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ASSESSMENT OF THE OCCURRENCE AND MOLECULAR DIVERSITY OF *AZOTOBACTER* IN POLISH SOILS

SUMMARY

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The research presented in this dissertation concerned soil bacteria belonging to the genus *Azotobacter*, characterized by e.g. the ability to assimilate atmospheric nitrogen. These studies were aimed at finding out: - the occurrence and abundance of *Azotobacter* spp. in the soils of Poland (1140 samples), - the relationship between properties of soil and their colonization by these bacteria, and - the differentiation and systematic affiliation of the isolated *Azotobacter* spp. isolates.

The conduced research demonstrated that the bacteria belonging to the genus *Azotobacter* inhabit about 43% of soils in Poland. These bacteria are present in all of the studied soil groups (types, species), but within these groups the percentage of soils colonized by *Azotobacter* spp. is very differentiated and ranges from 8% and 19% in the case of loose sands and weakly loamy sands to about 80% and 90% in the case of clay loam and cambisols. The high correlation coefficient ($r = 0.944$) between the content of floatable parts (< 0.02 mm) in various soil species and the percentage of their colonization by *Azotobacter* spp. proves that the silt-clay fraction of the soils is favorable for their colonization by these bacteria.

The total number of *Azotobacter* spp. in soils inhabited by these bacteria varies greatly within each analyzed soil group and ranges from a few cells (CFU) to several hundred thousand CFU g^{-1} dm of soil. The highest abundance was found in some loess, silts and weakly loamy sands, respectively 297 750, 45 800 and 40 000 CFU g^{-1} dm of soil. The highest mean numbers of *Azotobacter* spp. are found in loess, clays, cambisols and clayey silts, respectively 13 676, 2 213, 2 035 and 1 950 CFU g^{-1} dm of soil, and the lowest in gravels, weakly loamy sands and loose sands, respectively 44, 158 and 204 CFU g^{-1} dm of soil.

In line with the results of previous studies, the current studies have also shown that soil pH is a very important factor influencing the occurrence of *Azotobacter* spp. in the soils. These bacteria are present only in about 4% of very acidic soils ($pH \leq 4.5$) and in 27% of acidic soils

(pH 4.6-5.5), while the occurrence of bacteria belonging to the genus *Azotobacter* in neutral (pH 6.6-5.5) and alkaline (pH> 7.2) soils were 74% and 84%, respectively. The soil pH also significantly influences the population of *Azotobacter* bacteria in the soil environment. The highest average number of these bacteria is found in neutral soil.

Within some groups of soils, such as gleyic luvisols, cambisols, loess and loamy sands, the soils inhabited by the discussed bacteria contained much greater amounts of available P and Mg forms and total forms of Ca, Fe and Mo than soils with a similar pH and structure, but without *Azotobacter* spp. Therefore, it seems that apart from the pH, $C_{org.}$ and N_{total}, also other chemical properties of the soil have a significant influence on the colonization of soils by *Azotobacter* bacteria.

The studied isolates of *Azotobacter* spp. were characterized by a large diversification in terms of the effectiveness of atmospheric nitrogen binding in laboratory conditions. However, no significant influence of soil properties on the N_2-fixing capacity of the *Azotobacter* bacteria inhabiting them was found, because both from fertile soils with a neutral pH and from acidic soils, intensively N_2-fixing isolates and strains that were not very active in this respect were isolated.

The genomic polymorphism of the studied strains was determined by the ITS-PCR/RFLP technique (HaeIII, MspI). The conducted analyzes allowed to distinguish four genetic profiles within the studied *Azotobacter* spp. isolates, and based on the results of 16S rRNA gene sequencing, 30 isolates representing the above-mentioned profiles, it was found that they all belong to the species *Azotobacter chroococcum*, which indicates that this species is dominant in the soils of Poland.

Moreover, this research indicates that a relatively simple laboratory determination of the presence of *Azotobacter* spp. in soils may be a good biological indicator of soil quality assessment, e.g. for the purposes of monitoring changes in their fertility and pH.