faba* ssp. minor and *Trifolium pratense*. *Acta Mycol*. 32: 226–235.

Singh U.P. and B. Prithiviraj. 1997. Biological control of plant diseases in India. p. 17–21. In: A. Ogoshi, K. Kobayashi, Y. Homma, F. Kodama, N. Kondo, S. Akino (eds), Plant Growth-Promoting Rhizobacteria. Present Status and Future Prospects. Sapporo, Japan.

Strzelczyk E., K. Gorlach and H. Różycki. 1990. Chitinolytic and proteolytic activity of streptomyces isolated from root-free soil, rhizosphere and mycorhizosphere of pine (*Pinus silvestris* L.). *Biol Fertil. Soils*, **9**: 268–272.

Święcicka I. and T. Hauschild. 1996. Genus *Bacillus* – existence and importance in natural environments. (in Polish with English summary). *Post. Mikrobiol.* **35**: 27–43.

Toyota K., K. Miyashita, M. Kimura. 1994. Introduction of a chitinase gene into *Pseudomonas stutzeri* A18 isolated from the surface of chlamydospores of *Fusarium oxysporum* F. sp. *raphani*. *Soil Biol. Biochem*. **26**: 413–416.

Tsuchiya K. 1997. Current status and future prospects in research and practice of biological control of plant diseases in Japan. p. 30–35. In: A. Ogoshi, K. Kobayashi, Y. Homma, F. Kodama, N. Kondo, S. Akino (eds), Plant Growth-Promoting Rhizobacteria. Present Status and Future Prospects. Sapporo, Japan.

Uoti J. 1995. Commercial biofungicides: facts or fantasies. p. 113–120. In: M. Mańka (ed.), Environmental biotic factors in integrated plant disease control. European Foundation for Plant Pathology, Poznań, Poland.

van Loon L.C. 1998. Biotechnology as a means to improve biological control of plant diseases *Med. Fac. Landbouww. Univ. Gent* **63/4b**: 1657–1666.

Voisard C., C. Keel, D. Haas and G. Defago. 1991. Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. *EMBO J.* **8**: 351–358.

Weller D.M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Ann. Rev. Phytopathol.* **26**: 379–407.

Wenhua T. and Y. Hetong. 1997. Research and application of biocontrol of plant diseases and PGPR in China. p. 2–9. In: A. Ogoshi, K. Kobayashi, Y. Homma, F. Kodama, N. Kondo, S. Akino (eds), Plant Growth-Promoting Rhizobacteria. Present Status and Future Prospects. Sapporo, Japan.

Wong P.T.W. and R. Baker. 1984. Suppression of wheat take-all and ophiobolus patch by fluorescent pseudomonads from a fusarium-suppressive soil. *Soil Biol. Biochem.* **16**: 397–403.