Antagonistic activity of selected bacteria occurring in the soil after root chicory cultivation

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ABSTRACT

Chicory (*Cichorium intybus* L.) is a rich source of polysaccharides (intibin and inulin), vitamins, acids and mineral salts. The present studies determined the antagonistic activity of selected bacteria of the soil environment of root chicory towards fungi pathogenic towards this plant: *Alternaria alternata*, *Botrytis cinerea*, *Fusarium culmorum*, *F. oxysporum*, *Thanatephorus cucumeris* and *Sclerotinia sclerotiorum*. Root chicory was cultivated with using cover crops (oats, tansy phacelia and common vetch). The use of cover crops, especially oats, in the cultivation of root chicory had a positive effect both on the total population of bacteria and the population of *Bacillus* spp. and *Pseudomonas* spp. in the soil. Antagonistic *Bacillus* spp. and *Pseudomonas* spp. displayed differentiated activity towards the studied fungi. The greatest antagonistic effect was found out after the mulch of oats. After common vetch, the antagonistic activity of bacteria was more than twice, and after phacelia three times as low as after oats. In the traditional cultivation of root chicory this activity was nearly twelve times lower.

Keywords: soil-borne bacteria; *Bacillus* spp.; *Pseudomonas* spp.; *Cichorium intybus*; intercrop plants

Chicory (*Cichorium intybus* L.) is a green plant that includes about 10 species, growing in moderately warm zones of Europe, Asia and Africa (Geldenhuis et al. 2006). It contains considerable quantities of inulin – a glycoside which has a positive influence on *Lactobacillus* sp. and *Bifidobacterium* sp. It has a positive effect on kidneys and liver as well as inhibiting cancerous processes (Koo et al. 2003).

The agriculture uses different manners of cultivation with the aim of creating good conditions for the growth and development of plants. The most significant is the system of conserving cultivation (Borowy 2013, Patkowska and Konopiński 2013a, 2014). 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 (Kołota and Adamczewska-Sowińska 2013).

Mulching plants – through their root exudates and products of decomposition of their organic substance – can inhibit the development of plant pathogens and stimulate the growth and development of antagonistic microorganisms (Bendig and Lincoln 2000, Patkowska and Konopiński 2013b). The greatest antagonistic activity is found for bacteria *Bacillus* spp. and *Pseudomonas* spp. (Jeyaseelan et al. 2012, Sivanantham et al. 2013, Patkowska and Konopiński 2014).

The cultivation of root chicory as a high-inulin plant, with good nutritious and dietary values is not very popular. The effect of cover crops on the antagonistic activity of microorganisms in the soil environment of this is not known, either. Therefore, the purpose of the studies was to determine the

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antagonistic activity of selected bacteria of the soil environment of root chicory cultivated with using cover crops.

MATERIAL AND METHODS

Fieldwork. The field experiment was conducted in the years 2007–2008 in Felin Experimental Station belonging to the University of Life Sciences in Lublin, district of Lublin (22°56'E, 51°23'N, Central Eastern Poland, 200 m a.s.l.), on a grey-brown podzolic soil. The object of the studies were selected bacteria isolated from the soil sampled (every year during the first 10 days of June) from the depth of 5–6 cm of the plough layer of the field where root chicory (*Cichorium intybus* L. var. *sativum* Bisch.) cv. Polanowicka was cultivated. The cultivation of this plant took into consideration soil mulching with intercrop cover plants such as oats, tansy phacelia and common vetch. Chicory was sown during the first 10 days of May, whereas cover plants were sown in the first half of August of each year preceding the setting up of the experiment. Before winter, oats, phacelia and common vetch created an abundant green mass, constituting the natural mulch on the surface of the land. The conventional cultivation, i.e. without any cover crops, was the control. The experiment was established in a split-plots scheme, in four replications.

Laboratory analyses. The microbiological analysis of the soil of particular experimental treatments was made according to the method described by Czaban et al. (2007) and Patkowska and Konopiński (2014). It served to establish the total populations of bacteria, the populations of *Pseudomonas* spp. and the spores of genus *Bacillus* in 1 g dry weight (DW) of the soil. After preparing the proper dilutions of the soil solution, the total number of bacteria was marked on the Nutrient agar. Tryptic soy agar was used to determine the number of *Bacillus* spp. bacteria, and Pseudomonas agar F was 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. (300 isolates of each genus) were used to determine their antagonistic effect towards such fungi as *Altenaria alternata*, *Botrytis cinerea*, *Fusarium culmorum*, *F. oxysporum*, *Sclerotinia sclerotiorum* and *Thanatephorus cucumeris* (isolated from the infected root chicory plants). Pathogenicity of those fungi towards the plants of root chicory was found out in earlier studies conducted in a growth chamber (Patkowska and Konopiński 2008).

In order to establish the antagonistic effect of bacteria towards pathogenic fungi, laboratory tests were carried out and the scale described by Martyniuk et al. (1991) and Patkowska and Konopiński (2013a) was used. Besides, antagonistic activity of all antagonistic isolates of *Bacillus* spp. and *Pseudomonas* spp. was determined towards the studied fungi in the cultivation environment of root chicory.

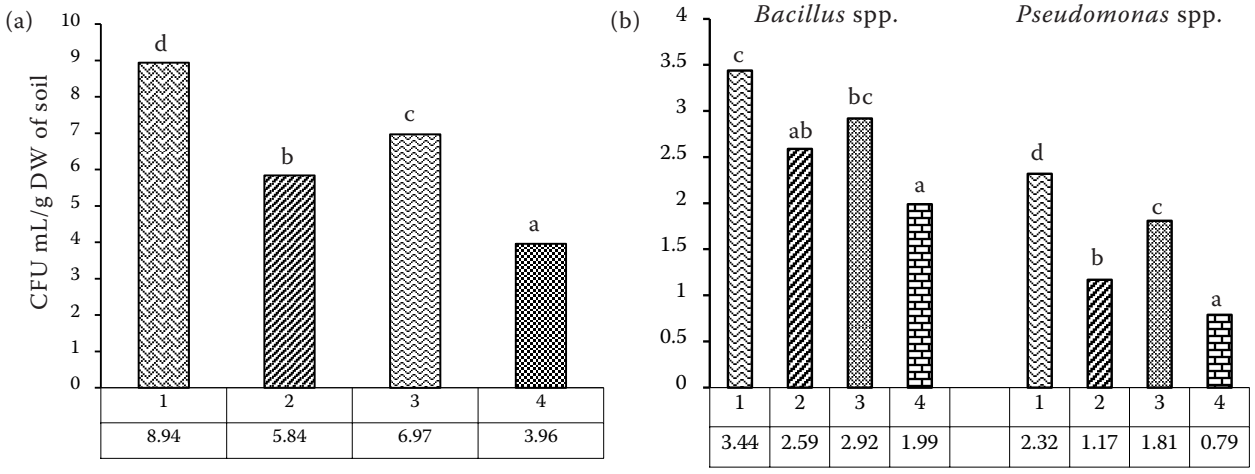

Figure 1. Total number of (a) bacteria and (b) *Bacillus* spp. and *Pseudomonas* spp. isolated from the soil of particular experimental treatment (means from the years 2007–2008). 1 – soil after oat cultivation; 2 – soil after tansy phacelia cultivation; 3 – soil after spring vetch cultivation; 4 – soil without cover crops cultivation. Means differ significantly ($P < 0.05$) if they are not marked with the same letter. CFU – colony forming units; DW – dry weight

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

Genus of bacteria	Number of antagonistic isolates	*Altenaria alternata*	*Botrytis cinerea*	*Fusarium culmorum*	*F. oxysporum*	*Thanatephorus cucumeris*	*Sclerotinia sclerotiorum*	Total effect of antagonistic activity
2007								
Bacillus spp.	35	105	140	175	175	105	140	840
Pseudomonas spp.	48	96	48	192	192	144	144	816
Total effect of antagonistic activity		201	188	367	367	249	284	1656
2008								
Bacillus spp.	27	81	81	135	81	135	135	648
Pseudomonas spp.	38	76	114	152	152	152	152	798
Total effect of antagonistic activity		157	195	287	233	287	287	1446
Total effect of antagonistic activity		358	383	654	600	536	571	3102

Statistical analysis. The 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 1 g DW of the soil ranged from 3.96×10^6 to 8.94×10^6 CFU/g of soil DW (colony forming units/g DW of soil). The most of total bacteria were obtained after mulching the soil with oats, and that was twice as much as in the control, i.e. without any cover crops. A little fewer total bacteria were obtained after mulching the soil with common vetch or phacelia (Figure 1a). The population of *Bacillus* spp. ranged from 1.99×10^6 to 3.44×10^6 CFU/g DW of soil, that of *Pseudomonas* spp. from 0.79×10^6 to 2.32×10^6 CFU/g DW of soil. The most of those bacteria occurred after mulching the soil with oats (3.44×10^6 and 2.32×10^6 CFU/g DW of soil, respectively), and the fewest in the traditional cultivation of root chicory (Figure 1b).

Table 2. Antagonistic activity of *Bacillus* spp. and *Pseudomonas* spp. isolated from the soil after tansy phacelia cultivation towards pathogenic fungi

Genus of bacteria	Number of antagonistic isolates	*Altenaria alternata*	*Botrytis cinerea*	*Fusarium culmorum*	*F. oxysporum*	*Thanatephorus cucumeris*	*Sclerotinia sclerotiorum*	Total effect of antagonistic activity
2007								
Bacillus spp.	18	18	36	72	54	36	54	270
Pseudomonas spp.	22	44	44	44	66	66	44	308
Total effect of antagonistic activity		62	80	116	120	102	98	578
2008								
Bacillus spp.	11	22	33	44	44	44	33	220
Pseudomonas spp.	21	21	21	63	42	21	63	231
Total effect of antagonistic activity		43	54	107	86	65	96	451
Total effect of antagonistic activity		105	134	223	206	167	194	1029

Table 3. Antagonistic activity of *Bacillus* spp. and *Pseudomonas* spp. isolated from the soil after spring vetch cultivation towards pathogenic fungi

Genus of bacteria	Number of antagonistic isolates	*Altenaria alternata*	*Botrytis cinerea*	*Fusarium culmorum*	*F. oxysporum*	*Thanatephorus cucumeris*	*Sclerotinia sclerotiorum*	Total effect of antagonistic activity
2007								
Bacillus spp.	23	46	69	115	92	46	92	460
Pseudomonas spp.	36	72	36	180	144	72	72	576
Total effect of antagonistic activity		118	105	295	236	118	164	1036
2008								
Bacillus spp.	20	40	60	80	80	80	80	420
Pseudomonas spp.	29	58	58	58	87	58	87	406
Total effect of antagonistic activity		98	118	138	167	138	167	826
Total effect of antagonistic activity		216	223	433	403	256	331	1862

Studies conducted by Pięta et al. (2002) showed that cereals can stimulate the growth and development of bacteria. Moreover, oats used for soil mulching in the cultivation of scorzonera had a similar effect on the formation of the populations of *Bacillus* spp. and *Pseudomonas* spp. in the cultivation environment of this plant (Patkowska and Konopiński 2013a).

Antagonistic *Bacillus* spp. and *Pseudomonas* spp. showed differentiated activity towards the studied fungi pathogenic towards root chicory. The greatest total antagonistic effect of those bacteria towards plant pathogens was observed after the mulch of oats, and it was 3102 (Table 1). The total antagonistic effect of *Bacillus* spp. and *Pseudomonas* spp. was three times as low after phacelia (1029) (Table 2) and over twice as low after common vetch (1862) (Table 3). The smallest total antagonistic effect of those bacteria was observed in the traditional cultivation of root chicory, i.e. without any cover plants (270) (Table 4). It was nearly twelve times smaller than in the treatment with oats.

Antagonistic *Bacillus* spp. and *Pseudomonas* spp. isolated from the soil after root chicory cultivation were the most effective in reducing the growth

Table 4. Antagonistic activity of *Bacillus* spp. and *Pseudomonas* spp. isolated from the soil without cover crops cultivation towards pathogenic fungi

Genus of bacteria	Number of antagonistic isolates	*Altenaria alternata*	*Botrytis cinerea*	*Fusarium culmorum*	*F. oxysporum*	*Thanatephorus cucumeris*	*Sclerotinia sclerotiorum*	Total effect of antagonistic activity
2007								
Bacillus spp.	6	6	12	6	12	18	24	78
Pseudomonas spp.	8	8	8	16	24	16	16	88
Total effect of antagonistic activity		14	20	22	36	34	40	166
2008								
Bacillus spp.	2	2	2	4	6	8	4	26
Pseudomonas spp.	6	6	12	12	18	12	18	78
Total effect of antagonistic activity		8	14	16	24	20	22	104
Total effect of antagonistic activity		22	34	38	60	54	62	270

and development of *Fusarium culmorum* and *F. oxysporum.* Those bacteria proved to be the poorest antagonists towards *Alternaria alternata* and *Botrytis cinerea* (Tables 1–4).

The presented studies showed, that oats and common vetch had the most positive effect on the populations of antagonistic bacteria and their antagonistic activity towards pathogenic fungi. According to Gamliel et al. (2000), the development of *Bacillus* spp. and *Pseudomonas* spp. can be favored by secondary metabolites of the mulching plants introduced into 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 or siderophores. Those compounds have a fungistatic or fungicidal effect.

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