Antagonistic bacteria in the soil after cover crops cultivation

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ABSTRACT

The purpose of the studies was to establish the quantitative composition of bacteria *Bacillus* spp. and *Pseudomonas* spp. and their antagonistic effect towards soil-borne fungi after the cultivation of oats, spring vetch and tansy phacelia as intercrop cover plants. The total population of bacteria in 1 g dry weight of the soil from the experimental combination where mulch of oats was used was larger than in the combination with spring vetch or tansy phacelia. Totally, approximately three times as much *Bacillus* spp. was obtained from soil samples as compared to *Pseudomonas* spp. Tests showed that the most isolates of antagonistic bacteria out of the enumerated genera occurred in the soil after oats cultivation, and the least in the soil after the cultivation of tansy phacelia. Antagonistic *Bacillus* spp. and *Pseudomonas* spp. inhibited the growth and development of *Fusarium oxysporum*, *Haematonectria haematococca* and *Thanatephorus cucumeris* in the most effective way. The greatest total antagonistic effect of *Bacillus* spp. and P*seudomonas* spp. towards *Altenaria alternata*, *F. culmorum*, *F. oxysporum*, *H. haematococca*, *P. irregulare* and *T. cucumeris* was found out after managing the mulch of oats. The smallest total antagonistic effect of bacteria was observed after managing the mulch of tansy phacelia.

Keywords: *Bacillus* spp.; *Pseudomonas* spp.; soil fungi; antagonistic activity; mulching

In the soil environment different interactions between microorganisms on the one hand and between plants and microorganisms on the other take place. Soil bacteria and fungi have a positive or a harmful effect on the growth and development of plants (Czaban et al. 2007, Abdulkadir and Waliyu 2012, Patkowska and Konopiński 2013a). Antagonistic bacteria *Pseudomonas* spp. and *Bacillus* spp. play a big role in limiting the occurrence of soil-borne fungi (Patkowska 2009, Sivanantham et al. 2013). The enumerated bacteria are distinguished by a big ability to inhibit the development of pathogens through the competition for nutrients elements and the space, antibiosis, production of lytic enzymes, HCN production and decomposition of toxins (Krid et al. 2010, Abdulkadir and Waliyu 2012).

Cover crops, which have an important protective and conserving function, are more and more often used in the integrated field cultivation of vegetables (Lithourgidis et al. 2011, Patkowska and Konopiński 2013a,b). Mulching the soil with intercrop plants, left in the field for winter, protects the soil from erosion, excessive surface flow of the water, washing away of nutrients elements, and it increases the biological activity of the soil. Depending on the species, and even the cultivar, intercrop plants – through their root exudates and products of decomposition of their organic substances – can inhibit the development of plant pathogens and stimulate the growth and development of antagonistic microorganisms.

The role of cover crops at soil cultivation of high-inulin root vegetables and their effect on the

Supported by the Ministry of Science and Higher Education of Poland, Project No. 2 P06 R 01429.

formation of soil-borne antagonistic microorganisms are little known. That is the reason why the present studies were undertaken. The purpose of the studies was to determine the quantitative composition of bacteria *Bacillus* spp. and *Pseudomonas* spp. as well as their antagonistic effect towards soil-borne fungi after the cultivation of oats, spring vetch and tansy phacelia as cover crops.

MATERIAL AND METHODS

Fieldwork. The object of the studies conducted in the years 2006–2008 was the soil sampled each year in the second 10-days' period of June at the depth of 5–6 cm of the plough layer of the field where scorzonera (*Scorzonera hispanica* L.) cv. Duplex was cultivated. The cultivation of this vegetable considered mulching the soil with cover crops such as oats, spring vetch and tansy phacelia.

Laboratory analyses. In order to determine the number of antagonistic bacteria in the soil after the cultivation of oats, spring vetch and tansy phacelia, a microbiological analysis was conducted according to the method described by Czaban et al. (2007). Dilutions of soil ranging from 10^{-1} to 10^{-7} served to determine the total population of bacteria and the population of *Pseudomonas* spp., and *Bacillus* spp. in CFU/g DW of soil (colony forming units/g dry weight (DW) of soil). The total population of bacteria was marked on the nutrient agar, using soil solutions with the dilutions of 10^{-5}, 10^{-6} and 10^{-7}. In the case of bacteria from genus *Bacillus* spp., tryptic soy agar and the dilutions of 10^{-4}, 10^{-5}, 10^{-6} were used, whereas Pseudomonas agar F and the dilutions of 10^{-2}, 10^{-3}, 10^{-4} were used for *Pseudomonas* spp. For isolation of *Bacillus* spp. soil dilutions were heated for 20 min at 80°C. In each studied year, the obtained isolates of *Bacillus* spp. and *Pseudomonas* spp. (500 isolates from each genus) were used to establish their antagonistic effect towards such fungi as *Altenaria alternata*, *Fusarium culmorum*, *F. oxysporum*, *Haematonectria haematococca*, *Pythium irregulare* and *Thanatephorus cucumeris* (isolated from the infected scorzonera plants). Pathogenicity of those fungi for scorzonera plants was observed in the tests carried out in the laboratory and the growth chamber (Patkowska and Konopiński 2008). While establishing the antagonistic effect of bacteria on pathogenic fungi, laboratory tests were conducted and the method and scale described by Martyniuk et al. (1991) were used. It takes into consideration five degrees, i.e. 0° – no inhibition zone; 1° – inhibition zone of 1–2 mm; 2° – inhibition zone of 3–5 mm; 3° – inhibition zone of 6–10 mm; 4° – inhibition zone of over 10 mm. In order to fully determine the effect of bacteria on the pathogenic fungus, the studies also used the degrees of growth inhibition of plant pathogens as provided by Pięta and Kęsik (2007). It comprised the following: 0° – no fungus growth inhibition; 1° – colony growth inhibited to 20%; 2° – colony growth inhibited to 50%; 3° – colony growth inhibited to 80%; 4° – colony growth inhibited to 100%.

Statistical analysis. The total population of bacteria was statistically analyzed and the significance of differences was determined on the basis of the Tukey's confidence intervals ($P < 0.05$). Statistical calculations were carried out using the Statistica program, version 6.0 (StatSoft Inc., Krakow, Poland).

RESULTS AND DISCUSSION

The total population of bacteria in the soil from the experimental combination where mulch of oats was used was approximately twice as big as in the combination with tansy phacelia, and it was 5.02×10^6 and 3.32×10^6 CFU/g DW of soil, respectively (Figure 1). A lot of total bacteria were also obtained from the soil after the cultivation of spring vetch (on average, 4.28×10^6 CFU/g). Totally, approximately three times as many *Bacillus*

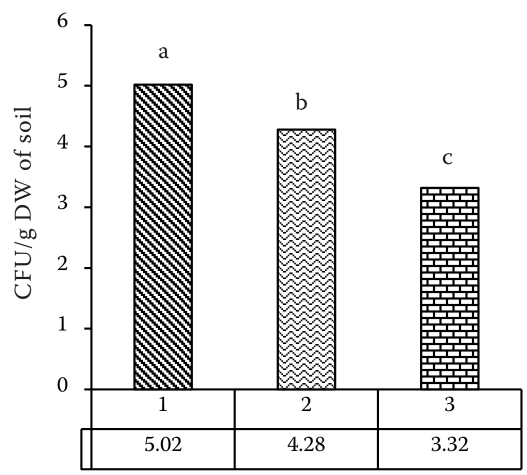

Figure 1. Total number of bacteria isolated from the soil after oat (1), spring vetch (2) and tansy phacelia (3) cultivation (means from the years 2006–2008). Means differ significantly ($P < 0.05$) if they are not marked with the same letter. DW – dry weight

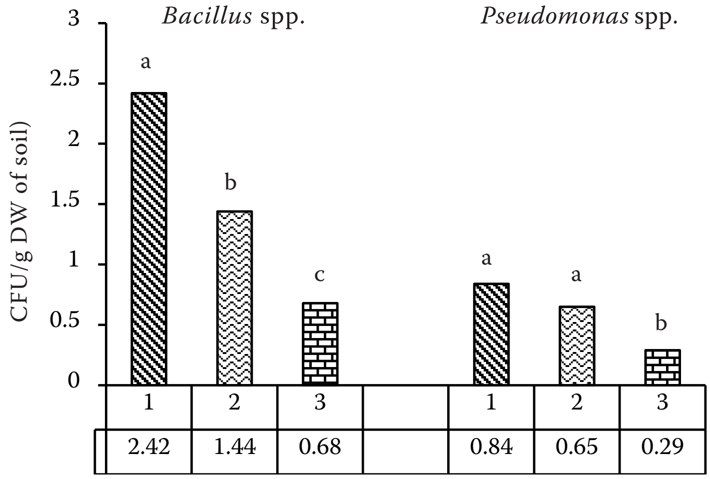

Figure 2. Total number of *Bacillus* spp. and *Pseudomonas* spp. isolated from the soil after oat (1), spring vetch (2) and tansy phacelia (3) cultivation (means from the years 2006–2008). Means differ significantly (P < 0.05) if they are not marked with the same letter. DW – dry weight

spp. were obtained from particular experimental combinations as *Pseudomonas* spp. The most bacteria from genera *Bacillus* and *Pseudomonas* occurred in the soil after the cultivation of oats (2.42×10^6 and 0.84×10^6 CFU/g, respectively), and the least in the soil after the cultivation of phacelia (0.68×10^6 and 0.29×10^6 CFU/g, respectively) (Figure 2). A similar relation in the composition of microorganisms was observed by Patkowska and Konopiński (2013b), who used oat and spring vetch in the conserving cultivation of root chicory and salsify.

In the soil there were more antagonistic isolates of *Pseudomonas* spp. than isolates of *Bacillus* spp. As reported by Kunze et al. (2011), the development of those bacteria could be supported by secondary metabolites introduced into the soil of cover crops.

Antagonistic *Bacillus* spp. and *Pseudomonas* spp. isolated from the soil after oat cultivation were the most effective in inhibiting the growth and development of *F. oxysporum* and *T. cucumeris* because the obtained value of the antagonistic effect from three-year-long studies totally was 658 and 651, respectively (Table 1). *Bacillus* spp. and *Pseudomonas* spp. obtained from this experimental combination proved equally effective in limiting the growth of *H. haematococca*, *F. culmorum* and

Table 1. Antagonistic activity of *Bacillus* spp. and *Pseudomonas* spp. isolated from soil after oat cultivation towards pathogenic fungi

	Genus of bacteria	Number of antagonistic isolates	*Alternaria alternata*	*F. culmorum*	*F. oxysporum*	*Haematonectria haematococca*	*Pseudomonas irregulare*	*Thanatephorus cucumeris*	Total effect of antagonistic activity
2006	*Bacillus* spp.	20	60	60	100	100	60	100	480
	Pseudomonas spp.	34	68	68	136	102	68	102	544
	total effect of antagonistic activity		128	128	236	202	128	202	1024
2007	*Bacillus* spp.	16	32	48	80	64	48	80	352
	Pseudomonas spp.	37	37	74	111	111	74	111	518
	total effect of antagonistic activity		69	122	191	175	122	191	870
2008	*Bacillus* spp.	30	90	120	150	120	50	150	780
	Pseudomonas spp.	36	36	108	108	108	+108	108	576
	total effect of antagonistic activity		126	228	258	228	258	258	1356
	total effect of antagonistic activity		323	478	685	605	508	651	3250

Table 2. Antagonistic activity of *Bacillus* spp. and *Pseudomonas* spp. isolated from soil after spring vetch cultivation towards pathogenic fungi

Year	Genus of bacteria	Number of antagonistic isolates	*Alternaria alternata*	*F. culmorum*	*F. oxysporum*	*Haematonectaria haematococca*	*Pseudomonas irregulare*	*Thanatephorus cucumeris*	Total effect of antagonistic activity
2006	*Bacillus* spp.	9	18	27	36	36	18	36	171
	Pseudomonas spp.	16	16	32	48	32	16	32	176
	total effect of antagonistic activity		34	59	84	68	34	68	347
2007	*Bacillus* spp.	18	18	36	72	54	54	72	306
	Pseudomonas spp.	25	50	25	25	50	25	50	225
	total effect of antagonistic activity		68	61	97	104	79	122	531
2008	*Bacillus* spp.	16	32	48	64	48	48	64	304
	Pseudomonas spp.	20	20	40	40	40	40	40	220
	total effect of antagonistic activity		52	88	104	88	88	104	524
	total effect of antagonistic activity		154	208	285	260	201	294	1402

P. irregulare since the total value of the antagonistic effect was 605, 478 and 508, respectively. On the other hand, those bacteria were the poorest antagonists towards *A. alternata* (+323). Within the group of the tested bacteria isolated from the soil after the cultivation of spring vetch, the smallest antagonistic effect was found out towards *A. alternata* (154 the total antagonistic effect) (Table 2). *Bacillus* spp. and *Pseudomonas* spp. isolated from the soil after the cultivation of spring vetch inhibited the growth and development of *T. cucumeris*, *F. oxysporum* and *H. haematococca*. The number of antagonistic *Bacillus* spp. and *Pseudomonas* spp. isolated from the soil after the cultivation of

Table 3. Antagonistic activity of *Bacillus* spp. and *Pseudomonas* spp. isolated from soil after tansy phacelia cultivation towards pathogenic fungi

Year	Genus of bacteria	Number of antagonistic isolates	*Alternaria alternata*	*F. ulmorum*	*F. oxysporum*	*Haematonectaria haematococca*	*Pseudomonas irregulare*	*Thanatephorus cucumeris*	Total effect of antagonistic activity
2006	*Bacillus* spp.	5	10	10	15	20	5	15	75
	Pseudomonas spp.	10	10	20	30	20	10	20	110
	total effect of antagonistic activity		20	30	45	40	15	35	185
2007	*Bacillus* spp.	9	9	9	18	18	18	27	99
	Pseudomonas spp.	18	18	18	18	18	18	18	108
	total effect of antagonistic activity		27	27	36	36	36	45	207
2008	*Bacillus* spp.	9	9	18	27	9	18	27	108
	Pseudomonas spp.	18	18	36	36	36	36	36	198
	total effect of antagonistic activity		27	54	63	45	54	63	306
	total effect of antagonistic activity		4	111	144	121	105	143	698

tansy phacelia was the lowest, and they inhibited the growth of *A. alternata* colonies the worst (74 the total antagonistic effect). Those bacteria proved to be the most effective in inhibiting the growth of *F. oxysporum*, *H. haematococca* and *T. cucumeris* since the value of the antagonistic effect was 144, 121 and 143, respectively (Table 3). The largest total antagonistic effect of *Bacillus* spp. and *Pseudomonas* spp. towards pathogenic fungi was found out after managing the mulch of oats; it was 3250. The total antagonistic effect was more than twice as low after the cultivation of spring vetch (1402). The smallest total antagonistic effect of *Bacillus* spp. and *Pseudomonas* spp. was observed after managing the mulch of tansy phacelia (698) (Table 3).

The big antagonistic effect of bacteria *Bacillus* spp. and *Pseudomonas* spp. in the soil, especially after the cultivation of oats and spring vetch testifies to their large biological activity contributing to the improvement of the phytosanitary condition of the soil. As reported by Krid et al. (2010) and Verma et al. (2012), high antagonistic activity of those bacteria is connected with their ability to produce antibiotics of siderophores. Those compounds have a fungistatic or fungicidal effect.

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Received on October 29, 2013
Accepted on February 6, 2014

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