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INTERACTIONS BETWEEN PHYSICO-CHEMICAL CHARACTERISTICS OF SOILS AND POPULATIONS OF BACTERIA FIXING ATMOSPHERIC NITROGEN

INTERAKCJE POMIĘDZY FIZYCZNO-CHEMICZNYMI WŁAŚCIWOŚCIAMI GLEB A POPULACJAMI BAKTERII WIĄŻĄCYCH AZOT ATMOSFERYCZNY

Abstract: Population densities of bacteria from the genus *Azotobacter* strongly depend on soil reaction. These bacteria are usually absent in acid soils (pH below 6). Our studies have shown that among the researched soil physical and chemical characteristics, mechanical soil structure (particularly clay content) and soil reaction have the greatest influence on numbers of the symbiotic bacteria in the soils. Symbionts of red clover and alfalfa were usually more abundant in soil richer in clay and having pH about 7. On the contrary, such soils are not conducive for lupine and serradella symbionts.

Keywords: *Azotobacter*, symbiotic bacteria, soil properties, agricultural practices

The process of biological nitrogen fixation (BNF) is carried out by various physiological groups of bacteria. From an ecological point of view these bacteria can be divided into: - free-living diazotrophic bacteria (*Azotobacter*, *Clostridium*), fixing atmospheric N in soil, or in associations with plant roots (*Azospirillum*), and – symbiotic bacteria (*Bradyrhizobium*, *Rhizobium*, *Sinorhizobium*), fixing N_2 in root nodules of leguminous plants or some trees (*Frankia*) [1, 2]. On the global scale, BNF contributes a major source of nitrogen to the biosphere. Total world biological N_2 fixation is estimated to be as high as $17.2 \cdot 10^7$ tons per year. Particularly, symbiotic nitrogen fixation is an important source of N to leguminous plants and soil. For example, a field alfalfa crop may fix up to 460 kg N/ha, of which 60–70 % may be derived from the symbiosis with specific rhizobia [1]. Amounts of N_2 fixed by free-living diazotrophic bacteria in soil are much lower and range from 1 kg to 30 kg N/ha [1–3]. In this short review soil and agrotechnical factors affecting populations of free-living Azotobacter spp. and root-nodule bacteria in soils are discussed.

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Occurrence of Azotobacter in soils

Important ecological feature of bacteria from the genus *Azotobacter* is their sensitivity to soil reaction; in acid (pH < 6.0) soils these bacteria are generally absent or occur in very low numbers [3–5]. Soil populations of *Azotobacter* spp. in neutral or alkaline soils rarely exceed several thousands cells per gram of soil. With respect to Polish soils, in 1923 Ziemięcka [3] published results of her pioneer studies on the occurrence of *Azotobacter* spp. in soil samples collected in 1917 and 1918 from 28 locations in the former Polish Kingdom. These studies showed that 50 % of the examined soils contained *Azotobacter* spp.. Most of the soils studied by Ziemięcka were also characterized for their chemical properties, such as soil reaction and contents of: humus, total N, P_2O_5 and CaO. Among these parameters, soil reaction was found to be the most important environmental factor influencing the occurrence and numbers of *Azotobacter* spp. in the soils studied. In 2000 Martyniuk & Martyniuk [5] examined population densities of *Azotobacter* spp. in 31 soils from Poland and compared their results with those reported by Ziemięcka [3], to see whether intensification of agricultural practices that took place during the course of the past century has caused any changes in the colonization of Polish soil by *Azotobacter* spp. In this study various populations of *Azotobacter* spp. were detected in 16 out of 31 soils examined, thus the percentage of soils with *Azotobacter* (51.6 %) was slightly higher than that (50 %) reported by Ziemięcka. This comparison indicates that intensification of agricultural practices during the course of the past century did not change significantly colonization of Polish soils by the bacteria studied. Detailed discussion on changes in Polish agriculture taking place during the course of the 20th century, and on the effects of these changes on soil quality, is beyond the scope and volume of this paper. However, the use of mineral fertilizers and its effect on soil chemical properties, particularly soil pH, deserves short consideration in relation to *Azotobacter*. It has been well documented in long-term field experiments that mineral N fertilizers may cause substantial acidification of soil, particularly when these fertilizes are used in high doses without liming. Since *Azotobacter* is very sensitive to soil acidity one could expect that intensification of the use of mineral N fertilizers might cause acidification of agricultural lands resulting in a reduction of soil populations of *Azotobacter* spp. Condensed description of the changes in Polish agriculture, including the use of mineral fertilizers, in the 19th and 20th centuries, is given by Krasowicz [6]. For example, application of mineral N increased in Poland (on average) from less than 5 kg N ha^{-1} in the forties of the 20th century to about 70 kg N in the eighties of that century. However, data presented by Lipiński [7] prove that acidification of Polish soils did not increase between 1955 and 1999. On the contrary, slight improvement of this soil property could be seen in that period. For example, the area of acidic soils (pH < 5.5) decreased from 58 % in the decade 1955–1964 to 55 % in the period of 1994–1999, and simultaneously, the area of soils having the pH > 6.5 increased from 17 % to 19 % in the respective periods [7]. Thus, these data seem to correspond with a slightly higher percentage of soils colonized by *Azotobacter* spp. found in the year 2000, as compared with that presented by Ziemięcka [3].

Populations of rhizobia in soils as influenced by environmental factors and agrotechnical practices

Root-nodule bacteria, fixing atmospheric nitrogen in the symbiosis with leguminous plants, are members of the following genera: *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azorhizobium* and *Allorhizobium* within the family *Rhizobiaceae* [8]. These bacteria, commonly known as 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 of legumes in a given area or field [9–12]. Based on a long-term plot experiment Martyniuk et al [11] 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 or pea were found, though these legumes were not grown in this experiment for over 20 years, but in the unlimed soils populations of the rhizobia were markedly lower. Contrary to pea and clover rhizobia, almost no microsymbionts of alfalfa were detected in the studied soils, both limed and unlimed, indicating that populations of alfalfa rhizobia in soil strongly depend on cultivation of their host plant. Similar results were obtained by Nutman and Hearne [9] for rhizobia occurring in soils of the UK. In France, Amarger [13] 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 smaller in soils with the pH below 6.0 than in neutral or alkaline soils, but the reverse was true for lupine rhizobia. Similar studies were conducted for 80 soil samples collected from different sites in Poland [2]. Rhizobia nodulating lupine and serradella, (*Bradyrhizobium* sp.) were not detected in 19 out of 80 Polish soils analyzed and in the other 27 soils populations of these bacteria were low. Moderate numbers of *Bradyrhizobium* sp. were found in 10 soils and 24 soils contained high numbers of these rhizobia. Among the soils tested, only 4 soils originated from fields in which lupine or serradella were grown in the year of sampling or in the previous growing season. These soils contained the highest numbers ($1.7–2.8 \cdot 10^4$) of *Bradyrhizobium* sp., proving that cultivation of the host-plants had beneficial effect on soil populations of these root-nodule bacteria. It should be added, however, that many soils harbouring moderate or high populations of *Bradyrhizobium* sp. were not planted to lupine or serradella for long period of time, in some cases even more than 30 years. This fact indicate that bradyrhizobia have the capacity to survive in soil for many years in the absence of their host-plants in crop rotation. The numbers of *Bradyrhizobium* sp. were significantly (negatively) correlated with the contents of silt-clay fractions, with the total N contents and with the pH (in water) of the examined soils, indicating that the light-textured, acid soils are beneficial for the proliferation and survival of these root-nodule bacteria. Rhizobia forming the symbiosis with roots of vetch, pea and faba-bean, (*Rhizobium leguminosarum* bv. *viciae*) were found almost in all the examined soils and most of these soils (70) contained high or moderate populations of *R.l. viciae*. Common occurrence of these bacteria in Polish soils could be explained, at least partially, by relatively frequent cultivation of the host plants of *R.l. viciae* by farmers, since in

23 cases the studied soil originated from fields on which these crops (pea or faba-bean) were included in the crop rotation. These soils contained generally high populations of *R.l. viciae*, but also many soils not planted to peas or faba-bean were also rich in these root nodule bacteria. The soils with no detectable or very low populations of *R.l. viciae* were very acid (pH = 3.7–4.2). Highly significant correlation coefficients between populations of these rhizobia and pH of the soils indicate that soil reaction is an important factor influencing the occurrence of *R.l. viciae* in soils [2]. Symbionts of clover, (*R. leguminosarum* bv. *trifolii*), similarly to *R.l. viciae*, were found in high or moderate populations almost in all Polish soils examined. Only 4 soils contained no detectable populations of *R.l. viciae* and in 7 soils these populations were assessed as very low. The majority of the researched soils were not planted to red clover for many years (with the exception of 3 soils) indicating that rhizobia nodulating this crop can survive in soils for a long period of time, even in the absence of the host plant. 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 these rhizobia [2, 9, 12].

Rhizobia nodulating beans (*R. leguminosarum* bv. *phaseoli*), were examined in 76 soils and of this number in 15 soils these bacteria were not detected. In the other soils tested populations of the bean rhizobia varied from very low (in 3 soils) to high in 25 soils. Field bean was grown only on 3 of the tested soils and the numbers of *R.l. viciae* in these soils were high. Soil populations of the bean rhizobia showed significant correlation with the contents of total N as well as with the contents of 0.02 mm soil mechanical fraction in the soils. Root nodule bacteria of alfalfa (*Sinorhizobium meliloti*), was the only species of the rhizobia which was not detected in the majority of the studied soil. Soils with no, very low and low populations of the alfalfa rhizobia made up almost 93 % of all the studied soils. Only 3 soils originated from fields with the stands of alfalfa and all these soils contained high numbers of *S. meliloti*. These results clearly indicate the presence and population levels of the alfalfa rhizobia in Polish soils are, similarly to soils in other countries [2, 9, 12, 13] strongly dependent on the cultivation of the host crop. Soil texture as represented by the contents of silt-clay fractions is also an important factor influencing the survival of *S. meliloti* in soil.

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Abstrakt: Liczebność populacji bakterii z rodzaju *Azotobacter* w glebie uzależniona jest przed wszystkim od odczynu środowiska glebowego. Bakterie te na ogół nie występują w glebach o odczynie kwaśnym (poniżej pH 6,0). Przedstawione badania wykazały, że spośród analizowanych właściwości fizycznych i chemicznych gleb, skład granulometryczny, a zwłaszcza zawartość części spławianych, oraz odczyn gleb mają największy wpływ na liczebności glebowych populacji bakterii symbiotycznych (brodawkowych). Symbionty koniczyny i lucerny są na ogół liczniejsze w glebach zwięźlejszych i odczynie obojętnym lub zbliżonym do obojętnego, natomiast takie gleby nie sprzyjają symbiontom łubinu i seradeli.

Słowa kluczowe: *Azotobacter*, bakterie symbiotyczne, właściwości gleby, zabiegi agrotechniczne