Polish Journal of Environmental Studies Vol. 12, No. 3 (2003), 371-374

Letter to Editor

Occurrence of *Azotobacter* Spp. in Some Polish Soils

S. Martyniuk*, M. Martyniuk

Department of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation, Czartoryskich 8, 24-100 Puławy, Poland

Received: 18 October, 2002
Accepted: 20 November, 2002

Abstract

In the year 2000 the occurrence of *Azotobacter* spp. in 31 Polish soils was examined and results of this study were compared with those published by Ziemięcka for 28 soils analysed in 1917-18. Almost 52% of the soils tested in 2000 were colonised by *Azotobacter* spp. indicating that intensification of agricultural practices that took place during the past century has not significantly changed (50% soils with *Azotobacter* examined in 1917-18) the colonisation of Polish soils by the studied bacteria. When present, numbers of these bacteria varied widely, from several (soils No. 17 and 31) to almost 10,000 cfu g-1 of the soil No. 25.

Keywords: *Azotobacter*, occurrence, soil, soil properties

Introduction

Aerobic bacteria belonging to the genus *Azotobacter* represent a diverse group of free-living diazotrophic (with the ability to use N_2 as the sole nitrogen source) microorganisms commonly occurring in soil. The genus *Azotobacter* includes 6 species, with *A. chroococcum* most commonly inhabiting various soils all over the world [1-9]. The occurrence of other *Azotobacter* species is much more restricted in nature, e.g. *A. paspali* can be found only in the rhizosphere of a grass, *Paspalum notantum* [8, 9]. Soil populations of *Azotobacter* spp. rarely exceed several thousand cells per gram of neutral or alkaline soils, and in acid (pH < 6.0) soils these bacteria are generally absent or occur in very low numbers [1-7]. With respect to Polish soils, in 1923 Ziemięcka [1] published results of her pioneer studies on the occurrence of *Azotobacter* spp. in soil samples collected in 1917 and 1918 from 28 locations in Poland. These studies showed that 50% of the examined soils contained *Azotobacter* spp. Most of the soils (23) studied by Ziemięcka [1] were also characterised by 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 studied soils. Zawiślak [3] detected *Azotobacter* spp. more frequently, and in higher numbers, in cultivated soils than in uncultivated (sodded) soils collected from hills in Olsztyn province, suggesting that agricultural practices might create environmental conditions more favourable for the development and survival of *Azotobacter* in soil.

In 2000 we examined populations of various groups of microorganisms, including *Azotobacter* spp., in 31 different soils from Poland. Therefore, it seemed interesting to compare our data with those reported by Ziemięcka [1] to see whether intensification of agricultural practices that took place during the course of the past century have caused any changes in the colonisation of Polish soil by *Azotobacter* spp.

Material and Methods

In October of 2000 samples from 0-25 cm layer of 31 soils from different regions of Poland were collected.

*Corresponding author

Numbers of colony-forming units (cfu) of *Azotobacter* spp. in the examined soils were assessed by the dilution-pour plates method [10] on N-free agar medium containing: K_2HPO_4 0.5 g, $MgSO_4$ 0.2 g, NaCl 0.2 g, $CaCO_3$ 5 g, sucrose 10 g, agar 12 g and H_2O dist. 1000 ml and traces of Mn, Fe and Mo. *Azotobacter* spp. after 48-72 hours of incubation at 28°C formed on this medium large, moist colonies. All colonies turned dark brown after 5-7 days of incubation indicating that they belonged to *A. chroococcum* [8, 9]. However, we did not aim at isolation and exact identification of these bacteria, and for this reason no species name of *Azotobacter* is used throughout this paper. Analyses of basic soil properties were done by standard methods at the certified Central Chemical Laboratory of

Table 1. Some physical and chemical properties of the tested soils and their colonisation with *Azotobacter* spp. in the year 2000.

Number and origin of soil	C org. %	N total **%**	Soil fraction <0.02mm	pH (H_2O)	pH (KCl)	Numbers of *Azotobacter* (cfu in 1 g of soil d.m.)
1.ODR Szepietowo	0.71	0.074	18	6.5	5.9	0
2. "	0.83	0.094	29	7.3	6.9	250
3.ODR Radom	2.14	0.210	19	7.3	7.0	810
4. "	1.44	0.151	17	6.5	6.2	97
5. ODR Barzkowice	0.86	0.095	15	6.2	5.7	0
6. "	0.73	0.082	15	6.8	6.6	200
7. ODR Strzelino	1.26	0.128	18	5.8	4.5	0
8. "	1.08	0.112	17	5.8	4.5	0
9. ODR Sielinko	1.84	0.234	24	7.7	7.5	760
10. "	0.63	0.068	14	6.2	5.6	0
11. ODR Bratoszewice	0.96	0.080	14	5.5	4.9	0
12. "	3.27	0.228	8	5.2	4.6	0
13. ODR Przysiek	0.92	0.098	20	5.5	5.0	0
14. "	0.79	0.088	21	5.6	5.4	0
15. ODR Kalsk	0.77	0.070	11	6.6	6.2	0
16. "	1.06	0.098	13	5.4	4.9	0
17. ODR Leszno	0.93	0.101	18	7.1	6.9	8
18. "	0.74	0.079	17	4.4	3.8	0
19. ODR Łosiów	0.77	0.085	31	7.6	7.2	180
20. "	1.46	0.159	45	6.2	5.5	0
21. "	0.83	0.094	40	7.3	7.0	150
22. ODR Jelenia Góra	0,87	0.082	25	6.1	4.7	0
23. "	1.25	0.144	48	7.1	6.4	140
24. ODR Karniowice	0.94	0.088	34	6.7	6.2	380
25. "	1.29	0.135	43	6.8	6.5 –	9900
26. ODR Kościerzyn	0.83	0.093	16	5.4	4.4	0
27. "	1.54	0.184	21	7.5	7.2	490
28. RZD Borusowa	1.55	0,140	53	7.3	6.5	540
29. "	0.99	0.105	36	7.5	6.3	97
30. "	1.66	0.120	51	6.9	6.3	8
31. RZD Grabów	0.75	0.052	19	7.0	6.1	130

Table 2. Correlation coefficients between numbers of cfu of *Azotobacter* spp. in the tested soils and their physical and chemical properties.

	% org. C	% total N	Soil fraction <0.02 mm	pH(H_2O)	pH(KCl)
All soils tested	0.095	0.152	0.293	0.149	0.195
Excluding soil No. 25	0.372*	0.584**	0.235	0.621**	0.646**

*, **Significant at P = 0.05 and P = 0.01, respectively

the Institute of Soil Science and Plant Cultivation (IUNG) in Puławy. The soil samples were provided by Regional Agriculture Advisory Centres (ODR) or by IUNG Agriculture Experimental Stations (RZD) shown in Table 1.

Results and Discussion

We detected various populations of *Azotobacter* spp. in 16 out of 31 different Polish soils examined in the year 2000 (Table 1). Thus, the percentage of soils with *Azotobacter* (51,6%) found in our studies was only slightly higher than that (50%) reported by Ziemięcka [1]. When present, numbers of these bacteria varied widely, from several cells (soils No. 17 and 31) to almost 10,000 cells (cfu) in soil No. 25. However, the extreme numbers were rare and in the majority of the soils with *Azotobacter* spp. populations of these bacteria varied within the range of about 100 to 1,000 cfu g^{-1} (Table 1). It is interesting to note that even though Ziemięcka used different methodology (liquid media) to detect and assess the most probable numbers (MPN) of *Azotobacter* spp., her estimates of soil populations of these bacteria were very similar to those presented in our work. For example, only in one soil was the MPN of *Azotobacter* spp. assessed as being "higher than 1,000 cells", while in other soils examined by Ziemięcka populations of these bacteria (when present) were most often placed within the range of 100-1,000 cells g^{-1}. Numbers of *Azotobacter* spp. reported by Zawiślak [3] also rarely exceeded 1,000 cfu per 1 g of soil.

Our work was not aimed at the detailed and exact comparison of the obtained results with those published by Ziemięcka [1]. This was impossible for several reasons. For example, besides the above-mentioned differences in the methods used to count populations of *Azotobacter* spp., we could not analyse the same soils as Ziemięcka because their exact locations were not given by the author. Having these restrictions in mind, comparison of our results with those described by Ziemięcka [1] seemed to us interesting and justified, particularly with respect to the percentage of soils colonised by *Azotobacter* spp. at the first two decades of the 20th century and over 80 years later. This comparison indicates that intensification of agricultural practices during the course of the past century did not significantly change the colonisation of Polish soils by the studied bacteria. 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 fertilisers 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 fertilisers may cause substantial acidification of soil, particularly when these fertilises are used in high doses without liming [11, 12, 13]. Since *Azotobacter* is very sensitive to soil acidity [1-9] one could expect that intensification of the use of mineral N fertilisers might cause acidification of agricultural lands and thus reduce soil populations of *Azotobacter* spp. Condensed description of changes in Polish agriculture, including the use of mineral fertilisers, in the 19th and 20th centuries, is given by Krasowicz [14]. For example, application of mineral N increased in Poland (on average) from less than 5 kg N ha^{-1} in the 1940s to about 70 kg N in the 1980s [14]. However, data presented by Lipiński [15] prove that acidification of Polish soils did not occur in the period between 1955 and 1999. On the contrary, a slight improvement of this property of Polish soils could be seen in that period. For example, the area of acidic soils (pH < 5.5) decreased from 58% in the decade 1955-64 to 55% in the period of 1994-99, and simultaneously, the area of soils having pH > 6.5 increased from 17% to 19% in the respective periods [15]. Thus, these data seem to correspond with a slightly higher percentage of soils colonised by *Azotobacter* spp. found in our studies as compared to that presented by Ziemięcka [1].

In order to study interactions between the occurrence of *Azotobacter* spp. in the tested soils and their properties we calculated correlation coefficients between some basic physical and chemical characteristics (shown in Table 1) of the soils and the numbers of the bacteria detected (Table 2). When all soils were included in the calculations no significant correlation was found between the analysed factors. However, when soil No. 25 intensively colonised by *Azotobacter* spp. was excluded from the calculations, numbers of these bacteria in the rest of the soils were highly (P = 0.01) correlated with soil pH (both measured in H_2O and KCl) and with total N content, and to a lower extent with organic C content in the soils (P = 0.05). It is not known why soil No. 25 contained the highest numbers of *Azotobacter* spp. The tested properties of this soil were within the range of characteristics presented for other soils harbouring markedly lower populations of *Azotobacter* spp. (Table 1). Soil No. 25 was the only one on which red clover was grown at the time of the collection of the samples. Strzelczyk [16] has shown that some

crops, e.g. radish and legumes, stimulate proliferation of *Azotobacter* spp. in their rhizosphere. On the other hand, Ziemięcka [1] also analysed two soils on which red clover was grown but no *Azotobacter* was detected in those soils. More research is needed on the effects of different crops and soil edaphic factors on populations of *Azotobacter* in the soil environment.

High correlation coefficients between reaction (pH) of the tested soils and the occurrence of *Azotobacter* spp. in soils confirm the well-known sensitivity of these bacteria to low soil pH [1, 2, 4, 5, 7], indicating that soil reaction is the main factor controlling colonisation of soils by these bacteria. In our study, bacteria from the genus *Azotobacter* were not detected in soils having pH(KCl) below 6.0. Similar results for Polish soils were reported by Zawiślak [3] and Szember *et al*. [6], and for soils in other countries by Geinley [2] and Mahmoud *et al.* [4]. Significant correlation between numbers of *Azotobacter* spp. and the contents of organic C and total N in the examined soils (Table 2) indicates that soil fertility is also an important factor influencing colonisation of soils by *Azotobacter*.

References

1. ZIEMIĘCKA J. Występowanie azotobaktera w glebach polskich. Rocz. Nauk Roln. **10**, 1, **1923**.
2. GEINLEY P.L. Influence of the absolute reaction of a soil upon *Azotobacter* flora and nitrogen fixing ability. J. Agric. Res. **24**, 907, **1923**.
3. ZAWIŚLAK K. Występowanie azotobaktera w glebach na stokach w województwie olsztyńskim. Rocz. Glebozn. **24**, 344 , **1973**.
4. MAHMOUD S.A.Z., EL-SAWY M., ISHAC Y.Z., EL-SAFTY M. M. The effect of salinity and alkalinity on the distribution and capacity of N_2-fixation by *Azotobacter* in Egiptian soils. In: Granhall U. (ed) Environmental Role of Nitrogen-fixing Blue-green Algae and Asymbiotic Bacteria, Uppsala, Sweden, pp. 99-109, **1978**.
5. MARTYNIUK S., WAGNER G.H. Quantitative and qualitative examination of soil microflora associated with different management systems. Soil Sci. **125**, 343, **1978**.
6. SZEMBER A., K. KRÓLIKOWSKA, J. KUSYK, J. NIEDZIELSKA, B. TYCZYNSKA, E. WIELGOSZ. Występowanie w glebach Kielecczyzny bakterii niesymbiotycznie wiążących azot atmosferyczny. W: Maliszewska W. (ed.) Mikrobiologiczne przemiany związków azotowych w glebie w różnych warunkach ekologicznych, IUNG, Puławy, pp. 113-121, **1981**.
7. BALANDREAU J. Ecological factors and adaptive processes in N_2-fixing bacterial populations of the plant environment. Pant and Soil **90**, 73, **1986**.
8. TCHAN Y-T., NEW P.B. Genus I. *Azotobacter* Beijerinck 1901. In: Holt J.G., Krieg N.R. (eds) Bergey's Mannual of Systematic Bacteriology, Vol. 1, Williams and Wilkins, Baltimore, pp. 220-229, **1984**.
9. DÖBEREINER J. Isolation and identification of aerobic nitrogen-fixing bacteria from soil and plants. In: Alef K., Nannipieri P. (eds) Methods in Applied Soil Microbiology and Biochemistry, Academic Press, London, pp. 134-141, **1995.**
10. FENGLEROWA W. Simple method for counting *Azotobacter* in soil samples. Acta Microb. Pol. **14**, 615, **1965**.
11. MYŚKÓW W., WRÓBLEWSKA B., STACHYRA A., PERZYŃSKI A. Effect of long-term organic and mineral fertilization on the biological fixation of atmospheric nitrogen and the productivity of light soils. Pam. Puł., **93**, 131, **1988**.
12. MERCIK S. Most important soil properties and yielding in long-term static fertilising experiments in Skierniewice. Rocz. Glebozn., **44(**Supl**)**, 71, **1994**.
13. FILIPEK T., KACZOR A. Dynamika antropogenicznej presji zakwaszenia rolniczej przestrzeni produkcyjnej w Polsce. W: Kukuła S. *et al*. (eds) Ochrona i wykorzystanie rolniczej przestrzeni produkcyjnej Polski, IUNG, Puławy, pp. 35-43, **1997**.
14. KRASOWICZ S. Produkcja roślinna na ziemiach polskich w XIX i XX wieku - rys historyczny. Pam. Puł. **130/I**, 11, **2002**.
15. LIPIŃSKI W. Odczyn i zasobność gleb w świetle badań stacji chemiczno-rolniczych. Nawozy i Nawożenie, **3(4)**, 89, **2000**.
16. STRZELCZYK E. Wpływ różnych roślin uprawnych na liczebność azotobaktera i *Clostidium* w ich rizosferze. Acta Microb. Polon. **7**, 112, **1958**.