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Development and Metabolic Activity of Bacteria Isolated from Polyhumic Stęgwica Lake

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Abstract

This study concerns developmental intensity and metabolic activity of heterotrophic bacteria isolated from water of the littoral and pelagial zones of the polyhumic lake Stęgwica. The experiments were carried out with humic substances and [^{3}H]-glucose in media with pH 6.0 and 8.0. It was found that the development and metabolic activity of most strains in the presence of humic substances were higher in the medium with pH 8.0 than in that with pH 6.0. It was found that humic substances in acid medium had an inhibitory effect on the development and metabolic activity of 90% of the strains of bacteria isolated from littoral zone and of 50% of the strains isolated from the pelagial zone.

Keywords: heterotrophic bacteria, polyhumic lake, humic substances, littoral, pelagial.

Introduction

There is still little information about the role of humic substances in aquatic ecosystems and their effect on the microorganisms living in them. According to Jones and Salonen [1], the amount of organic matter created in the process of primary production in humic lakes is too small to maintain a high level of secondary production. According to Tranvik and Höffle [2] and Tranvik [3], humic substances in the total amount of dissolved organic matter (DOM) constitute an important source of carbon for heterotrophic bacteria. According to those researchers, allochtonous organic matter, including humic substances is the main source of carbon for bacteria in dystrophic lakes, providing up to 90% organic carbon required to maintain the secondary production noted in those lakes. Hessen [4] thinks that humic substances are too poor in nutrients, particularly nitrogen and phosphorus, to be the only source of nutrient substance for heterotrophic bacteria. According to Jones [5], the organic carbon coming from photosynthesis does not play a significant quantitative role relative to the bacterial biomass produced in a humic lake. However, that source of organic matter may constitute a qualitatively important pool of nutrient substrates necessary for optimal growth of bacteria. According to Wetzel [6] and Hobbie [7], in humic lakes refractory DOM (including humic substances) stabilizes the development of bacterial plankton, enabling it to grow slowly and steadily, independent of the seasonal appearance of autochthonous organic matter from photosynthesis. The object of the present work was to find out how humic substances affect the metabolic activity of heterotrophic planktonic bacteria, the intensity of their development, and how their effect depends on the pH of the medium.

Materials and Methods

Study Area

The studies concerned heterotrophic bacteria isolated from the surface water of the littoral and the pelagial zone of lake Stęgwica. This lake, which formed during the last glaciation is situated in the Iława Lakeland, about 5 km north of the town of Iława. Lake Stegwica is a highly eutrophized lake of the alloiotrophic type. Its high humic substance content gives the water brown coloring, and the inflow of calcium-saturated humic substances induces the formation of the so-called "sweet humus", which besides containing a large amount of organic matter does not have any significant effect on the composition and quantitative fluctuations of the basic nutrients [8].

Sampling

Water samples for microbiological studies were taken in the summer (20.07.1996) from a depth of 20-30 cm in the littoral and pelagial zones of lake Stęgwica using a Ruttner

sampler. The water was poured into sterile glass bottles, then placed in a container with ice (the temperature inside did not exceed +7°C), taken to the laboratory and analyzed immediately. Generally, the time between sample collection and analysis did not exceed 6 hours.

Isolating Bacterial Strains

The strains of bacteria were isolated using the spread plates method and iron-pepton media after Ferrer, Stapert and Sokolski [9]. Water sample diluted with sterile buffer water [10] was plated over the medium surface, and after 10 days of incubation at 20°C, bacterial colonies were randomly isolated and transferred to a semisolid iron-peptone medium. After checking the purity of the cultures, the strains were stored in a refrigerator at +4°C for further study. Every two months they were transferred to a fresh semisolid medium.

Measurement of Development Intensity of Bacteria in the Presence of Humic Substances

The bacterial strains were multiplied on slants of iron-pepton medium at 20°C for 72 hours. Next the bacteria were washed off with 3 cm^3 physiological salt solution (NaCl 0.85%), centrifuged at 15,000 rpm for 10 minutes, and the bacterial sediment was washed twice with physiological salt solution. The washed bacteria were resuspended in physiological salt solution, adjusting the optical density of the suspension to 0.15 value at wavelength λ = 560 nm (corresponding to about 10^9 bacterial cells per cm^3) using a Spekol 221 spectrophotometer (Carl Zeiss, Jena). In order to determine the intensity of bacterial development in the presence of humic substances as the only source of organic carbon and nitrogen, one loopful of bacterial suspension (prepared as above) was used to inoculate mineral medium B containing: K_2HPO_4 – 1.0 g, KNO_3 – 0.5 g, $MgSO_4 \cdot 7H_2O$ – 0.4 g, $CaCl_2$ – 0.2 g, NaCl – 0.1 g, $FeCl_3$ – 0.01 g, H_2O (dist.) – 1000 cm^3, to which humic substances sodium salt (Fluka) were added until a concentration of 100 mg $\cdot$ dm^{-3} was obtained. The intensity of bacterial development was estimated after 3 and 6 days of culturing at 20°C by measuring the turbidity of the medium compared with a blank (uninoculated medium) by means of a Spekol 221 spectrophotometer (Carl Zeiss, Jena) at wavelength λ = 560 nm.

Determination of Metabolic Activity of Bacteria in the Presence of Humic Substances

The metabolic activity of the bacteria was determined by measuring the rate of incorporation of [^{3}H]-glucose as cometabolite of humic substances. The bacterial strains were multiplied at 20°C for 72 hours in fluid medium A after Lechead and Chase [12], with pH 6.0 and 8.0. Then 9 cm^3 bacterial suspension was collected from each culture (in duplicate) and centrifuged at 15,000 rpm for 10 min. The bacterial sediment was washed three times with mineral medium B (as above) and finally the washed bacteria from the first replication were resuspended in 5 cm^3 mineral medium B with a supplement of 100 mg $\cdot$ dm^{-3} humic substances sodium salt (Fluka). From these series, 1.2 cm^3 bacterial suspension was taken in six replications and transferred to Eppendorf flasks (3 samples for study and 3 controls). To each sample for study were added 0.1 cm^3 [^{3}H]-glucose aquatic solution (0.1 μCi = 1.2 ng glucose) and 0.1 cm^3 isotope unlabelled glucose solution (40.8 ng glucose). After being stirred, the samples were incubated at 20°C for 2 hours. The incubation was stopped by adding 0.1 cm^3 40% formalin to each sample. To the control samples 0.1 cm^3 40% formalin was added at start of the experiment (at time t_0), and after 15 minutes 0.1 cm^3 [^{3}H]-glucose solution and 0.1 cm^3 isotope unlabelled glucose solution were added to each sample. The formalin-fixed samples, both experimental and control, were centrifuged at 15,000 rpm for 20 minutes, the sediment was washed three times with mineral medium B, then 1 cm^3 scintillation liquid Opti Phase Hi Safe was poured over the sediment and the whole was stirred thoroughly. Bacterial cell radioactivity was measured in a Wallac 1409 Liquid Scintillation Counter. The amount of glucose incorporated was calculated from the formula:

$$PG = \frac{(R_{exp} - R_{cont})}{(R_{gluc})} \times (S) \times \frac{1}{T}$$

where:

- PG – rate of glucose uptake by the bacterial suspension (μg glucose $\cdot$ cm^3 $\cdot$ h^{-1})
- R_{exp} – radioactivity in experimental samples (DPM)
- R_{cont} – radioactivity of control samples (DPM)
- R_{gluc} – total radioactivity of the added [^{3}H]-glucose (DPM)
- S – total concentration of substrate, i.e. isotope + unlabelled glucose (30 μg $\cdot$ dm^{-3})
- T – incubation time (2h).

Considering the different numbers of bacterial cells in the particular samples, the results were normalized to one cell according to the formula:

$$SPG = \frac{PG}{N}$$

where:

- SPG – μg glucose $\cdot$ $cell^{-1}$ $\cdot$ h^{-1}
- PG – rate of glucose uptake by bacterial suspension (μg glucose $\cdot$ dm^{-3} $\cdot$ h^{-1})
- N – number of bacteria in the suspension used in the test (cells $\cdot$ dm^{-3}).

Results

The development of planktonic bacteria isolated from the pelagial and the littoral zones of lake Stęgwica in the presence of humic substances is illustrated in Figs. 1 and 2. It follows from them that except for strains L1 and P1, which developed better in the medium with pH 6.0, all the remaining bacteria showed more intensive development in the medium with pH 8.0.

From the studies carried out in alkaline medium (with pH 8.0), it also follows that most of the strains isolated from the littoral showed greater metabolic activity in the presence of humic substances than the cultures kept in a mineral medium containing glucose as the only source of

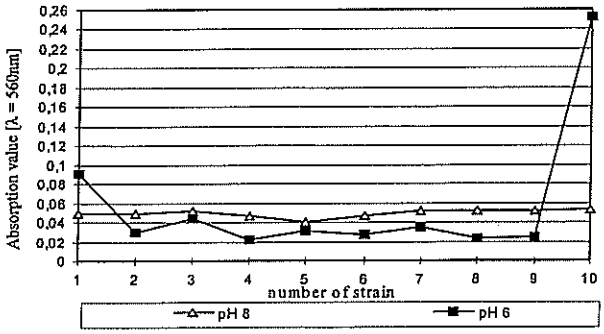


Fig. 1. Intensity of development of bacteria isolated from the littoral zone of Stęgwica lake in medium B with humic substances added.

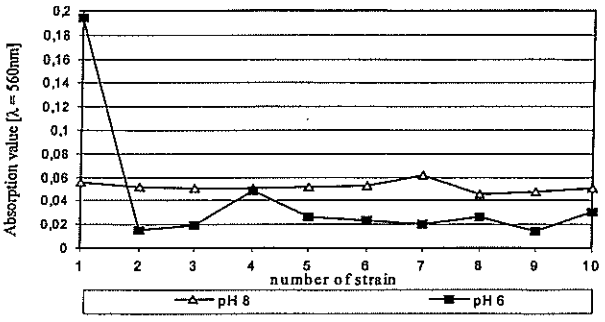


Fig. 2. Intensity of development of bacteria isolated from the pelagic zone of Stęgwica lake in medium B with humic substances added.

Table 1. Values of SPG bacterial strains isolated from the littoral zone of lake Stęgwica.

Studied strain	pH 8.0		pH 6.0	
	Medium 1*[1]	Medium 2*[2]	Medium 1	Medium 2
L1	2.26831E-07*[3]	7.13186E-07	1.01464E-06	5.30019E-07
L2	6.15952E-06	6.23382E-06	2.3785E-06	2.9817E-07
L3	4.86492E-07	5.20704E-07	3.80936E-07	3.06787E-08
L4	3.68522E-07	3.69334E-07	2.40741E-06	2.0665E-07
L5	3.87631E-07	7.67561E-07	1.49626E-07	2.32235E-08
L6	3.32238E-06	3.61813E-06	3.69228E-07	2.98639E-07
L7	5.87758E-06	5.27206E-06	6.0536E-06	4.39017E-06
L8	1.08882E-06	1.30559E-06	6.09117E-07	7.85265E-07
L9	5.22817E-07	3.80005E-06	3.87569E-06	3.75981E-06
L10	8.3815E-07	7.87644E-07	1.31098E-06	3.44751E-07

Explanations:
*[1] – Medium 1 - medium B without humic substances,
*[2] – Medium 2 - medium B with humic substances added,
*[3] – values SPG per 1000 bacterial cell

carbon and energy (Table 1, Fig. 3). A similar phenomenon was found in bacteria isolated from the pelagial of the lake under study: only in the case of strain P6 a drop in metabolic activity was observed in that pH (Table 2, Fig. 4).

In acid medium (pH 6.0) humic substances had an inhibitory effect on most bacteria isolated from the littoral zone, causing a drop in their metabolic activity. The behaviour of strains isolated from the pelagial zone was not so explicit. Half of them showed an increase in metabolic

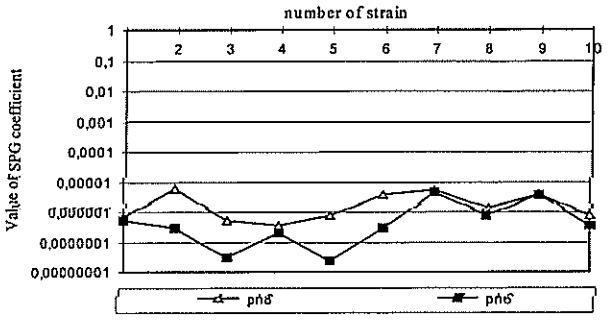


Fig. 3. Metabolic activity of bacteria isolated from the littoral zone of Stęgwica lake in medium B with humic substances added.

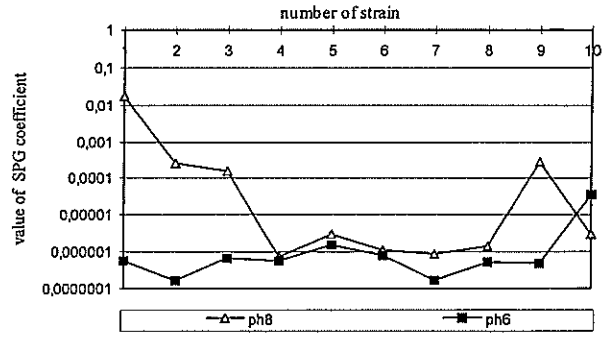


Fig. 4. Metabolic activity of bacteria isolated from the pelagic zone of Stęgwica lake in the medium B with humic substances added.

Table 2. Values of SPG bacterial strains isolated from the pelagic zone of lake Stęgwica.

Studied strain	pH 8.0		pH 6.0	
	Medium 1*[1]	Medium 2*[2]	Medium 1	Medium 2
P1	0.00256391*[3]	0.0172505	5.57915E-07	5.62116E-07
P2	6.54747E-06	0.0002524	4.0568E-07	1.64779E-07
P3	0.000120949	0.000152862	6.19422E-06	6.779225E-07
P4	3.74211E-07	6.9797E-07	5.17399E-07	5.66497E-07
P5	2.83641E-06	2.95559E-06	9.83288E-07	1.48536E-06
P6	2.87251E-06	1.0841E-06	2.98875E-06	8.07667E-07
P7	3.54963E-07	8.75328E-07	3.86128E-07	1.70551E-07
P8	2.67938E-08	1.42463E-06	2.96212E-06	5.02096E-07
P9	8.20272E-05	0.000285291	1.2376E-06	4.84047E-07
P10	2.00237E-06	3.030004E-06	3.15998E-05	3.57351E-05

Explanations: as in Table 1.

activity in the presence of humic substances, while the remaining showed a decrease.

It follows from the present studies that the effect of humic substances on developmental intensity and metabolic activity of bacteria clearly depends on the pH of the medium. In the alkaline medium (pH 8.0), humic substances inhibit the development and metabolic activity of bacteria to a lesser degree than in the acid medium (pH 6.0), and in some cases they even stimulate the processes in question. Acid medium (pH 6.0) causes an increase in inaccessibility, and even an effect of toxicity of humic substances for

most microorganisms, which is reflected by a drop in their metabolic activity and rate of development.

Discussion

Heterotrophic bacteria constitute the most important group of organisms participating in the transformation of matter and energy in ecosystems. In lakes they mineralize more than half the organic matter [12]. The metabolic activity and growth rate of the number and biomass of bacteria depend to a large extent on environmental factors such as: temperature, oxygen content, concentration of hydrogen ions, and on the quantity and quality of accessible nutrient substances.

The organic substances contained in dissolved organic matter (DOM) occur both in the form of simple, easily assimilable compounds (amino acids, monosaccharides) and in the form of macromolecular compounds. While the lowmolecular substances constitute the nutrient base for many groups of microorganisms, the macromolecular compounds (> 20kD) cannot be assimilated directly but must first undergo enzymatic hydrolysis by exoenzymes. That process is carried out most effectively by heterotrophic bacteria. Considering that in polyhumic lakes humic substances constitute the major part of DOM, those substances are a quantitatively essential source of carbon for microorganisms [7].

The present results indicate that the alkaline medium (pH 8.0) is most favourable to humic substance biodegradation by heterotrophic bacteria. Considering that most polyhumic lakes are strongly acid, this may provide a partial explanation of the occurrence of those substances and their inaccessibility to bacteria. However, in the case of lake Stęgwica the problem seems to be more complicated, since its pH ranges around 8. The favourable effect of that pH obtained in the present studies may be partly due to adaptation of the microorganisms living there. The pH of the medium as an external factor has a definite effect on microorganisms, and in particular on the activity of their exoenzymes. In studies concerning the activity of bacterial exoenzymes, the optimal pH for five of them has been established by Munster et al. [13]. According to those authors, glucosidases (α- and β-glucosidases and exocellobiohydratases) showed maximum activity at pH 5.4, while leucino-aminopeptidase at pH 7.8. In the case of phosphatases, two optima of activity have been found: a higher one at pH 5.4 and a slightly lower one at pH 8.5. It follows that the bacteria should show maximum metabolic activity in acid medium.

In our study of samples with humic substances supplement, however, higher metabolic activity was noted at pH 8.0 (Figs. 3 and 4). James [14], while studying the effect of humic substances on the rate of bacterial biomass production, noticed a favourable effect of these substances on the parameters under study, but only at concentrations not exceeding 20 mg · dm^{-3}. At higher concentrations humic substances were found to have an inhibitory effect on the development of microorganisms due to the large amounts of phenol components occurring in them. The humic substance concentration used in the present study was 100 mg · dm^{-3}, since in our earlier work [15] it was found that that was the concentration at which microorganisms showed maximum biomass increment. It must be stressed that James [14] used natural humic substances isolated from a lake (which are known to be characterized with great variability), while in the present study commercial humic substances were used (humic substances sodium salt, Fluka), which maybe contain fewer phenol components.

There are also good grounds for presuming that phenol groups contained in humic substances may show different inhibitory effects on heterotrophic bacteria. According to Steelink [16], in alkaline solutions the humic substances macromolecule twist is stronger than in acid solution. With this kind of conformation, components of hydrophobic character are closed inside the macromolecule, while mainly polar aliphatic hydrocarbons are exposed externally. In this situation the latter can be enzymatically cut off and subsequently metabolized by bacteria. Lowering of pH causes loosening of the macromolecule's structure and a reduction of the number of twists. Such conformation should, on the one hand, facilitate microorganism access to the particular components, but on the other hand it makes phenol groups exposed outside cause inhibition of enzymatic activity [17]. That presumption was confirmed when after adding polyvinylpirolidyne (PVP — which inactivates phenol groups) to the humic substances solution, the activity of the particular enzymes was found to be 10-23% higher compared with control samples [13].

The higher metabolic activity of bacteria in the presence of humic substances and in the medium with pH 8.0 observed in the present study is probably a derivative of the changes in conformation of humic substance macromolecules. Only in the case of strain P10 was metabolic activity found to be higher in acid medium. However, that particular strain showed similar properties in samples devoid of humic substances, which suggests that acid medium is optimal for its development. That interpretation is supported by the fact that many of the remaining 19 strains in samples devoid of humic substances showed higher metabolic activity in the medium with pH 6.0. That rules out adaptation to natural reaction as the main factor increasing the strains; activity in pH 8.0 in the presence of humic substances. Moreover, eight of the strains from the littoral zone showed increased metabolic activity in alkaline medium in the presence of humic substances compared with samples with the same pH devoid of humus. That suggests that under proper conditions humic substances may have a favourable effect on the activity of bacterial cells and constitute an important source of nutrient substances.

Though a similar result was obtained for only one half of the strains isolated from the pelagial, it must be taken into account that humic substances get into lake Stęgwica mainly from the catchment area, where probably a large proportion of microorganisms are capable of biodegradation of humic substances. That is why more bacteria previously adapted to the presence of humic substances may occur in the littoral than in the pelagial zone. Adaptation of bacteria to humic substances has been found by Tranvik and Höffle [2]. In their experiment, strains coming from humic lakes developed much better in the presence of phenol components than those isolated from non-humic lakes. In Kalinowska's [15] experiment, in the presence of humic substances as the sole source of carbon and nitrogen bacteria showed a slow, gradual increase in biomass, followed by a slight decrease in numbers and biomass in the sample, then another increase. According to Tranvik [3], the increase rate of the bacterial biomass in humic lakes is directly proportional to the amount of humic substances occur-

ring in them. Tranvik and Höffle [2] found that bacteria isolated from polyhumic lakes were capable of growth in the presence of phenol, which imitated humic substances in the experiment.

They also found that, although the curves of growth rate from strains from humic and non-humic lakes were similar, the production of bacterial biomass in the presence of phenol was twice as much in cultures isolated from humic lakes. Moreover, they observed that the increase in biomass bacteria isolated from humic lakes was due mainly to the decomposition of phenol, and only partly to metabolizing glucose, while with the cultures of strains from non-humic lakes the reverse took place [2]. Those data confirm our results, indicating that for bacteria of polyhumic lakes humic substances constitute a substantial pool of assimilable organic matter.

Likewise, the results of the experiment carried out in this study, aimed at estimating the intensity of bacterial development in the presence of humic substances as the only source of carbon, lend support to the presumption that HS can constitute an important source of accessible organic matter. As follows from Figs. 1 and 2, the medium with pH 8.0 provided better conditions for bacterial development than the medium with pH 6.0, which lends additional support to the above data. The results of the present studies confirm earlier observations concerning the ability of heterotrophic bacteria to use humic substances as nutrient substrate, particularly at higher pH.

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