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Populations of rhizobia in some Polish soils not planted with legumes

Stefan Martyniuk,

Jadwiga Oroń

Department of Agricultural Microbiology,
Institute of Soil Science and
Plant Cultivation,
State Research Institute,
Czartoryskich 8,
24-100 Pulawy, Poland
E-mail: sm@iung.pulawy.pl

Using the soil dilution – plant infection method, populations of several species of root-nodule bacteria (rhizobia) were enumerated in 46 soil samples collected throughout Poland. To exclude effects of host-plant cultivation on the interactions between soil physico-chemical properties and populations of the rhizobia, only soils not planted with legumes during at least the last 10 years were analysed. *Rhizobium leguminosarum* bv. *viciae* (symbionts of pea, faba bean, vetch) and *R. leguminosarum* bv. *trifolii* (symbionts of clover) were detected in 44 and 42 soils, respectively. Most of these soils contained moderate and high numbers of these species rhizobia. Symbionts of beans, *R. leguminosarum* bv. *phaseoli*, and symbionts of lupine, *Bradyrhizobium* sp. (*Lupinus*) were less frequent in the examined soils, of which 11 contained non-detectable populations of these bacteria. *Sinorhizobium meliloti*, rhizobia nodulating alfalfa, were sparse in the test soils, with 32 soil samples containing no detectable numbers of *S. meliloti* and only 3 samples harbouring moderate populations of this species. The estimated numbers of rhizobia were also related to the following properties of the soils: organic C and total N contents, pH in water and in KCl, and the content of soil mechanical fractions <0.02 mm and <0.002 mm. Results of this study have shown that soil texture (particularly clay content) and soil reaction have the greatest influence on populations rhizobia in soils.

Key words: rhizobia, population, soil, leguminous plants

INTRODUCTION

Root-nodule bacteria, commonly known as rhizobia, form an important group of soil microorganisms with the ability to fix atmospheric nitrogen in the symbiosis with leguminous plants (*Fabaceae*). These bacteria are members of the following genera: *Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium* and *Sinorhizobium,* within the family *Rhizobiaceae* (Małek and Sajnaga, 1999). Rhizobia survive in soil between symbiotic phases as saprophytes, and their populations depend on many physical and chemical properties of the soil environment and on the frequency of planting legumes in a given area or field (Sadowsky and Graham, 1998; Martyniuk et al., 1999). In a long-term plot experiment, Martyniuk et al. (1999) assessed numbers of various species of rhizobia in two soils cropped to cereals and treated with different rates of mineral fertilisers (NPK), with or without liming. In the limed soils, relatively high populations of rhizobia nodulating red clover were found, though this crop had not been grown in this experiment for more than 20 years, but in the non-limed soils populations of these bacteria were markedly lower. Contrary to clover rhizobia, almost no microsymbionts of alfalfa were detected both in the limed and non-limed soils, indicating substantial differences in the ability of various rhizobial species to survive in soils, particularly in the absence of their host plant. Nutman and Hearne (1979) presented similar results for rhizobia in the UK soils. In France, Amarger (1980) analysed populations of various species of root nodule bacteria in 60 different soils and related numbers of these bacteria to soil pH. It was found in this study that populations of alfalfa rhizobia were much lower in soils with the pH below 6.0 than in neutral or alkaline soils, but the reverse was true for lupine rhizobia.

The aim of this work was to assess populations of several species of rhizobia in 46 soil samples collected throughout Poland. Since we were interested in the interactions between soil physico-chemical properties and populations of rhizobia, only soils not planted to legumes during at least the last 10 years were analysed. It has been hypothesised that species of rhizobia differ with respect to their response to various soil characteristics.

MATERIALS AND METHODS

Soils

In September 2005, a total of 46 soils from different regions (voivodeships) of Poland were sampled. Samples were immediately sent to the Institute of Soil Science and Plant Cultivation in Pulawy by workers of the Regional Agriculture Advisory Centres in Poland. Each soil sample, weighing about 1–1.5 kg, consisted of several cores (3 cm × 20 cm) taken from farmer's fields on which leguminous plants had not been grown for at least 10 years.

The soils were sieved through a 2-mm sieve and stored moist in a refrigerator for a period no longer than 2 weeks before assessing the numbers of root nodule bacteria. Sub-samples of the soils were air-dried and analysed at the Certified Central Chemical Laboratory of the Institute of Soil Sc. and Plant Cultivation for the following physical and chemical properties: silt-clay content (sedimentation technique), organic C (dichromate digestion), total N (Kjeldahl) and pH (in H_2O and KCl).

Counting of rhizobia

The soils were assessed for the numbers of five species of root-nodule bacteria (rhizobia) listed in Table 1. Although root-nodule bacteria are culturable on different synthetic or semi-synthetic media, there is no selective medium available for making plate counts of these bacteria in soil or other contaminated materials. For this reason, the soil dilution – plant infection method is used to assess the most probable numbers (MPN) of rhizobia in soils (Brockwell, 1963; Toomsan et al., 1984; Martyniuk et al., 2000). This technique, modified by Martyniuk et al. (2000), was also used in the present studies. Shortly, in this method, the seedlings of the test leguminous plants were grown under semi-aseptic conditions in plastic pouches filled with sterile sand moistened with N-free nutrient solution to support plant growth. The seedlings were inoculated with 1 ml of 10-fold soil dilutions in water and grown in a plant growth chamber (Hereus HPS). In these tests, we used six soil dilution steps (from 10^1 to 10^6) and two or four replicated pouches (seedlings) for each dilution. The growth chamber was set at a 16 h / 8 h light–dark regime and at 22 °C day / 15 °C night temperature. After 4–6 weeks of growth, the roots of the seedlings were gently washed in tap water and inspected for the presence of nodules in each dilution, and the total number of positive cases was counted. Based on these scores, the most probable numbers (MPN) of rhizobia in the test soils and 95% confidence limits were calculated from mathematical tables (Vincent, 1970). Log-transformed numbers of rhizobia in the test soils were expressed per 1g of soil dry matter.

RESULTS AND DISCUSSION

***Bradyrhizobium* sp. (*Lupinus*)**, rhizobia nodulating lupine and serradella, were not detected in 11 out of 46 soils examined, and in other 17 soils populations of these bacteria were low or very low (Table 2). Moderate numbers of *Bradyrhizobium* sp. (log 2.15–2.96) were found in 6 soils, and 12 soils contained high numbers (over 1000 cells in 1 g soil d.m.) of these rhizobia. The soils with the highest numbers of lupine rhizobia contained between 7% and 20% of <0.02 mm fraction and had pH (KCl) between 4.4 and 6.2. As Table 3 shows, the numbers of *Bradyrhizobium* sp. were significantly (negatively) correlated with the contents of silt-clay fractions in the soils, indicating that light-textured soils are beneficial for the proliferation and survival of these root-nodule bacteria. Interestingly, in Poland (Barbacki, 1972) and in other countries (Slattery and Coventry, 1989) lupine is cultivated preferably on soils with similar characteristics. Moreover, Gołębiowska and Sypniewska (1962) have demonstrated that the symbiotic process between lupine and *Bradyrhizobium* sp. was markedly disturbed or even inhibited in loamy and alkaline soils.

***Rhizobium leguminosarum* bv. *viciae* (*R.l.v.*)**, rhizobia forming a symbiosis with roots of vetch, pea and faba-bean, were found almost in all the test soils. Most of these soils (39) contained high and moderate (log 2.23–5.84) populations of *R.l.v*. (Table 2). Similar results were reported for French and British soils (Amarges, 1980; Nutman and Hearne, 1979). Only in five soils tested in our studies, low or very low numbers of *R.l.v.* were found and in other two soils no *R.l.v.* was detected. The soils with no detectable or very low populations of *R.l.v*. were very acid (pH 3.7–4.5), and the highly significant correlation coefficients between soil populations of these rhizobia and the pH of the soils (Table 3) indicate that soil reaction is an important factor influencing the persistence (survival) of *R.l.v.* in soils.

***R. leguminosarum* bv. *trifolii* (*R.l.t.*)**, symbionts of clover, similarly to *R.l.v.,* were found in almost all the soils. High and

Table 1. **Species of the rhizobia, their host-plants and host species (cultivars) used in plant infection tests to assess most probable numbers of rhizobia**

Species of rhizobia	Host legumes	Host species and cultivars used
Bradyrhizobium sp.	*Lupinus, Ornithopus*	*Ornithopus sativus* L., cv. Libella
Rhizobium leguminosarum:		
biovar *trifolii*	*Trifolium*	*Trifolium repens* L., cv. Hruszowska
biovar *viciae*	*Vicia, Lathyrus, Pisum*	*Pisum arvense* L., cv. Fidelia
biovar *phaseoli*	*Phaseolus*	*Phaseolus vulgaris* L., cv.Aura
Sinorhizobium meliloti	*Medicago, Melilotus*	*Medicago sativa* L., cv. Socza

Table 2. **Populations of the rhizobia in the soils (1g soil d. m.) and numbers of soils in which rhizobia were detected**

Population of rhizobia*	Estimated numbers (log) of the tested species of rhizobia and numbers of soils (in brackets)				
	***Bradyrhizobium* sp. (*Lupinus*)**	***R. leguminosarum* biovar *vicae***	***R. leguminosarum* biovar *trifolii***	***R. leguminisarum* biovar *phaseoli***	***Sinorhizobium meliloti***
High	3.21–3.78 (12)	3.23–5.84 (29)	3.04–4.86 (19)	3.23–5.34 (11)	(0)
Moderate	2.15–2.96 (6)	2.23–2.85 (10)	2.0–3.0 (12)	2.23–2.83 (10)	2.34 2.69 (3)
Low	1.23–1.97 (11)	1.28–1.82 (3)	1.23–1.76 (8)	1.23–1.84 (13)	1.04–1.87 (3)
Very low	0.08–0.88 (6)	0.82–0.83 (2)	0.78–0.87 (3)	0.84 (1)	0.7–0.95 (8)
Not detected	(11)	(2)	(4)	(11)	(32)

* Populations the rhizobia were grouped by the authors into: high = > 1000, moderate = > 100 – ≤1000, low > 10 – ≤100, very low = ≤10 rhizobial cells in 1 g soil d.m.

Table 3. **Correlation coefficients between numbers of rhizobia in the examined soils and some chemical and physical characteristics of these soils**

Species of rhizobia	C org. (%)	N total (%)	pH(H_2O)	pH(KCl)	Soil fraction <0.02 mm (%)	Soil fraction <0.002 mm (%)
	Range in the soils					
	0.55–3.27	0.042–0.30	4.8–7.7	3.7–7.1	7–61	1–27
Bradyrhizobium sp. (*Lupinus*)	–0.067	–0.165	–0.251	–0.162	–0.465**	–0.441**
Rhizobium leguminosarum:						
biovar *viciae*	–0.261	–0.184	0.515**	0.581**	0.133	0.023
biovar *trifolii*	0.099	0.291*	0.534**	0.385*	0.646**	0.583**
biovar *phaseoli*	0.274	0.451**	0.278	0.174	0.424**	0.348*
Sinorhizobium meliloti	0.106	0.129	0.385*	0.453**	0.308*	0.250

* Significant at $p = 0.05$; ** Significant at $p = 0.01$;
n = 46.

moderate populations (log 2.0–4.86) of the clover rhizobia occurred in 31 soils, and low numbers of these bacteria were detected in 18 soils. Only four soils contained no detectable populations of *R.l.t.*, and in three soils these populations were assessed as very low (Table 2). These results indicate that this species of rhizobia can survive in soils for a long time, even in the absence of their host plant. The highly significant correlation coefficients with soil pH and with the contents of silt–clay fractions suggest that medium or heavy soils heaving slightly acid or neutral pH are beneficial for the proliferation and survival clover rhizobia (Table 3).

***R. leguminosarum* bv. *phaseoli* (*R.l.ph.*)**, rhizobia nodulating beans, were not detected in 11 soils (Table 2). In other soil populations of bean, rhizobia varied from very low (in one soils) to moderate or high in 21 soils (log 2.23–5.34). Soil populations of bean rhizobia showed a significant correlation with the contents of the total N as well as with the contents of the silt–clay fraction in the soils (Table 3). These results indicate that these rhizobia are moderately sensitive to the absence of their host plant, and that the survival of these bacteria is favoured in fertile and heavier soils (Nutman and Hearne, 1979).

Sinorhizobium meliloti, the root nodule bacterium of alfalfa, was the only species of the rhizobia that was not detected in the majority (32) of the studied soils (Table 2). Soils with no, very low and low populations of the alfalfa rhizobia made up almost 93% of all the test soils. Only three soil samples contained moderate populations, and none of the soils was colonised with high numbers of *S. meliloti*. These results clearly indicate that the presence and population levels of the alfalfa rhizobia in Polish soils are, similarly to soils in other countries (Amarger, 1980; Nutman and Hearne, 1979), strongly dependent on the cultivation of the host crop. Soil texture as represented by the contents of silt fractions (< 0.02 mm) and the pH are also important factors influencing the persistence (survival) of *S. meliloti* in the soil (Table 3).

CONCLUSIONS

1. *Rhizobium leguminosarum* bv. *viciae* (*R.l.v.*) and *R. leguminosarum* bv. *trifolii* (*R.l.t.*) occur commonly in Polish soils. Most of the soils examined contained moderate or high populations of *R.l.v.* and *R.l.t.*

2. *R. leguminosarum* bv. *phaseoli,* and *Bradyrhizobium* sp. (*Lupinus*) were less frequent in the soils. Moderate or high numbers of these species were found in about 40–46% of the examined soils, and 24% of the soils contained non-detectable populations of these bacteria.

3. *Sinorhizobium meliloti*, rhizobia nodulating alfalfa, were sparse in the examined soils. Moderate populations of this bacterial species were detected only in three soils out of 46 soils tested, and in 32 soils no alfalfa rhizobia were found.

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Stefan Martyniuk, Jadwiga Oroń

GUMBELINIŲ BAKTERIJŲ POPULIACIJOS LENKIJOS DIRVOŽEMIUOSE, NEUŽSĖTUOSE ANKŠTINIAIS AUGALAIS

S a n t r a u k a

Dirvožemio skiedimo–augalo užkrėtimo metodu buvo ištirtos šaknų gumbelinių bakterijų keleto rūšių populiacijos Lenkijoje surinktų keturiasdešimt šešių dirvožemių ėminiuose. Žirnių, pupelių, vikių simbiontai *Rhizobium leguminosarum* bv. *viciae* ir dobilų simbiontai *R. leguminosarum* bv. *trifolii* buvo rasti atitinkamai 44 ir 42 dirvožemiuose. Daugumoje dirvožemių šių gumbelinių bakterijų rūšių pupelių simbiontai *R. leguminosarum* bv. *phaseoli* ir lubinų simbiontai *R. Bradyrhizobium* sp. *lupinu* dirvožemiuose buvo retesni, o 11 dirvožemių jų visai nerasta. Liucernos simbiontai *Sinorhizobium meliloti* tirtuose dirvožemiuose buvo reti: 32 dirvožemiuose jų nerasta ir tik 3 dirvožemiuose šių rūšių bakterijų kiekis buvo vidutinis. Gumbelinių bakterijų gausa tirtuose dirvožemiuose priklausė ir nuo jų fizikinių bei cheminių savybių.

Raktažodžiai: populiacija, dirvožemis, ankštiniai augalai