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The Utilization of N-acetyloglucosamine and Chitin as Sources of Carbon and Nitrogen by Planktonic and Benthic Bacteria in Lake Jeziorak

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Abstract

It follows from the research conducted on the number of chitinolytic bacteria among planktonic and benthic bacteria that they occur in a higher number in bottom sediments than in water. However, the percentage of chitinolytic bacteria among the total number of heterotrophic bacteria was higher in the water than in the bottom sediments. Chitinolytic bacteria and the bacteria unable to decompose chitin most readily utilized N-acetyloglucosamine as additional sources of carbon and nitrogen. Chitinolytic bacteria developed well on a substrate with colloidal chitin as the only or an additional source of carbon and nitrogen. However, bacteria unable to decompose chitin displayed very weak growth on a substrate with colloidal chitin as the only source of carbon and nitrogen.

Keywords: chitinolytic bacteria, planktonic bacteria, benthic bacteria, N-acetyloglucosamine, chitin, chitinases

Introduction

Chitin is a polysaccharide, a linear polymer composed of N-acetyloglucosamine monomers connected by glycoside β - 1, 4 bonds [10, 3]. It makes part of outer arthropod skeletons and also is one of components of fungi and some yeats cell walls [11]. Biological degradation of chitin is realized with the use of endo- and egzo- enzymes known as chitinases (EC 3.2.1.114) and β - N – acetylohexaminidases (EC 3.2.1.52) [15]. Tronsmo and Harman [19] isolated three groups of chitinolytic enzymes: chitinases cutting the chitin at random at different points of N-acetyloglucosamine polymer: chitobiosidases operating after chitinases and releasing chitobiasis off spilt fragments, and N-acetyl - β - glucosamidases hydrolyzing chitobiases for monomers. N-acetyloglucosamine and its decomposition products: glucosamin, acetic acid and ammonia are released during the process of chitin decomposition [14]. They are used by many different organisms as a source of carbon and nitrogen.

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Materials and Methods

Study Area

The study was carried out in Lake Jeziorak's Moty Bay. Lake Jeziorak is located in the Iławskie Lake District and makes part of the Vistula-Drwęca catchment area. It is a post-glacial lake of a meridian-like placement. The lake surface is 32.3 km^2 , length - 27.5 km, the mean width - about 1.2 km and maximum depth - 12.0 m. Mean depth is about 5.7 m. The lake is eutrophic [8].

Sampling

The study material contained bacteria isolated from water and bottom sediments of the sublittoral and pelagic zones. The water was sampled from the deph of 10-20 cm with a sterile pipette by means of a pipette automatic pump (Pipet – boy, De Ville), and then poured into sterile bottles sealed with rubber stoppers. The bottom sediments were sampled by means of a self-constructed tube scoop and their surface layer (down to 5 cm) was aseptically transferred into sterile, top-twisted jars. All samples were put into thermoisolated containers with ice (temperature inside did not exceed 7^0C) and then transferred into a laboratory, where they were immediately analyzed. The study material was collected between June and November 1999.

Number of Heterotrophic Bacteria

The number of heterotrophic bacteria (CFU) in the samples of water and bottom sediments were determined by means of the spread plates method, inoculating the material from respective solutions in three parallel repetitions on the iron-peptone agar medium, according to Ferrer, et al. [7]. After 6 days of incubation at 20°C, the grown colonies were counted by converting the result into 1 cm^3 of water and g dry weight of the sediments.

Number and Isolation of Chitinolytic Bacteria

The number of chitinolytic bacteria was determined by means of spread plates methods, inoculating the samples of water and bottom sediments respective solutions onto medium containing the following components: peptone (peptobak) - 1.0 g; iron sulphate - 0.1 g; ammonium sulphate - 0.1 g; iron gluconate - 0.1 g; yeast extract - 0.1 g; colloidal chitin - 7.0 g dry mass; agar - 15.0 g; tap water - 1.0 dm3, pH 7.2-7.4. After 14 days of incubation at 20°C, the diameter of the lightened zone around the colony was measured, this being evidence of the ability of the bacteria to decompose chitins. These strains were isolated on semi-liquid medium with the addition of colloidal chitin and stored in the refrigerator, being transferred every 2 months on to new medium as above. Colloidal chitin was prepared after Lingappa and Lockwood [12].

Fig. 1. Zones of clearing of chitinolytic bacteria grown on a colloidal chitin – agar plate isolated from Lake Jeziorak.

Identification of Chitinolytic Bacteria

Identification of the investigated chitinolytic strains was performed following the pattern suggested by Allen, et al. [1], and Shewan, et al. [17] for freshwater bacteria.

The Utilization of N-acetyloglucosamine and Colloidal Chitin by Planktonic and Benthic Bacteria

For each test, 6 chitinolytic strains and 6 strains incapable of decomposing chitin, isolated from the water and the bottom sediments, were used. These bacteria were preincubated for 72 h at 26°C on slants iron-peptone agar medium and washed off with 5 cm^3 of sterile physiological salt solution. The resulting suspension was centrifuged at 10,000 rpm for 10 min. After centrifuging, the supernatant was poured off while the deposite was suspended in 3 cm^3 of sterile physiological salt solution and centrifuged once again. Then the bacterial deposit was suspended in such a volume of physiological salt solution as to obtain an optical density of the suspension of E = 0.15 at a wavelength of 560 nm. The optical density of the bacterial suspension was measured against a blank test (physiological salt solution) on a Marcel s 330 pro spectrophotometer. This gave an inoculum corresponding to 107 cells/cm3, which was used for further tests on the influence of N-acetyloglucosamine and colloidal chitin on the development of the bacteria under investigation. The tests were conducted in a medium modified after Lochhead and Chase [13], containing glucose as the source of carbon, potassium nitrate as the source of nitrogen, and N-acetyloglucosamine and colloidal chitin (as source of C and N). The bacterial culture was conducted at 20°C and after 2, 4, 6 and 8 days of incubation, the concentration of bacterial protein was determined using Bradford's method [2].

Results

The results of the studies on the number of heterotrophic and chitinolytic bacteria in Moty Bay in Lake Jeziorak are presented in Table 1. It follows from them that heterotrophic and chitinolytic bacteria occurred in significantly higher numbers in the bottom sediments than in the water. In the water of the sublittoral zone and the pelagic zone, the highest number of chitinolytic bacteria occurred in June and July, with their number falling

Table 1. Number of heterotrophic and chitinolytic bacteria in water and bottom deposits in Lake Jeziorak.

Date of	Surface water •				Bottom sediments ••			
sampling	Sublittoral		Pelagial		Sublittoral		Pelagial	
17.06.1999	16.1 *		15.0		140.0		120.0	
	3.8 **	(23.3)	2.3	(15.0)	17.5	(12.5)	5.5	(4.6)
24.07.1999	15.2		12.3		198.0		172.0	
	2.4	(16.1)	1.0	(8.1)	22.8	(11.5)	6.9	(4.0)
22.08.1999	2.3		2.3		940.0		840.0	
	0.23	(10.0)	0.07	(3.3)	94.0	(10.0)	35.3	(4.2)
21.09.1999	4.4		4.4		172.1		168.1	
	0.59	(13.3)	0.6	(6.7)	34.4	(20.0)	5.5	(3.3)
20.10.1999	4.4		1.9		169.2		152.0	
	0.73	(16.7)	0.6	(3.3)	16.9	(10.0)	0.0	(0.0)
25.11.1999	2.3		1.7		112.1		104.0	
	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Average	7.45		6.3		288.6		259.4	
	1.3	(13.2)	0.76	(6.1)	30.9	(10.7)	8.9	(2.7)

Explanations: * - number of heterotrophic bacteria , ** - number of chitinolytic bacteria, • - number of bacteria x 10^3cells/cm^3, •• - number of bacteria x 10^3cells/dry weight, ( ) – bacteria in percents

Table 2. The utilization of N-acetylglucosamine as an additional source of carbon and nitrogen by planktonic and benthic bacteria.

Name of bacteria	Habitat	Incubation time [days]				
		0	2	4	6	8
CHITINOLYTIC BACTERIA						
Pseudomonas sp.	Surface water	* 0.012	0.091	0.138	0.232	0.267
Alcaligenes denitrificans		0.013	0.099	0.163	0.189	0.237
Agrobacterium sp.		0.006	0.130	0.151	0.177	0.215
Vibrio fluvialis		0.012	0.109	0.169	0.249	0.298
Aeromonas sp.		0.013	0.064	0.174	0.204	0.251
Aeromonas hydrophila		0.010	0.103	0.148	0.202	0.295
Vibrio fluvialis	Bottom sediments	0.010	0.085	0.070	0.166	0.227
Cytophaga hutchinsonii		0.011	0.126	0.160	0.180	0.247
Aeromonas hydrophila		0.010	0.109	0.161	0.218	0.265
Agrobacterium sp.		0.009	0.149	0.207	0.238	0.268
Aeromonas hydrophila		0.009	0.169	0.216	0.250	0.272
Alcaligenes denitrificans		0.012	0.110	0.164	0.213	0.264
NON CHITINOLYTIC BACTERIA						
Pseudomonas sp.	Surface water	* 0.012	0.108	0.168	0.204	0.249
Bacillus megaterium		0.013	0.118	0.165	0.203	0.266
Alcaligenes sp.		0.010	0.133	0.156	0.190	0.250
Acinetobacter sp.		0.013	0.134	0.170	0.222	0.270
Micrococcus varians		0.012	0.114	0.130	0.177	0.220
Micrococcus sp.		0.012	0.103	0.121	0.178	0.227
Bacillus subtilis	Bottom sediments	0.013	0.097	0.166	0.207	0.248
Bacillus megaterium		0.013	0.096	0.159	0.248	0.262
Flavobacterium sp.		0.011	0.119	0.173	0.199	0.244
Micrococcus sp.		0.012	0.044	0.139	0.165	0.225
Bacillus megaterium		0.015	0.114	0.177	0.222	0.265
Aeromonas sp.		0.012	0.102	0.171	0.213	0.254

Explanation: * - concentration of bacterial protein in mg/cm^3

Table 3. The utilization of N-acetylglucosamine as the only source of carbon and nitrogen by planktonic and benthic bacteria.

Name of bacteria	Habitat	Incubation time [days]				
		0	2	4	6	8
CHITINOLYTIC BACTERIA						
Pseudomonas sp.	Surface water	* 0.018	0.079	0.164	0.173	0.182
Alcaligenes denitrificans		0.013	0.104	0.169	0.179	0.204
Agrobacterium sp.		0.017	0.069	0.109	0.123	0.135
Vibrio fluvialis		0.019	0.056	0.089	0.089	0.155
Aeromonas sp.		0.020	0.079	0.133	0.141	0.172
Aeromonas hydrophila		0.018	0.090	0.201	0.204	0.220
Vibrio fluvialis	Bottom sediments	0.014	0.045	0.122	0.146	0.178
Cytophaga hutchinsonii		0.011	0.045	0.140	0.177	0.206
Aeromonas hydrophila		0.009	0.071	0.135	0.164	0.180
Agrobacterium sp.		0.010	0.090	0.156	0.175	0.203
Aeromonas hydrophila		0.012	0.092	0.182	0.184	0.212
Alcaligenes denitrificans		0.012	0.073	0.158	0.177	0.213
NON CHITINOLYTIC BACTERIA						
Pseudomonas sp.	Surface water	* 0.017	0.067	0.079	0.112	0.151
Bacillus megaterium		0.018	0.099	0.103	0.122	0.211
Alcaligenes sp.		0.013	0.090	0.211	0.211	0.230
Acinetobacter sp.		0.011	0.089	0.102	0.118	0.196
Micrococcus varians		0.014	0.096	0.099	0.104	0.219
Micrococcus sp.		0.010	0.084	0.107	0.147	0.196
Bacillus subtilis	Bottom sediments	0.013	0.027	0.032	0.145	0.198
Bacillus megaterium		0.012	0.038	0.044	0.133	0.145
Flavobacterium sp.		0.015	0.080	0.092	0.122	0.175
Micrococcus sp.		0.014	0.093	0.123	0.156	0.200
Bacillus megaterium		0.011	0.071	0.101	0.198	0.212
Aeromonas sp.		0.011	0.073	0.105	0.136	0.185

Explanation: * - concentration of bacterial protein in mg/cm^3

rapidly in subsequent months. In the bottom sediments of the sublittoral zone and the pelagic zone, the highest number of chitinolytic bacteria was found in August, with their number decreasing over the following months. The percentage of chitinolytic bacteria was higher in the water than in the bottom deposits (on average: in the water, 13.2 % in the sublittoral zone and 6.1 % in the pelagic zone, and in the bottom deposits, 10.7 % in the sublittoral zone and 2.7 % in the pelagic zone).

The results concerning the utilization of N-acetyloglucosamine and colloidal chitin as sources of carbon and nitrogen are presented in Tables 2-5 and in Figs. 2-3. As follows from the data presented, both chitinolytic bacteria and the bacteria unable to decompose chitin isolated from the water and bottom sediments developed the best in a medium with N-acetyloglucosamine as an additional source of carbon and nitrogen. Chitinolytic bacteria developed equally well in a medium with colloidal chitin as an additional source of carbon and nitrogen, and slightly worse on a substrate with N-acetyloglucosamine as the only source of carbon and nitrogen. The bacteria incapable of decomposing chitin developed well in a medium with N-acetyloglucosamine as the only source of carbon and nitrogen, but worse in a medium with colloidal chitin as an additional source of carbon and nitrogen, while they did not develop at all on a substrate with chitin as the only source of carbon and nitrogen. The growth of both chitinolytic bacteria and the bacteria incapable of decomposing chitin was proportional to the duration of incubation.

Discussion

In animal organisms, chitin constitutes the rigid structure of the exoskeleton, and in plants it supports the cell walls, mainly in fungi [9]. The quantity of chitin produced in the biosphere is enormous. It is estimated that 1011 tonnes of it is formed annually. This quantity and the absence of an accumulation of this component suggests that it is quickly degraded. It is above all bacteria and fungi that are capable of decomposing chitin. Having the appro-

Table 4. The utilization of colloidal chitin as an additional source of carbon and nitrogen by planktonic and benthic bacteria.

Name of bacteria	Habitat	Incubation time [days]				
		0	2	4	6	8
CHITINOLYTIC BACTERIA						
Pseudomonas sp.	Surface water	* 0.012	0.128	0.163	0.206	0.237
Alcaligenes denitrificans		0.011	0.103	0.142	0.179	0.215
Agrobacterium sp.		0.012	0.187	0.201	0.289	0.333
Vibrio fluvialis		0.012	0.108	0.164	0.208	0.251
Aeromonas sp.		0.014	0.085	0.141	0.190	0.227
Aeromonas hydrophila		0.012	0.102	0.144	0.210	0.227
Vibrio fluvialis	Bottom sediments	0.014	0.100	0.118	0.171	0.204
Cytophaga hutchinsonii		0.012	0.111	0.135	0.178	0.215
Aeromonas hydrophila		0.010	0.100	0.145	0.212	0.259
Agrobacterium sp.		0.010	0.137	0.171	0.222	0.251
Aeromonas hydrophila		0.011	0.138	0.214	0.269	0.321
Alcaligenes denitrificans		0.013	0.106	0.151	0.217	0.260
NON CHITINOLYTIC BACTERIA						
Pseudomonas sp.	Surface water	* 0.011	0.056	0.100	0.121	0.130
Bacillus megaterium		0.012	0.045	0.122	0.125	0.130
Alcaligenes sp.		0.014	0.039	0.111	0.115	0.121
Acinetobacter sp.		0.012	0.042	0.109	0.122	0.126
Micrococcus varians		0.011	0.042	0.099	0.111	0.118
Micrococcus sp.		0.010	0.035	0.101	0.125	0.130
Bacillus subtilis	Bottom sediments	0.011	0,032	0.056	0.035	0.083
Bacillus megaterium		0.012	0.024	0.078	0.064	0.128
Flavobacterium sp.		0.014	0.023	0.066	0.160	0.212
Micrococcus sp.		0.010	0.033	0.045	0.166	0.197
Bacillus megaterium		0.012	0.045	0.087	0.131	0.214
Aeromonas sp.		0.014	0.026	0.069	0.137	0.225

Explanation: * - concentration of bacterial protein in mg/cm^3

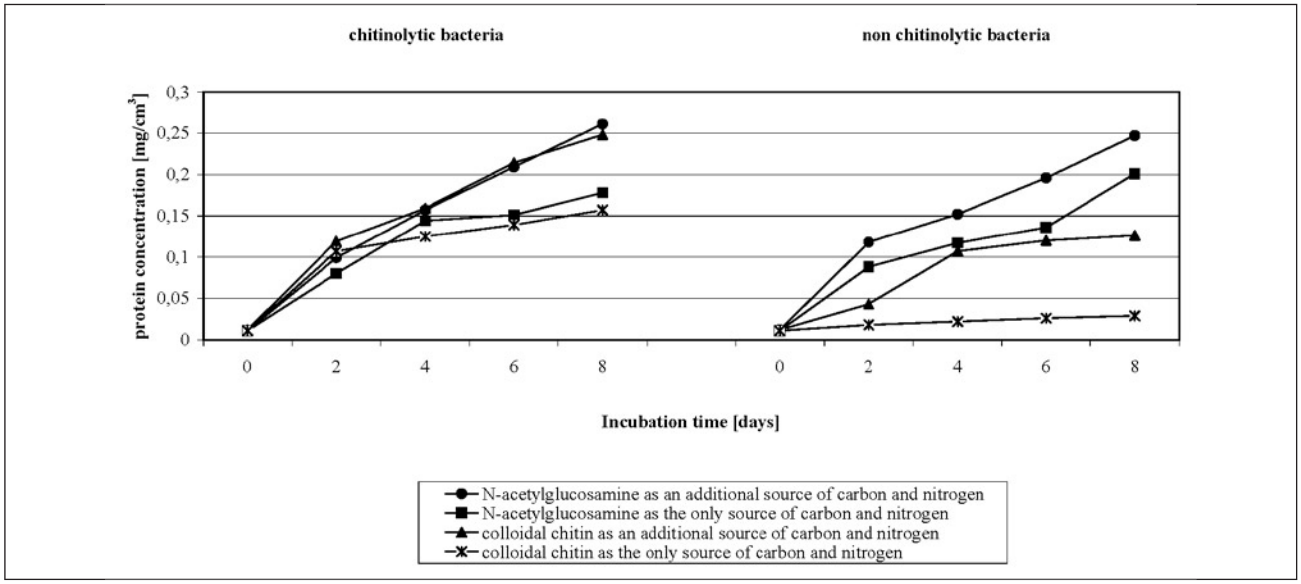

Fig. 2 The utilization of N-acetyloglucosamine and colloidal chitin as sources of carbon and nitrogen by planktonic bacteria in Lake Jeziorak.

Table 5. The utilization of colloidal chitin as the only source of carbon and nitrogen by planktonic and benthic bacteria.

Name of bacteria	Habitat	Incubation time [days]				
		0	2	4	6	8
CHITINOLYTIC BACTERIA						
Pseudomonas sp.	Surface water	* 0.010	0.099	0.110	0.115	0.116
Alcaligenes denitrificans		0.009	0.089	0.110	0.120	0.1350
Agrobacterium sp.		0.010	0.146	0.173	0.203	0.268
Vibrio fluvialis		0.012	0.103	0.118	0.132	0.135
Aeromonas sp.		0.011	0.099	0.118	0.127	0.140
Aeromonas hydrophila		0.014	0.107	0.119	0.135	0.146
Vibrio fluvialis	Bottom sediments	0.009	0.099	0.112	0.116	0.122
Cytophaga hutchinsonii		0.009	0.103	0.130	0.155	0.160
Aeromonas hydrophila		0.010	0.106	0.130	0.138	0.147
Agrobacterium sp.		0.013	0.099	0.113	0.121	0.151
Aeromonas hydrophila		0.012	0.152	0.251	0.205	0.321
Alcaligenes denitrificans		0.010	0.103	0.121	0.132	0.135
NON CHITINOLYTIC BACTERIA						
Pseudomonas sp.	Surface water	* 0.011	0.020	0.023	0.028	0.030
Bacillus megaterium		0.011	0.013	0.019	0.022	0.025
Alcaligenes sp.		0.013	0.020	0.024	0.029	0.030
Acinetobacter sp.		0.010	0.023	0.025	0.030	0.032
Micrococcus varians		0.012	0.015	0.020	0.025	0.026
Micrococcus sp.		0.011	0.014	0.018	0.022	0.030
Bacillus subtilis	Bottom sediments	0,013	0.019	0.022	0.026	0.030
Bacillus megaterium		0.012	0.020	0.025	0.030	0.030
Flavobacterium sp.		0.011	0.021	0.023	0.024	0.029
Micrococcus sp.		0.011	0.020	0.024	0.027	0.032
Bacillus megaterium		0.012	0.023	0.030	0.032	0.034
Aeromonas sp.		0.014	0.018	0.025	0.029	0.033

Explanation: * - concentration of bacterial protein in mg/cm^3

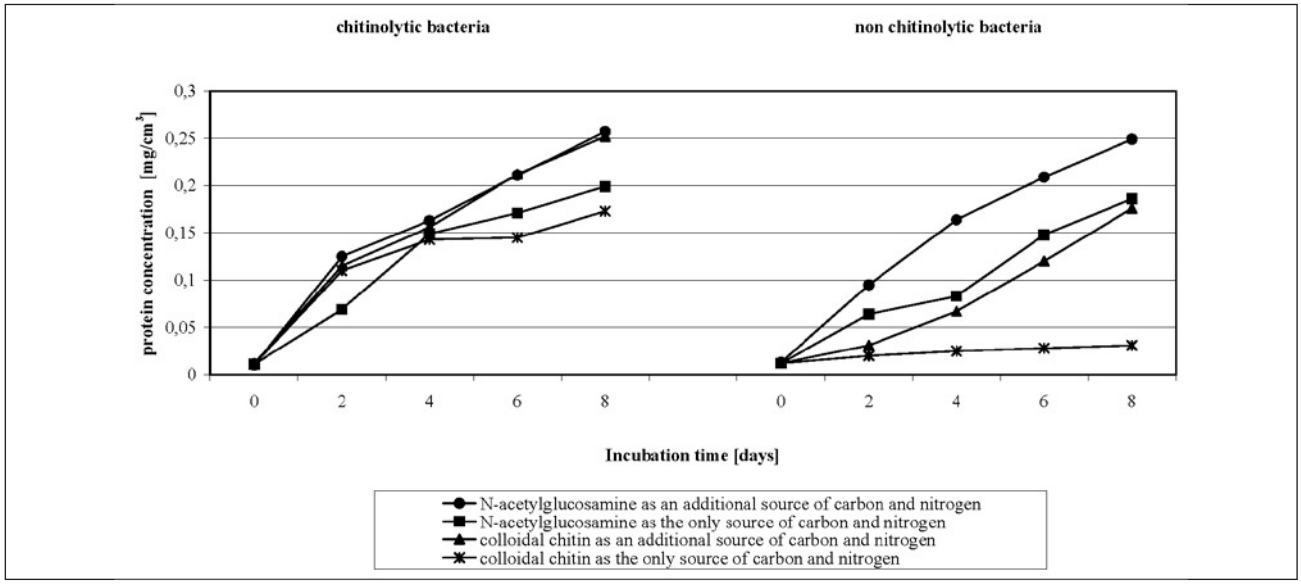

Fig. 3 The utilization of N-acetyloglucosamine and colloidal chitin as sources of carbon and nitrogen by benthic bacteria in Lake Jeziorak.

priate enzymatic system at their disposal, they release into the environment N-acetyloglucosamine, which is a source of carbon and nitrogen for them and other microbes. On account of the global distribution and supplies of chitin, the idea was born to exploit it and its derivatives as a source of carbon and nitrogen. However, as yet, not many researchers have studied this problem.

Considerable differences are observed in water bodies as to the occurrence of bacteria in different seasons. Many researchers have also found a certain conformity in the distribution of bacteria, both vertical and horizontal [16, 4]. In lakes, the number of heterotrophic bacteria is usually higher in the sublittoral zone than in the pelagic zone. Our results confirm this conformity. Irrespective of the season in the samples taken from both the water and the bottom sediments, the number of heterotrophic and chitinolytic bacteria was higher in the sublittoral zone than in the pelagic zone. This is probably connected with the concentration of organic material and the oxygen content. Both these factors reach the highest index in the sublittoral zone of the lake, as a result of the influence of the drainage basin and the presence of numerous macrophytes. Despite the fact that the content of organic material and oxygen is lower in the sublittoral zone than in the littoral zone, but greater than in the pelagic zone, the differences in the number of heterotrophic and chitinolytic bacteria in these zones are rather considerable.

As follows from the data presented in this paper the number of chitinolytic bacteria was decidedly higher in the bottom sediments than in the water. However, taking into account their percent contribution, there were more of them in the water. Studying the activity of chitinolytic bacteria in Moty Bay in Lake Jeziorak, Donderski and Trzebiatowska [6] found 13.9 % of these bacteria in the water and only 8.2 % in the bottom sediments. Donderski and Swiontek Brzezinska [5] found that the chitinolytic bacteria constituted 11.9 % and 8.81 % of the bacteria in the surface water of Lake Jeziorak and Lake Tynwałd, respectively, while in the bottom sediments of these lakes the number of bacteria was relatively lower: 1.3 % in Lake Jeziorak Mały and 2.4 % in Lake Tynwałd.

As follows from our data, N-acetyloglucosamine was readily and quickly mineralized by both chitinolytic bacteria and the bacteria incapable of decomposing chitin. Donderski [4] also found that N-acetyloglucosamine is well utilized as a substrate by both planktonic and benthic heterotrophs. Chitinolytic bacteria utilized colloidal chitin as an additional source of carbon and nitrogen better than N-acetyloglucosamine and colloidal chitin as the only source of carbon and nitrogen. This is probably connected with the fact that chitin is decomposed to N-acetyloglucosamine, thus constituting, apart from glucose, an additional source of carbon and nitrogen. Similar observations were made by Szajer and Koths [18] when studying the growth of the strain *Arthrobacter* "P 35" on different media. They observed the quickest development of the bacteria on a substrate with glucose with the addition of chitin, N-acetyloglucosamine and yeast extract. The strains developed somewhat more slowly on a substrate containing glucose with the addition of chitin alone and on a substrate with yeast extract with the addition of chitin. The strain developed even more slowly on a substrate containing chitin and N-acetyloglucosamine, and grew the slowest on a substrate with chitin alone. These consistent results can be utilized by the correlation between the complicated structure of the chitin compound, and thus its availability, and the preference for utilizing simple compounds (more easily available) in a situation where several sources of carbon and nitrogen are present. Hence, the chitinolytic strains, despite having the enzymatic equipment enabling them to metabolise chitin, will utilize it only after they have exhausted the other, more easily available sources of carbon and nitrogen. The results of the research on the strains unable to decompose chitin confirm these data. These strains developed better on a substrate with N-acetyloglucosamine and colloidal chitin as an additional source of carbon and nitrogen. However, they did not grow on a substrate with colloidal chitin as the only source of carbon and nitrogen. Their minimal growth was probably caused by residual mineral components present in the medium.

Conclusions

1. The number of heterotrophic and chitinolytic bacteria was higher in the bottom sediments than in the water.
2. The percentage of chitinolytic bacteria was higher in the water than in the bottom sediments.
3. The chitinolytic bacteria and the bacteria unable to decompose chitin isolated from the water and bottom sediments developed the best in a medium with N-acetyloglucosamine as an additional source of carbon and nitrogen.
4. The chitinolytic bacteria developed equally well in a medium with colloidal chitin as an additional source of carbon and nitrogen, and slightly worse on a substrate with N-acetyloglucosamine as the only source of carbon and nitrogen.
5. The bacteria incapable of decomposing chitin developed well in a medium with N-acetyloglucosamine as the only source of carbon and nitrogen, but worse in a medium with colloidal chitin as an additional source of carbon and nitrogen.
6. The bacteria incapable of decomposing chitin did not develop at all on a substrate with colloidal chitin as the only source of carbon and nitrogen.

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