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INFLUENCE OF SOME ABIOTIC FACTORS ON THE OCCURRENCE OF MYXOBACTERIA IN SELECTED FOREST SOILS OF PUSZCZA BIALA

WPŁYW CZYNNIKÓW ABIOTYCZNYCH NA WYSTĘPOWANIE MYKSOBAKTERII W WYBRANYCH GLEBACH LEŚNYCH PUSZCZY BIAŁEJ

Abstract: The aim of present paper was to establish the effect of some physical and chemical factors on the number and species composition of myxobacteria. The following chemical and physical factors were studied: pH, carbon and nitrogen content as well as terms of samples collection and depth of soil profile of selected forest soils (muck, gley) of Puszcza Biala The number of myxobacteria was determined by plate method using appropriate microbiological media. Isolated and purified strains were identified on the basis of their macro- and micromorphology using stereoscopic, light and scanning electron microscope.

The results showed that the highest number of myxobacteria was found in upper horizons of the examined soils. Higher pH and higher content of carbon as well as moderate humidity of muck soil have significant influence on higher number and species diversity of myxobacteria than in gley soil. It was found that two species of the cellulolytic myxobacteria: *Sorangium cellulosum* and *Polyangium compositum* dominated in whole profile of the investigated soils.

Keywords: myxobacteria, slime bacteria, soil microflora, forest soils, Puszcza Biala

Myxobacteria are microorganisms which have maintained, probably due to their unusual and complex life cycle as well as fascinating appearance, an unremitting attraction for many microbiologists all over the world. They are aerobic, unicellular, Gram-negative gliding bacteria with rod-shaped vegetative cells that mostly occur in many

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soil types. Myxobacteria frequently develop on decomposing plant material, the bark of living and dead trees or dung of herbivorous animals such as rabbit, hare, goat, deer and sheep [1–3].

They are unique among prokaryotes for their complicated multicellular behaviour. They use intercellular communication to engage in cooperative morphogenesis from which they produce unusual structures called fruiting bodies which contain myxospores [4]. Under starvation conditions the cells aggregate within the swarm, pile up and form fruiting bodies that allow the community to survive unfavourable environmental conditions [5–7]. Inside the maturing fruiting body the rod-shaped vegetative cells are converted into myxospores by shortening and fattening. Thick-walled myxospores are asexual, dormant cells that are responsible for the survival under hostile conditions such as desiccation, high and low temperature, high salt concentrations, anaerobic conditions, ultrasound and UV irradiation. They can survive in the environment for 10–25 years [2, 4, 8]. Myxospores germination is induced by favourable environmental conditions and in the laboratory by transfer to a suitable growth medium.

Myxobacteria also display other interesting features like social behaviour expressed by collective food uptake and cooperative motility [2, 9]. Their cells move by gliding over the surface of or within the substrate, so that colonies spread over the culture plate [10]. Cell motility plays an important role in development and morphogenesis in myxobacteria, especially in formation of fruiting bodies [11–13].

Myxobacteria are strictly aerobic organotrophs which prefer moderate temperature in the range between 9–38 °C. Myxobacteria specialize in the biodegradation of biomacromolecules. These organisms are divided into two sharply separated metabolic groups. Group 1 holds cellulose degraders. This group is capable of utilizing inorganic nitrogen compounds while growing on cellulose and glucose. Group 2, by far the majority of myxobacterial species, depend on an amino acid-containing medium such as peptone for growth. They obtain the required oligopeptides by hydrolyzing proteins [5]. In nature, these myxobacteria feed on other microorganisms like eubacteria or yeasts, that therefore have been called micropredators [2, 14].

Myxobacteria have some potential for various applications. They play a substantial role in natural environment in solubilizing large macromolecules, cell carcasses and other biological detritus. Their predatory activity can be very useful to control cyanobacterial water blooms by destroying cells of cyanobacteria in aqueous environments. Myxobacteria can also be used as pollution indicators which can inform us about environmental problems. A good opportunity for application may lie in the field of myxobacterial enzymes such as restriction endonucleases and special proteases which can be very useful in biochemistry and medicine. The most promising opportunities for biotechnological applications with myxobacteria is the production of secondary metabolites, especially compounds with biological activity such as antibiotics and other inhibitors like epothilon with antitumor activity. These substances are promising candidates for the development of useful drugs [2, 4].

The aim of present paper was to establish the effect of some physical and chemical factors on the number and species composition of myxobacteria. The following abiotic factors were studied: pH, carbon and nitrogen content as well as terms of samples collection and depth of soil profile of selected extremely acid, forest soils of Puszcza Biala.

Materials and methods

The soil samples were taken from Puszcza Biala from June to September 2006. The investigation was carried out on the muck and gley soils. The samples collected from all genetic horizons of investigated soils were analysed. The number of myxobacteria was determined by plate method by inoculation of appriopriate microbiological media with clumps of soil and soil suspensions 10–1, 10–2, 10–3. Inoculated microbiological media were incubated at 30 °C for 1–4 weeks and checked daily, beginning with the second and third day, under a dissecting microscope for the appearance of myxobacterial swarms and fruiting bodies. After an incubation period the colonies and fruiting bodies zones were counted to determine the number of myxobacteria in examined soils. Obtained results were expressed as CFU (colony forming units) in 1 g dry mass of soil.

For isolation and purification of bacterial strains the following media were used: VY/2 containing autoclaved yeasts (*Saccharomyces cerevisiae*) as the nourishment and B12 source [15, 16], water agar with mineral salts and filter paper as carbon source and CY containing casitone (Difco) as rich source of nitrogen [4]. To inhibit fungal growth 100 $\mu g \cdot cm^{-3}$ cycloheximide or nystatine to media, sterile water used to moisten clumps of soil and soil suspensions was added.

In order to obtain pure cultures isolated strains were purified by following methods [5]:

– direct purification from swarm or fruiting bodies to a suitable agar medium: predatory myxobacteria-CY, VY/2, MD1, CAS, CT, water agar with streaks of E. coli [5, 16]; cellulolytic myxobacteria-water agar with filter paper and mineral salts, ST21CX, CEL3, STAN-6 [5].

– heating of the suspension of the mature fruiting bodies at 58 °C for 10, 20 and 40 min.

– purification of the suspension of the mature fruiting bodies with mixture of antibiotics such as chloramphenicol, streptomycin, tetracycline, kanamycin, erythromycin, gentamycin (20–30 $mg \cdot 50\ cm^{-3}$ of water).

Isolated and purified strains of myxobacteria were identified on the basis of morphology of vegetative cells, myxospores, fruiting bodies and colonies using stereoscopic, light and scanning electron microscope. Furthermore for the identification of pure and enrichment cultures the determination keys of McCurdy and of Reichenbach and Dworkin were applied [5, 17].

Statistical methods were used to determine the influence of some abiotic factors of selected forest soils of Puszcza Biala (terms of samples collection, depth of soil profile as well as pH, carbon and nitrogen content) on the occurrence of myxobacteria. There were conducted one and two-factor analysis of variance as well as Tukey's studentized range test using SAS 9.1 software (SAS Institute INC., Cary, NC).

Results and discussion

Investigated soils from Puszcza Biala (muck and gley) have different chemical and physical properties which affected on the number and species composition of the population of myxobacteria (Table 1).

The higher number and higher biodiversity of myxobacteria were found in muck soil than in gley soil (Fig. 1, Table 2).

The results showed that myxobacteria mostly occurred in surface layers of soil-AM horizon. In deeper horizons the number of myxobacteria rapidly decreased reaching the lowest values in Dg horizon. These results are in good agreement with those of Krzemieniewska and Krzemieniewski [18], Gorny [19] and Reichenbach [3]. This fact was probably caused by shortage of oxygen and organic matter in deeper horizons of investigated soil which make unfavourable living conditions for this group of microorganisms.

Table 1

Characteristics of soil profiles of muck and gley.

Profile	Systematics of investigated soils	Genetic horizons	Depth [cm]	pH		%		C:N
				H2O	KCl	C	N	
21a	Type: muck soil Order: post-bog soils Division: hydrogenics soils	AM	5–10	5.1	4.6	36.50	2.09	17.46
		Mt	20–30	5.2	4.9	17.40	0.95	18.31
		Dg	50–60	6.3	5.8	1.10	0.07	15.71
35	Type: gley soil Order: bogged soils Division: semi-hydrogenics soils	Ol	0–1	4.8	4.2	18.59	1.77	10.50
		A	1–17	4.1	3.4	6.65	0.63	10.44
		Gor	17–50	6.4	5.2	0.09	0.01	6.92
		Gr	50–65	6.7	5.6	0.02	–	–

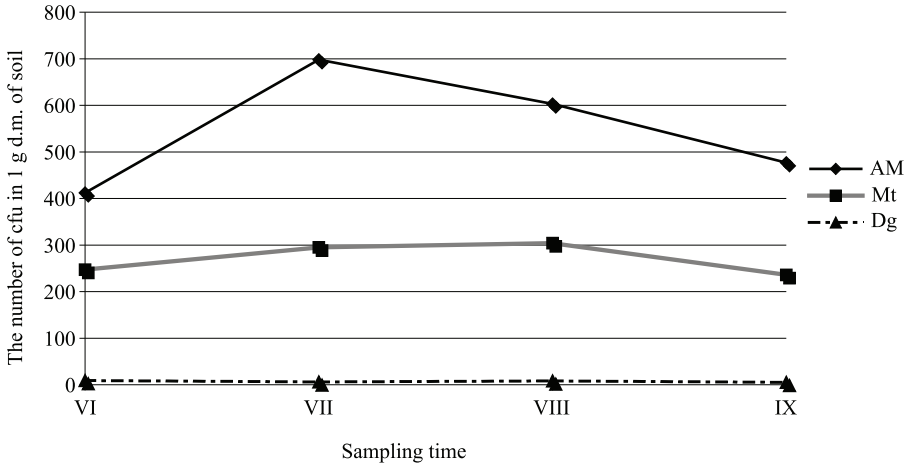

Fig. 1. The occurrence of myxobacteria in muck soil

Qualitative analyses also comprised dynamics of the number of myxobacteria in various seasons of the year. It was confirmed that the number of myxobacteria has increased from June with the maximum in July (AM horizon) and August (Mt horizon). The fall of the number of examined microorganisms was observed in September what

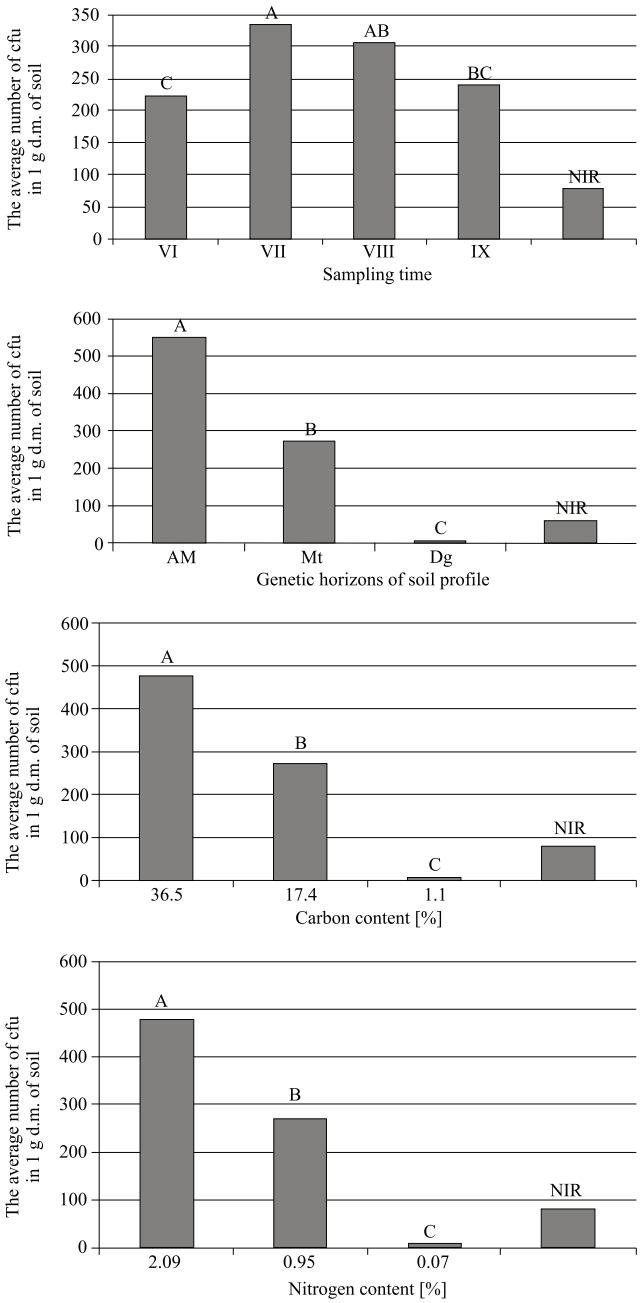

Fig. 2. Results of Tukey's test concerning the influence of abiotic factors on the number of myxobacteria in muck soil (Tukey's homogenous groups are represented by letters A through C. Bars in group A have the highest number of myxobacteria whereas bars in groups B to C have successively lower number of myxobacteria. Bars that have letters in common do not differ significantly from one another

was connected with drop of the air temperature as well as higher intensity of atmospheric falls.

Results of two-factor analysis of variance showed a significant influence of season and depth of soil profile on the number of myxobacteria in muck soil. Furthermore one-factor analysis of variance showed a significant influence of carbon and nitrogen content on the number of myxobacteria in examined soil.

Results of Tukey's test showed a statistically significant differences of the averages of the number of myxobacteria depending on season. There were identified three homogenous groups with the highest average of the number of myxobacteria in July and the lowest average in June.

Taking into consideration the influence of depth of soil profile there were observed a significant differences in each of three genetic horizons of muck soil. Tukey's test identified three homogenous groups with the highest average of the number of myxobacteria in AM horizon and the lowest average in Dg horizon.

Results of Tukey's test also showed a significant differences of the number of myxobacteria depending on carbon and nitrogen content. Tukey's test identified three homogenous groups with the highest average of the number of myxobacteria at the highest carbon and nitrogen content in muck soil.

Table 2

Species composition and frequency of isolation of myxobacteria from muck soil

Species of myxobacteria	Frequency of isolation [%]
Polyangium compositum	43.00
Sorangium cellulosum	34.67
Myxococcus fulvus	11.67
Angiococcus disciformis	6.33
Corallococcus coralloides	1.33
Polyangium septatum	1.00
Polyangium sorediatum	0.67
Cystobacter fuscus	0.67
Polyangium aureum	0.33
Polyangium luteum	0.33

The most often occurring myxobacteria were representatives of the *Sorangineae* suborder such as cellulolytic species *Polyangium compositum* and *Sorangium cellulosum* isolated from whole genetic horizons of examined soil profile. There were also observed myxobacteria belonging to the *Cystobacterineae* suborder such as *Myxococcus fulvus* and *Corallococcus coralloides*, especially in surface layers of soil. As pointed out by Krzemieniewska and Krzemieniewski [18, 20] and Dawid [2] in acid soils occur characteristic flora of myxobacteria with the majority of representatives belonging to *Sorangium* and *Polyangium* genuses as well as some species of *Myxococcus* genus, especially *Myxococcus fulvus*.

The second type of investigated soil-gley have dissimilar properties to muck soil. This soil was formed on the area with high level of groundwater which cause high humidity and anaerobic conditions creating negative influence on the occurrence of myxobacteria (Fig. 3, Table 3).

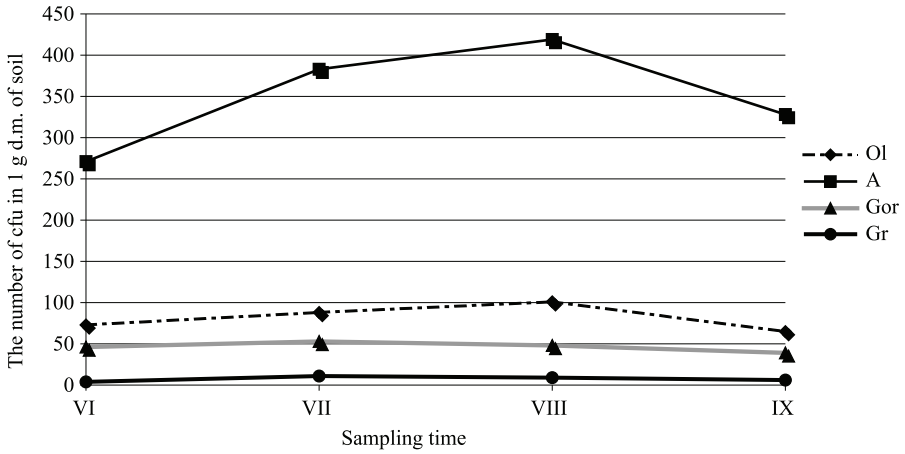

Fig. 3. The occurrence of myxobacteria in gley soil

Table 3

Species composition and frequency of isolation of myxobacteria from gley soil

Species of myxobacteria	Frequency of isolation [%]
Sorangium cellulosum	49.00
Polyangium compositum	21.33
Myxococcus fulvus	12.33
Corallococcus coralloides	9.33
Angiococcus disciformis	7.00
Cystobacter minus	0.67
Polyangium septatum	0.67
Polyangium luteum	0.34

The results showed that distinct majority of myxobacteria occurred in A horizon. There was observed the considerably lower number of myxobacteria in Ol horizon, even though it is upper horizon. It is directly connected with fact that dead leaves are not major and most convenient environment of living of these microorganisms, so that this horizon is settled by selected species. Furthermore, similar to muck soil, the number of myxobacteria decreased in deeper horizons of soil profile because of high saturation of soil with water causing anaerobic conditions as well as low content of organic matter, which is in agreement with the previous observations of Krzemieniewska and

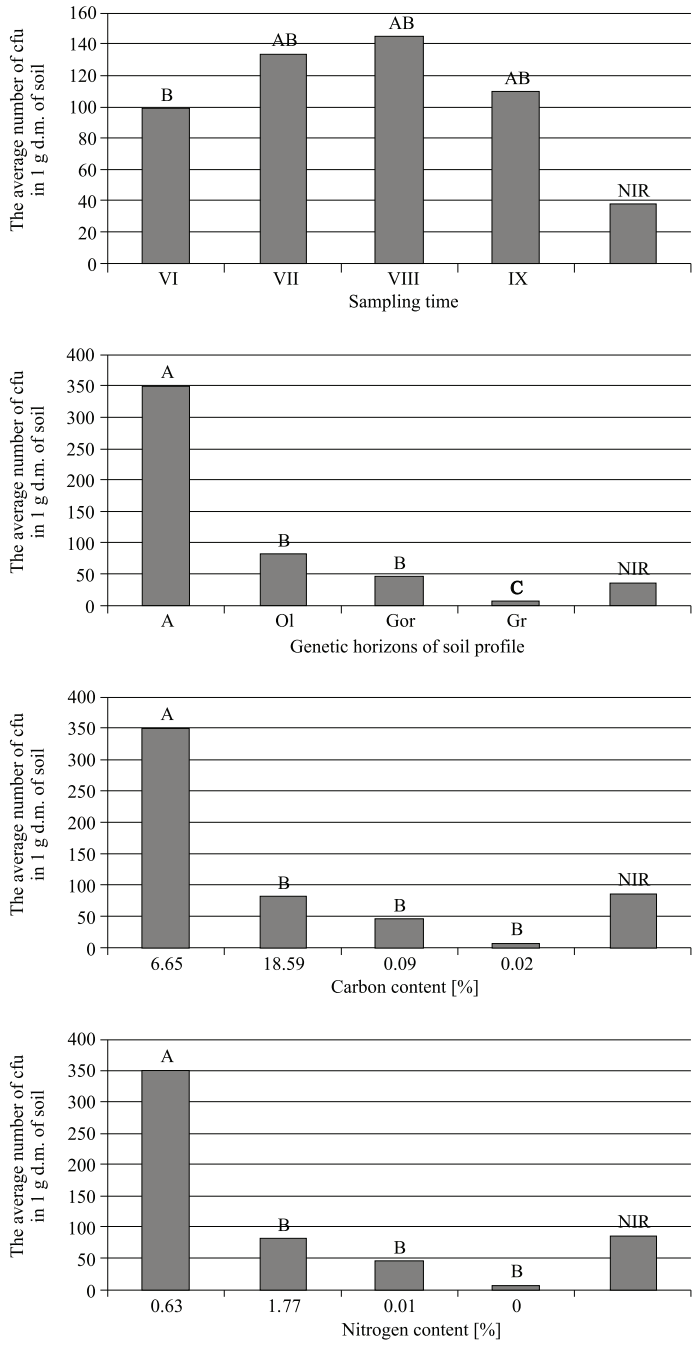

Fig. **4**. Results of Tukey's test concerning the influence of abiotic factors on the number of myxobacteria in gley soil.

Krzemieniewski [18, 20] and Reichenbach [3].

The number of myxobacteria in gley soil, similar to muck soil, increased from June reaching maximum in August. With early autumn in September there was observed the lower number of myxobacteria than in summer, what is probably connected with cooling and intensification of atmospheric falls causing high humidity of soil.

Similar to muck soil results of two-factor analysis of variance showed a significant influence of season and depth of soil profile on the number of myxobacteria in gley soil. Furthermore one-factor analysis of variance showed a significant influence of carbon and nitrogen content on the number of myxobacteria in examined soil.

Results of Tukey's test showed a statistically significant differences of the averages of the number of myxobacteria depending on season. There were identified two homogenous groups with the highest average of the number of myxobacteria in August and the lowest average in June.

There were also observed a significant differences of the number of myxobacteria depending of depth of soil profile. Tukey's test identified three homogenous groups with the highest average of the number of myxobacteria in A horizon and the lowest average in Gr horizon.

Taking into consideration the influence of carbon and nitrogen content in examined soils there were identified two homogenous groups. The influence of these factors on the number of myxobacteria were not so significant as in muck soil.

The results showed that dominating myxobacteria were *Sorangium cellulosum* and *Polyangium compositum*. There were also commonly found *Myxococcus fulvus*, *Corallococcus coralloides* and *Angiococcus disciformis* mainly isolated from Ol and A horizons. Obtained results confirm data published by Krzemieniewska and Krzemieniewski [18, 20] and Dawid [2].

Summarizing muck soil is more favorable environment of living of myxobacteria than gley soil. This fact is the result of higher reaction of upper horizons of muck soil, higher content of carbon, insignificant higher content of nitrogen and moderate humidity in comparison with gley soil.

Conclusions

1. Myxobacteria are strictly aerobic microorganisms occurring in surface layers of examined soils.
2. The number and species composition of myxobacteria are strictly dependent on type of soil, physicochemical properties of soil and atmospheric factors.
3. Dominating myxobacteria were representatives of cellulolytic species which play important role in carbon cycle.

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WPŁYW CZYNNIKÓW ABIOTYCZNYCH NA WYSTĘPOWANIE MYKSOBAKTERII W WYBRANYCH GLEBACH LEŚNYCH PUSZCZY BIAŁEJ

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Abstrakt: Celem pracy było określenie wpływu czynników fizykochemicznych, tj. pH, zawartości węgla i azotu oraz terminu pobrania próbek i głębokości profilu glebowego wybranych gleb leśnych Puszczy Białej (murszowa, gruntowo-glejowa) na liczebność oraz skład gatunkowy myksobakterii. Liczebność myksobakterii określono za pomocą metody płytkowej. Wyizolowane i oczyszczone szczepy zidentyfikowano na podstawie charakterystyki makro- i mikromorfologicznej z wykorzystaniem mikroskopii stereoskopowej, świetlnej i skaningowej mikroskopii elektronowej.

Wyniki analiz ilościowych wykazały, że myksobakterie najliczniej zasiedlają powierzchniowe poziomy genetyczne badanych profilów glebowych. Większe pH, większa zawartość węgla oraz umiarkowana wilgotność gleby murszowej wpływają znacząco na większą liczebność oraz większą różnorodność gatunkową myksobakterii w porównaniu z glebą gruntowo-glejową. W badanych glebach dominowały myksobakterie celulolityczne z gatunku *Sorangium cellulosum* i *Polyangium compositum*.

Słowa kluczowe: myksobakterie, bakterie śluzowe, mikroflora gleby, Puszcza Biała