Polish Journal of Environmental Studies Vol. 10, No. 5 (2001), 331-336

Occurrence of Chitinolytic Bacteria in Water and Bottom Sediment of Eutrophic Lakes in Iławskie Lake District

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Received: 2 May, 2001
Accepted 22 May, 2001

Abstract

A study was carried out on the occurrence of chitinolytic bacteria inhabiting surface water, water above the sediment and bottom sediments of lakes: Jeziorak, Jeziorak Maty and Tynwald. The greatest amounts of them were found in demersal water and in bottom sediment surface stratum. However, the percentage share of chitinolytic bacteria proved greater in deep water (9.35%) than in bottom sediments (2.16%). Surface water and the part just over the sediment contained most chitinolytic bacteria in a heavily eutrophicated lake Jeziorak Maty located in a town (11.9% and 12.6% respectively), whereas they inhabited bottom sediments in large amounts in lake Jeziorak (2.9%). The most abundant among chitinolytic bacteria were the genera of: *Achromobacter, Bacillus* and family of *Enterobacteriaceae.*

Keywords: chitinolytic bacteria, heterotrophic bacteria, bottom sediments, surface water, water above sediments

Introduction

Chitin makes an insoluble linear β-1,4 polymer of N-acetyloglucosamine (GlcNAc), one of most common polysaccharides occurring in nature [20]. It is the main structural component of the outer skeleton of insects and crustaceans [11, 15], being at the same time present in exoskeletons of the coelenterates, the platyhelminthes, protozoa, molluscs and in the cell wall of many fungi [18]. Great amounts of chitin are synthesized in the biosphere. In aqua biosphere alone, annual production of about 10^{10} - 10^{11} tons has been reported [5]. However, there is no substantial accumulation of chitin in the ocean sediments, which is due to its being effectively degraded and catabolised by bacteria [20].

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Chitinases (EC3.2.1.14) and N-acetyloglucosaminidases (EC3.2.1.30) make the basic enzymes degrading insoluble chitin into its monomelic components.These enzymes have been detected in various organisms including fungi, bacteria, insects, plants and animals [20].

The aim of the present paper is to find out about the occurrence of chitinolytic bacteria among heterotrophic bacteria inhabiting deep water and bottom sediments of eutrophic lakes of Uawskie Lake District, and their identification.

Material and Methods

Objective of the Study

The study was carried out in lakes Jeziorak, Jeziorak Mary and Tynwald. Those lakes make up part of Iławskie

Lake District in north-east Poland and are part of the Vistula-Drweca catchment area.

Lake Jeziorak is a post-glacial lake of a meridian-like placement fourth greatest lake in Poland [13]. The lake surface is 32.3 km^2, length - 27.5 km, the mean width - about 1.2 km and the maximum depth - 12.0 m; the mean depth is about 5.7 m. The lake is one of moderately eutrophic water bodies [8].

Lake Jeziorak Maty is situated in mid-town, and is heavily eutrophicated. It is connected to lake Jeziorak by a narrow link. Its surface is 26 ha. Length 805 m, max width 365 m and max depth 6.5 m.

Lake Tynwald is a water body surrounded by fields, about 10 km northeast of the town of Hawa and about 1 km due east of lake Jeziorak. The lake is heavily eutrophicated, one of the most productive ones of the type in the area. Its surface is 32 ha, max length 1175 m, max width 662 m, max depth 3.3 m [7, 21].

Sampling

The surface water (10-20 cm off the surface), water from over the sediments (10-20 cm off the sediment) and the surface layer of sediments (down to 10 cm) were sampled at two stations each time. In lakes Jeziorak and Jeziorak Maly water and bottom sediment samples were collected from the littoral (on the right muddy shore, about 2.0 m deep) and from the pelagialu zone (about 5.5 m deep). In the case of lake Tynwald, the samples were collected from the littoral (close to the right shore affected by the agricultural run-off, about 2 m deep), and from the pelagic zone (about 2.5 m deep). All samples were placed in an ice thermoinsulated container (the temperature inside was not higher than ± 7°C), and brought to a laboratory where they were immediately analysed. The material was sampled in spring (21st April 1994), in summer (5th July 1994), in autumn (3rd November 1994) and winter (27th February 1995).

Heterotrophic Bacteria Number

The number of heterotrophic bacteria in the samples of water and bottom sediments were determined by means of spread plates method, inoculating the material on the iron-peptone agar medium, according to Ferrer, Stapert, Sokolski [6]. The samples were diluted with sterile buffer water after Daubner [1]. After 6 days of incubation at 20°C, the grown colonies were counted by converting the result into 1 cm^3 of water or 1 g of fresh sediment matter.

Chitinolytic Bacteria Number

The number of chitinolytic bacteria in the examined samples were determined by means of spread plates, inoculating the material onto medium containing the following components: peptone (peptobak) - 1.0 g, iron sulphate - 0.1 g, ammonia sulphate - 0.1 g, iron gluconate - 0.1 g, yeast extract - 0.1 g, colloidal chitin - 7.0 g of dry mass, agar - 15.0 g, tap water - 1.0 dm^3, pH 7.2 -7.4. The colloidal chitin had been prepared according to Lingappa and Lockwood [12]. After 14 days of incubation at 20°C, the bright halo diameter around the colonies was measured to get information on the bacteria chitin decomposition ability. Those strains were then isolated onto semi-liquid substrate containing colloidal chitin and kept in a fridge. They were inoculated onto a fresh semi-liquid medium (see above) every 2 months.

Identification of Chitinolytic Bacteria

The identification of the chitinolytic bacteria under study was done according to the pattern suggested by Shewan and co-authors [17] and data published in papers by Hendrie [9], Thornley [19] and Holt et al. [10].

Table 1. Number of heterotrophic and chitinolytic bacteria in surface water of studied lakes.

Date of sampling	Lake Jeziorak		Lake Jeziorak Mały		Lake Tynwałd	
	littoral	pelagial	littoral	pelagial	littoral	pelagial
Spring (21.04.1994)	* 16.8 ** 4.5	20.0 3.7	22.3 7.9	17.0 7.7	2.0 0.0	3.3 0.3
Summer (5.07.1994)	11.2 0.6	27 0.1	21 0.1	24.0 1.5	11.0 0.0	15.5 1.2
Autumn (3.11.1994)	1.0 0.0	3.2 0.3	3.0 0.0	1.8 0.2	3.4 0.0	1.2 0.3
Winter (27.02.1995)	0.9 0.0	2.0 0.0	2.6 0.0	1.6 0.0	2.8 0.3	18.0 3.3
Average	7.47 1.27	13.05 1.02	12.22 2.0	11.10 2.35	4.8 0.075	9.5 1.27

Explanations: * - number of heterotrophic bacteria x 10^3 cells/cm^3, ** - number of chitinolytic bacteria x 10^3 cells/cm^3

Table 2. Number of heterotrophic and chitinolityc bacteria in water above sediment of studied lakes.

Date of sampling	Lake Jeziorak		Lake Jeziorak Mały		Lake Tynwald	
	littoral	pelagial	littoral	pelagial	littoral	pelagial
Spring (21.04.1994)	* 33.0 ** 10.0	37.5 0.0	210.0 87.0	337.0 152.5	10.0 0.0	6.0 0.0
Summer (5.07.1994)	65.0 5.0	30.0 0.0	100.0 0.0	465.0 30.0	30.0 1.5	75.0 1.0
Autumn (3.11.1994)	13.0 0.0	16.0 1.5	60.0 1.0	75.0 1.0	13.0 0.0	13.5 0.0
Winter (27.02.1995)	6.0 0.0	5.0 0.0	28.0 1.3	55.0 0.3	35.0 6.0	10.0 2.6
Average	29.25 3.75	22.13 0.38	99.5 22.3	233.0 45.95	22.0 1.9	26.13 0.9

Explanations: * - number of heterotrophic bacteria x 10^3 cells/cm^3, ** - number of chitinolytic bacteria x 10^3 cells/cm^3

Results

Tables 1-3 present the results of the study on the quantity of chitinolytic bacteria in surface water, over-sediment water and surface layer of bottom sediments in the littoral and pelagic zone of the lakes under investigation.

The presented data account for the fact that the occurrence of chitinolytic bacteria varies and is affected by sampling season, lake trophy, and sampling depth. In all the investigated lakes, the greatest amounts of chitinolytic bacteria were sampled from the surface layer of bottom sediments and the water layer just over the sediment, whereas the least quantity was found in surface water, except for lake Jeziorak Maly where the bacteria were most abundant in the water layer just over the sediment.

The surface water contained the most chitinolytic bacteria in lake Jeziorak Maly (2.0 x 10^3 cell/cm^3 in the littoral, and 2.35 x 10^3 cell/cm^3 in the pelagial zone on average). In lakes Jeziorak and Tynwald the amounts of chitinolytic bacteria were two times less.

In the water hanging just over the sediment, the most chitinolytic bacteria were found in lake Jeziorak Maty (22.3 x 10^3 cell/cm^3 in the littoral, and 45.95 x 10^3 cell/ cm^3 in the pelagic zone on average) and the least in lake Tynwald (1.9 x 10^3 cell/cm^3 and 0.9 x 10^3 cell/cm^3 respectively).

In the surface layer of bottom sediments the greatest amount of chitinolytic bacteria occurred also in lake Jeziorak Maly (9.55 x 10^3 cell/g wet sed. in the littoral and 16.0 x 10^3 cell/ g wet sed. in the pelagial part on average), and the least occurred in lake Jeziorak (5.35 x 10^3 cell/g wet sed. and 4.0 x 10^3 cell/g wet sed. respectively).

In the surface water and in the water just over the bottom sediments of lakes Jeziorak Maly and Jeziorak, the chitinolytic bacteria were most abundant in spring and least abundant in winter. In the bottom sediment of lake Jeziorak, the bacteria were most numerous in autumn, while in lake Jeziorak Maly - in summer. In lake Tynwald the chitinolytic bacteria were most abundant both in surface and over-sediment water, and in bottom sediments in winter.

The percentage share of chitin decomposing chitinoc-

Table 3. Number of heterotrophic and chitinolytic bacteria in bottom sediments of studied lakes.

Date of sampling	Lake Jeziorak		Lake Jeziorak Mały		Lake Tynwald	
	littoral	pelagial	littoral	pelagial	littoral	pelagial
Spring (21.04.1994)	* 203.0 ** 5.4	173.0 3.7	2895.0 25.2	2226.0 6.0	270.0 11.6	300.0 3.0
Summer (5.07.1994)	235.0 4.0	200.0 0.3	1285.0 8.0	1150.0 40.0	70.0 1.6	200.0 2.0
Autumn (3.11.1994)	150.0 10.0	150.0 10.0	1000.0 3.0	1100.0 7.0	200.0 3.0	185.0 5.0
Winter (27.02.1995)	92.0 2.0	92.0 2.0	180.0 2.0	360.0 11.0	576.0 10.0	316.0 14.0
Average	170.0 5.35	153.75 4.0	1340.0 9.55	1209.0 16.0	279.0 6.55	250.25 6.0

Explanations: * - number of heterotrophic bacteria x 10^3 cells/g wet wt., *• - number of chitinolytic bacteria x 10^3 cells/g wet wt.

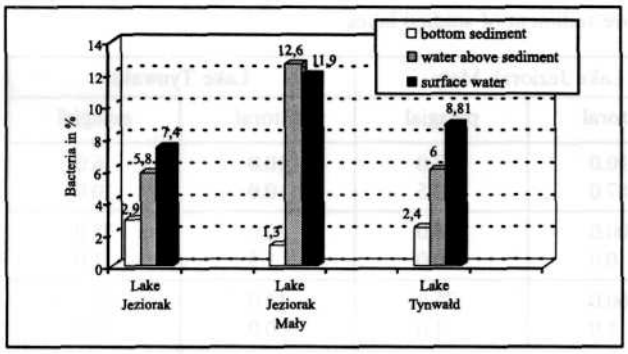

Fig. 1. Percentage share of chitinolytic bacteria among heterotrophic bacteria in the investigated water and bottom sediment samples.

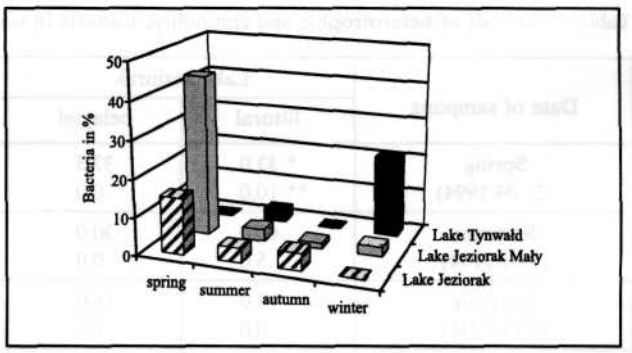

Fig. 3. Percentage share of chitinolytic bacteria among heterotrophic bacteria in the water above bottom sediment of the lakes under investigation.

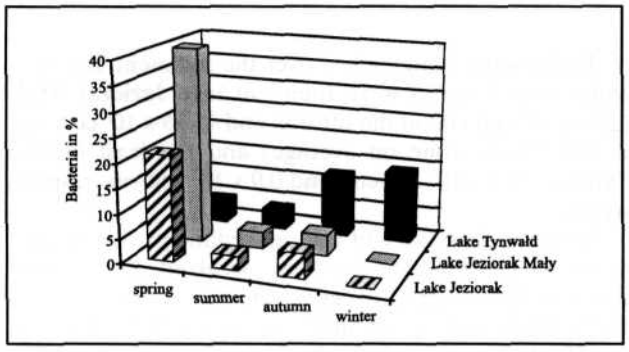

Fig. 2. Percentage share of chitinolytic bacteria among heterotrophic bacteria in the investigated surface water of the lakes under investigation.

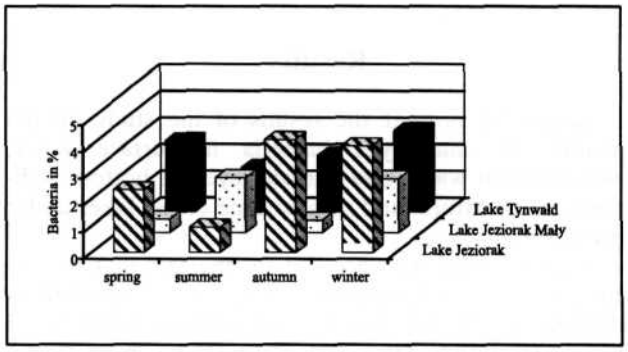

Fig. 4. Percentage share of chitinolytic bacteria among heterotrophic bacteria in the surface layer of bottom sediments samples.

lastic bacteria among the total of heterotrophic bacteria has been presented in Figures 1-4. In all the investigated lakes (Fig. 1), those bacteria displayed the smallest percentage share in bottom sediments, amounting to the following figures: 2.9% in lake Jeziorak, 2.4% in lake Tynwald and 1.3% in lake Jeziorak Maly. The chitinolytic bacteria were most abundant in surface and over-sediment water in lake Jeziorak Maty (11.9% and 12.6% respectively) and the least in lake Jeziorak (7.4% and 5.8% respectively). According to the data shown in Fig. 2, in lakes Jeziorak and Jeziorak Maly, the largest amounts of chitinolytic bacteria were recorded in spring (21.5% and 40% respectively), less in summer and none in winter. In lake Tynwald, the bacteria were most numerous in winter (14.4%) and the least in summer (3.85%). Similar data were recorded for the water over the sediment (Fig. 3), whereas in the bottom sediments surface layer (Fig. 4), the bacteria in lake Jeziorak were most abundant in autumn and winter, in lake Jeziorak Maly in summer and winter; and in lake Tynwald in spring and winter.

The identification of chitinolytic bacteria isolated from surface, over-sediment water and bottom sediment of the lakes under investigation (Fig. 5), confirmed the ability of the following bacteria genera to hydrolyse colloidal chitin: *Achromobacter, Bacillus, Chromobacterium, Pseudomonas* and *Vibrio,* along with bacteria from the

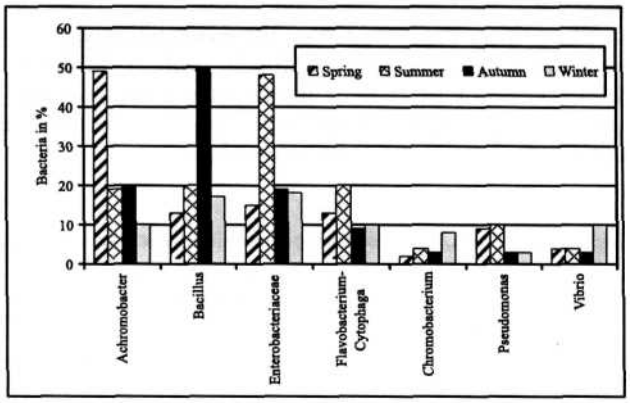

Fig. 5. Generic composition of chitinolytic bacteria (average values).

Flavobacterium-Cytophaga group and the *Enterobacterioceae* family. In spring, *Achromobacter* bacteria were most abundant (49%), in summer some of the *Enterobacteriacae* family (48%), in autumn *Bacillus* genus (50%) and in winter the *Enterobacteriacae* (18%) and *Bacillus* (17%) families. The genera of *Chromobacterium* and *Vibrio* were the least represented ones in all the seasons.

Discussion

Chitin makes the most frequent structural component of many invertebrates and fungi. In water bodies, it mainly originates from outer skeletons of insects and crustaceans [15]. Bacteria and fungi are quick at decomposing chitin. Most active seem to be the strains of *Pseudomomas* and *Vibrio* genera, while *Beneckea* and *Micromonospora* are noted less frequently. In the Western Baltic and its fiords, a few thousand the bacteria have been found that decompose chitin in 1 cm^3 of water. In aerobic sediments, several dozens of thousands of them have been recorded in one gram of dry mass. Those bacteria inhabit mainly on carapaces of dead cancers. The recorded bacteria were those of *Pseudomonas* and *Vibrio* genera [16]. Animals with chitin skeletons make a good feed for many fish species and for this reason chitin decomposing bacteria are able to inhabit their alimentary tracts [14].

Data acquired for this paper show that in surface water, the number of chitinolytic bacteria was dramatically lower than in the case of over-sediment water and bottom sediment. This may result from the fact that there occur fewer heterotrophic bacteria in surface water than in deeper lake layers and thus the chitinolytic bacteria are less abundant. However, when analysing the percentage share of chitinolytic bacteria among the total number of heterotrophic bacteria, it turns out that it was planktonic bacteria that were more eager to decompose chitin than the benthos ones. According to Donderski [2, 3], chitinolytic bacteria in compensation layer of lake Jeziorak made 15% microbes while in the bottom sediment surface layer only 7.5%. Then, in the Moty bay of lake Jeziorak, in the littoral surface water they made an average 18.5%, and 8.2% in bottom sediments [4]. Our data show that they made 7.4% of the surface water in lake Jeziorak, 11.9% in lake Jeziorak Maly and 8.81% in lake Tynwald. In bottom sediments in lake Jeziorak they made as little as 2.9%, in lake Tynwald 2.4% and in lake Jeziorak Maly 1.3%.

The frequent occurrence of chitinolytic bacteria among planktonic bacteria may indicate the fact that the outer casing of many invertebrates containing chitin undergoes decomposition in lakes; deep water. The conditions are more favourable due to their occurrence, better oxidation and usually higher temperature. Our results give evidence of the relation of chitinolytic bacteria number in surface and over-sediment water with maximum number of herterotrophic bacteria occurring there. In lakes Jeziorak and Jeziorak Maly those bacteria were most abundant in spring, while in lake Tynwald-in winter. No such correlation was found in reference to bottom sediments. Most probably, in the bottom sediments of the investigated lakes, there occurs much more other organic matter, which is absorbed more easily than chitin and therefore this polysaccharide undergoes metabolism at a later turn.

The percentage share of chitinolytic bacteria in the total heterotrophic bacteria depends to a significant extent on the lake trophy. Following our findings, the number of chitinolytic bacteria in the surface and over-sediment water was proportional to the water body trophy. They were most abundant in the most heavily eutrophicated lake Jeziorak Maly, and the least abundant in moderately eutrophicated lake Jeziorak. This relationship was not found in the case of bottom sediments. It may be associated with the fact there occurs more chitin in heavily eutrophicated lakes surface water, which is rich in plankton and, owing to the above conditions occurring there, the planktonic bacteria are more eager to inhabit it only to carry out the decomposition process. This, however, makes chitin less available for the bottom sediments and thus the percentage of chitinolytic bacteria occurring there is lower.

The study on genus composition of chitinolytic bacteria shows that *Achromobacter* and *Bacillus* genera, and *Enteriobacteriacae* family were represented most abundantly. A similar chitinolytic bacteria composition was recorded by Donderski [3], who found the following genera capable of chitinolytic activity: *Achromobacter, Pseudomonas, Chromobacterium* and *Nocardia.* Schlegel [15] found the following group and genera capable of chitilolytic activity among 50 bacteria isolated from soil: *Flavobacterium, Bacillus, Cytophaga* and *Pseduomonas.* The following bacteria found in water and bottom sediments of Lake Jeziorak by Donderski and Trzebiatowska [4] were capable of decomposing chitin: *Bacillus cereus, Bacillus pumilus, Bacillus firmus, Aeromonas sp., Aeromonas hydrophila.*

Conclusions

Summing up the results of the present work, the following conclusions can be drawn:

1. The chitinolytic bacteria were recorded at their most in demersal water and the surface layer of bottom sediments.
2. The percentage share of chitinolytic bacteria was greater in the case of deep water than bottom sediments.
3. In surface water and in over-sediment water layer, the greatest amount of chitinolytic bacteria were re corded for the heavily eutrophicated mid-city Lake Maly Jeziorak and in bottom sediments - in the moderately eutrophicated Lake Jeziorak.
4. Among the chitinolytic bacteria, the following oc curred most abundantly: *Achromobacter, Bacillus* and the family of *Enteriobacteriacae.*

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