Polish Journal of Environmental Studies Vol. 13, No. 5 (2004), 543-549

Physiological Properties and Taxonomy of Epiphytic Bacteria in Chełmżyńskie Lake in Poland

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Received: 24 November 2003
Accepted: 6 May 2004

Abstract

Research was carried out on the number of heterotrophic bacteria inhabiting the surface of macrophytes submerged in water that are dominant in the littoral zone of Chełmżyńskie Lake: common reed, cattail and hornwort. Bacteria were identified and their ability to decompose certain biopolimers was determined. It was found that the number of bacteria growing on helophytes, i.e. common reed and bulrush, increased from spring to summer, and then decreased in autumn. On the surface of hornwort the number of heterotrophic bacteria increased throughout the whole vegetative season, reaching its maximum in autumn. From among all of the isolated strains, lipo-, cellulo- and proteolytic bacteria were dominant. Strains isolated from the surface of cattail were characterized by the potentially highest physiological activity. The generic composition of the isolated strains changed with the development of the macrophytes (season) and depended on the species of plant.

Keywords: littoral zone, epiphytic bacteria, heterotrophic bacteria, biopolimer utilization, generic composition

Introduction

In lakes, especially eutrophic ones, one of the zones that plays a considerable role in the way these lakes function is the littoral. It constitutes the zone that links water depths with the land environment, and at the same time it is an important zone that holds back a considerable proportion of the contamination flowing into the water body from the drainage area. Thanks to the richness and variety of the plant and animal world that inhabits and develops in it, it constitutes a zone that slows the flow of nutrients into the aquatic environment, thus curbing the rate and progress of eutrophication in the central part of the water body. A key role in this process is played by heterotrophic bacteria, including epiphytic bacteria growing on the surface of various hydromacrophytes. Apart from the ability to utilize small-particle substances, heterotrophic bacteria produce ectoenzymes, which take part in the transformation of polimeric substrates [1]. According to Münster and Albrecht [2] it is precisely organic polimers that constitute a dominant fraction of organic matter in water bodies.

The aim of this study was to determine the number of heterotrophic epiphytic bacteria growing on the surface of selected hydromacrophytes in the littoral zone of Chełmżyńskie Lake, their ability to decompose certain biopolimers and their taxonomic status.

Material and Methods

Study Area

Microbiological studies were conducted in the north-western part of Chełmżyńskie Lake near an urbanized area. The lake belongs to the Chełmińsko-Dobrzyńskie Lake District and is part of the Fryba-Vis-

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tula catchment area. It is a channel-type water body. Its surface area is about 271.1 ha, average depth about 6 m., and maximum depth 27.1 m. The lake is situated 20 km from Toruń. Buildings from the town of Chełmża adjoin its north western shore. The lake's shoreline is not mainly flat and, to a large extent, covered with macrophytes. About 70% of the lake's drainage area is covered by agricultural land.

Sampling

15-centimetre sections of common reed shoots (*Phragmites australis* (Cav.) Trin. ex Steudel) and cattail shoots (*Typha latifolia L.*) lying beneath the water surface and whole hornwort shoots (*Ceratophyllum demersum* L.) were collected for microbiological tests. These plants grow abundantly in the littoral zone of Chełmżyńskie Lake. The sites where plants were collected are indicated in Fig. 1.

The plant matter was transferred into sterile glass jars and covered with lake water from the collection site. The samples were transported to the laboratory in containers filled with ice, inside which the temperature did not exceed + 7 °C. The time from the moment of collecting the samples to conducting the analyses did not exceed 2 hours.

Material for analysis was collected in accordance with the development cycle of the plant: in spring (23 May 02) - the period when young shoots appear; in summer (10 July 02) - the period when plants flower; and in autumn (16 October 02) - at the time when the shoots are growing old and dying.

At the same time, certain physico-chemical properties of the water were determined *in situ*: temperature and oxygen content were determined with an oxygen meter (DO_2 - Meter, Jenway - U.K., type 9071), the transparency of the water using a Secchi disc, the pH of the water using an electronic pH-meter N-5122 (Mera-Elwro) and the electrolytic conductivity of the water using a conductometer CM - 204 (Slandi).

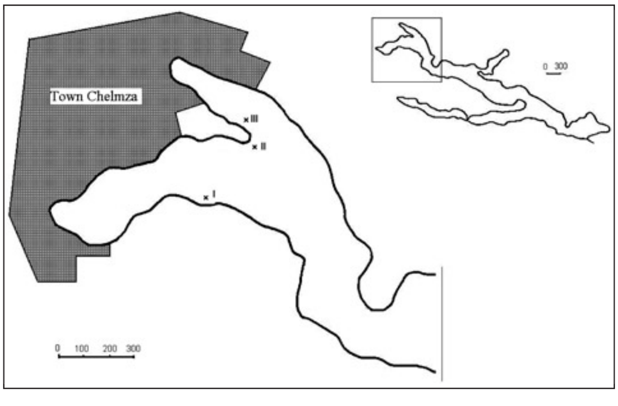

Fig. 1. Outline of Chełmżyńskie Lake.
Explanations: sites of occurrence and collection of hydromacrophytes, I - cattail, II - common reed, III - hornwort.

Estimating the Numbers of Heterotrophic Bacteria and Isolation of Bacterial Strains

In order to determine the number of heterotrophic bacteria inhabiting the surface of the studied plants 10 g of the epidermis of common reed and cattail and 10 g of hornwort shoots were collected. A volume of 90 cm^3 sterile buffer water [3] was added to these samples and they were homogenized in a homogenizer for 2 minutes at 4000 rpm. The resulting homogenates were diluted 10 times with sterile buffer water. These dilutions were seeded on the surface of iron-peptonic medium (IPA) according to Ferrer et al. [4]. After 10 days of incubating the plates at a temperature of 20°C the bacterial colonies that had grown were counted. The number of bacteria on the surface of the plants was expressed as the number of cells per g dry plant mass. Then 120 randomly selected epiphytic bacterial colonies from the whole surface of the plates or from particular sectors were transplanted onto semi-liquid iron-peptonic medium (5 g agar/l). These strains were grown for 6 days at 20°C. After checking the purity of the cultures, 100 strains were used each time for further tests. These bacteria were transplanted every 3 months onto fresh semi-liquid IPA medium and kept at a temperature of +4°C.

Physiological Properties

The isolated epiphytic bacteria were seeded onto a series of testing media containing various organic compounds. In the tests the decomposition of the following multi-particle compounds were taken into account: proteins (casein and gelatine), fat (tributyrin), starch, cellulose, pectin and chitin. The media were prepared after Donderski [5]. The coefficient of physiological activity (CA) of the bacteria was calculated according to the formula proposed by Dahlbäck et al. [6]:

$$CA = \frac{\sum_{i=1}^{n}}{n}$$

where a_i - percentage of strains displaying particular physiological properties, n - number of tests conducted.

Identification of Bacteria

Strains were identified based on the diagnosis schema proposed by Shewan et al. [7], Allen et al. [8] and Bergey's Manual et al. [9]. In the tests, apart from the ability to decompose selected polimers, the following tests were taken into account: morphology of bacteria, mobility, fluorescein production, oxidase production, arginine dihydrolase production, indole production, phosphatase production, ornithine decarboxylase production, citrate utilization, Tween degradation, maltose utilization, sodium formate utilization, lecithin degradation, aesculin hydrolysis, gluconate oxidation, methyl red test, Voges –Proskauer reaction, growth at 4ºC and

Table 1. Some physico-chemical properties of the water in Chełmżyńskie Lake (the range of variability of the parameters among the plant sites is given in brackets).

PARAMETER	SEASON		
	Spring	Summer	Autumn
Temperature [°C]	19.6 (19.4 – 19.8)	23.1 (22.4 – 24.2)	14.2(14.0 – 14.3)
Transparence [m]	3.25	2.00	2.50
Oxygen content [mg · L^{-1}]	8.3 (6.5 – 9.4)	4.1 (3.7 – 4.7)	8.4 (8.1 – 8.6)
Oxygen saturation of water [%]	91.7 (76.3 – 101.7)	48.9 (42.2 – 57.2)	84.7 (83.8 – 85.9)
Electrolytic conductivity [mS · cm^{-1}]	860.3 (859 – 862)	772.3 (761 – 787)	364.7 (350 – 392)
pH	8.8 (8.7 – 8.9)	8.5 (8.3 – 8.8)	9.1 (9.0 – 9.2)

37ºC, growth in a 4% and 6% NaCl solution, growth on TCBS agar, growth on McConkey agar and L-arabinose utilization.

Results

Changes in certain physico-chemical parameters of the water at the sites where macrophytes were collected are presented in Table 1. The highest temperature of the water was found in the surface layer of the littoral in summer, and the lowest in autumn. The differences between the temperature of the water at the three selected study sites varied depending on the season from 0.2 to 1.8°C.

The transparency of the water measured using a Secchi disc reached values of between 2 and 3.25 m during the studied periods. The greatest transparency of the water was observed in spring and the poorest in summer.

It follows from the tests on the content of oxygen dissolved in the water and the saturation of the water with oxygen that the amount of it in the water was considerably higher in spring and autumn than in summer. The average saturation of the water with oxygen during the studied seasons varied from 48.9% to 91.7%.

The electrolytic conductivity of the water in Moty Bay in the studied seasons was on average between 364.7 and 860.3 S· cm^{-1} and remained at a high level in spring and summer but fell in autumn.

The water of Chełmżyńskie Lake has an alkaline pH. The highest alkalinity of the water (pH = 9.2) was found in autumn and the lowest (pH = 8.3) in summer.

The results of the studies on the number of epiphytic bacteria inhabiting the surface of the studied hydromacrophytes are presented in Fig. 2. It follows from these data that the number of heterotrophic bacteria on the surface of common reed and cattail rose from spring to summer and then fell in autumn. On the surface of hornwort the highest number of heterotrophic bacteria was found in autumn and the lowest in summer. The surfaces of cattail shoots were inhabited by bacteria to the greatest degree, the number of bacteria varying depending on the season from $6.08 \cdot 10^7$ to $2.11 \cdot 10^9$ cells per g dry plant mass.

Among the isolated strains lipo-, cellulo- and proteolytic bacteria were dominant (Tab. 2). On average, in all the studied seasons, from about 52 to 93% of strains displayed lipolytic properties. These strains occurred in greater numbers on the surface of helophytes than on the surface of hornwort. Cellulolytic bacteria constituted on average from 22 to about 84% of all the studied strains. The highest percentage of them was observed on the surface of hornwort. From the two proteins, casein was utilized slightly more than gelatine. From about 26 to 56% of all the studied strains decomposed gelatine, while from about 18 to 53% of strains decomposed casein. Bacteria capable of hydrolysing starch, pectin and chitin belonged to less numerous physiological groups. The most active in the decomposition of all the studied compounds were strains isolated from the surface of cattail. This is testified to by the values of the physiological activity index, which, depending on the season, was from about 40 to 49% for strains isolated from the surface of this plant (Fig. 3).

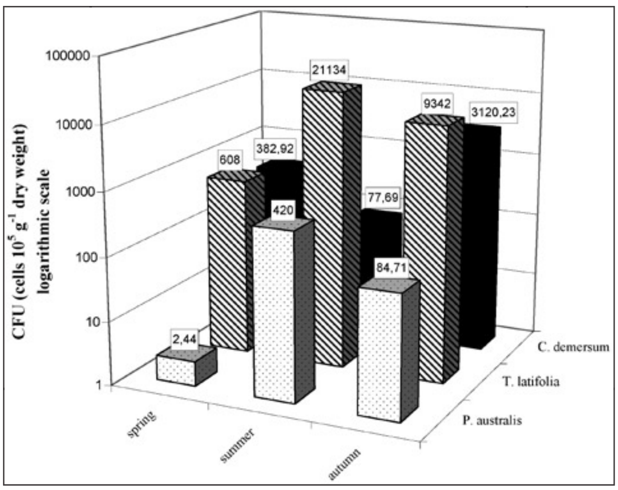

Fig. 2. Number of heterotrophic epiphytic bacteria inhabiting some macrophytes in littoral zone of Chełmżyńskie Lake.

Table 2. Physiological properties of strains of epiphytic bacteria.

Season	Plant	Bacteria decomposing (in %)						
		casein	gelatine	lipids	starch	cellulose	pectin	chitin
Spring	*P. australis*	43	46	45	26	74	0	7
	T. latifolia	70	70	90	71	21	3	25
	C. demersum	64	72	23	7	86	4	5
Summer	*P. australis*	2	16	92	14	85	6	12
	T. latifolia	50	52	93	45	21	3	22
	C. demersum	44	51	55	17	91	8	10
Autumn	*P. australis*	10	17	75	49	54	10	16
	T. latifolia	40	46	95	45	24	6	27
	C. demersum	13	31	79	25	76	19	11
Average	*P. australis*	18.3	26.3	70.7	29.7	71.0	5.3	11.7
	T. latifolia	53.3	56.0	92.7	53.7	22.0	4.0	24.7
	C. demersum	40.3	51.3	52.3	16.3	84.3	10.3	8.7

The generic composition of the isolated strains displayed distinct variability, linked to the species of plant and season. On the surface of common reed in spring and on the surface of hornwort in spring and summer, bacteria from the genus *Bacillus* were dominant, constituting from 56 to 80% of all the isolated species. Moreover, bacteria belonging to the group *Arthrobacter* - *Corynebacterium* and the genera *Alcaligenes* and *Flavobacterium* occurred in large numbers on the surface of the common reed. The surfaces of cattail were covered to the greatest degree by bacteria from the Enterobacteriaceae family and the genus *Aeromonas* (Tab. 3).

Discussion

One of the most significant groups of microorganisms in water bodies are bacteria inhabiting permanent abiotic and living substrates. They abundantly inhabit the surface of upper plants as well as sea and freshwater algae [10, 11, 12, 13, 14]. In comparison with planktonic or benthic bacteria, these bacteria are more metabolically active [15, 16]. Their occurrence and distribution depends on many physical, chemical and biological factors.

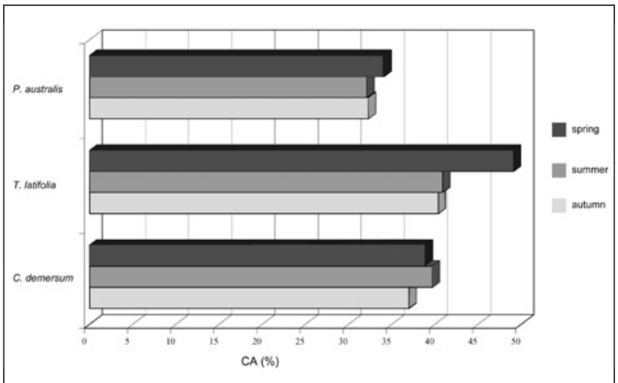

Fig. 3. Coefficient of physiological activity (CA) of epiphytic bacteria.

From the data obtained in the present study, it follows that the number of heterotrophic epiphytic bacteria was characterized by seasonal variability. The high number of bacteria in the summer period is probably linked with the stimulating effect of several different factors at the same time. These include an increase in the temperature of the environment and greater proportion of allochtonic contamination. Organic substances produced by phytoplankton and macrophytes also have a great effect on the number of bacteria in water bodies. For example, Baker and Farr [17] state that duckweed (*Lemna minor*) can secrete into the environment from 1.1 to 2.6% of the total amount of carbon involved in the process of photosynthesis.

Microorganisms occurring in the natural environment are capable of decomposing and utilizing particulate organic matter (POM) and dissolved organic matter (DOM). The rate of the secondary production of bacteria in water bodies indicates that they utilize polimeric compounds in metabolic processes alongside small-particle compounds [1].

One of the most important groups of biopolimers occurring in waters is lipids [2]. They are produced by both plants and animals. As follows from the research presented in this paper and our earlier observations [18, 19], lipolytic bacteria constitute the most numerous physiological group among epiphytic bacteria. It follows from research by Donderski and Strzelczyk [20] that lipolytic bacteria were one of the most numerous groups of bacteria occurring in the water and bottom deposits of Lake Jeziorak.

A compound that occurs universally in plants is cellulose. With lignin and acidic polysaccharides, it is the building material of plant cell walls. According to Zdanowski [21] environmental factors like temperature, the degree of eutrophication of the environment and the pH of the water, have an influence on the decomposition of cellulose. In the climatic zone of our study site, the decomposition of cellulose takes place most intensively at higher temperatures in the period from May to October. It follows from the present study that the ability to decompose cellulose was displayed by a high percentage of bacteria (on average from about 22% to 84% of epiphytic

Table 3. Epiphytic bacteria occuring at three macrophyte species in Chełmżyńskie Lake (bacteria in %).

Bacteria	Plant								
	P. australis			*T. latifolia*			*C. demersum*		
	a	b	c	a	b	c	a	b	c
Enterobacteriaceae	0	6	5	0	35	41	4	2	12
in them:									
Escherichia coli	0	4	1	0	10	25	2	0	6
Enterobacter aerogenes	0	1	2	0	20	16	1	0	2
Klepsiella sp.	0	1	2	0	0	0	0	0	4
Hafnia alvei	0	0	0	0	0	0	1	2	2
Erwinia stewartii	0	0	0	0	2	0	0	0	0
Serratia sp.	0	0	0	0	3	0	0	0	0
Bacillus sp.	56	1	4	3	4	6	80	62	7
in them:									
Bacillus cereus	10	1	2	0	4	1	10	25	0
Bacillus firmus	46	0	2	0	0	2	66	26	7
Bacillus pumilis	0	0	0	0	0	0	4	11	0
Bacillus megaterium	0	0	0	1	0	0	0	0	0
Flavobacterium sp.	14	7	25	4	4	3	0	1	15
Flexibacter- Cytophaga	0	11	8	10	5	7	0	7	18
in them:									
Cytophaga fermentas	0	0	2	0	0	0	0	6	7
Cytophaga salmonicolor	0	0	0	0	3	3	0	0	5
Aeromonas sp.	0	0	4	35	37	14	0	4	4
in them:									
Aeromonas hydrofila	0	0	0	6	2	5	0	4	2
Vibrio sp.	0	0	0	0	0	1	0	0	0
Alcaligenes sp.	25	1	32	26	3	7	7	0	0
in them:									
Alcaligenes denitryficans	0	0	0	6	0	1	4	0	0
Alcaligenes faecalis	9	1	6	4	1	2	2	0	0
Alcaligenes piechaudii	0	0	0	0	0	1	1	0	0
Pseudomonas sp.	1	3	3	0	2	3	3	6	5
in them:									
Pseudomonas fluorescens	1	2	1	0	2	0	1	4	2
Achromobacter sp.	4	6	0	5	0	6	6	7	9
Arthrobacter-Corynebacterium	0	58	15	13	4	8	0	5	30
Acinetobacter sp.	0	0	2	0	0	0	0	0	0
Bordetella bronchiseptica	0	4	1	0	0	2	0	0	0
Agrobacterium sp.	0	3	1	0	0	0	0	0	0
Staphylococcus sp.	0	0	0	4	6	2	0	0	0
Micrococcus sp.	0	0	0	0	0	0	0	6	0
in them:									
Micrococcus roseus	0	0	0	0	0	0	0	1	0
Micrococcus varians	0	0	0	0	0	0	0	2	0

Explanations: a – spring, b – summer, c - autumn

bacteria). However, epiphytic bacteria isolated from the submerged surfaces of common reed and cattail in the littoral zone of Lake Jeziorak displayed this property on average in only 20 to 31% of cases [18]. In the water of Chełmżyńskie Lake cellulolytic bacteria constituted, depending on the season and the site where the sample was taken, from about 13 to 21% of the total number of isolated strains [22].

Proteolytic bacteria constitute an important physiological group among microorganisms occurring in lakes. Paluch and Niewolak [23] drew attention to the numerous occurrence of bacteria decomposing protein in the water of Lake Jeziorak. The common occurrence of this group of bacteria results from the widespread presence of various types of proteins in waters. According to Donderski and Strzelczyk [20] proteolytic strains occur more often in bottom deposits of lakes than in the water depths. It follows from the study by Donderski and Kalwasińska [22] that proteolytic bacteria constituted on average about 35% of all heterotrophic strains in the water of Chełmżyńskie Lake. However, among bacteria inhabiting the surfaces of the plants studied in the present paper, this property was displayed on average, depending on the substrate used, by between 18 and 56% of heterotrophic bacteria.

One of the compounds least accessible to bacteria isolated from the surfaces of the studied plants was chitin-aminopolysaccharide, one of the building components of many organisms, mainly protozoa, nematodes, Annelida, Crustacea and insects. We also find it in the cell walls of fungi. Bacteria that decompose chitin utilize it as a source of carbon and nitrogen. According to Donderski [24], the activity of chitinase synthesized by aquatic bacteria is the highest in the pH range of 6-7. As follows from research by Donderski and Strzelczyk [20] and Donderski and Trzebiatowska [25], in bottom deposits of lakes chitinolytic bacteria constituted on average from 0.6 to 28.0% of heterotrophic bacteria. A relatively high percentage of chinolytic bacteria was found by Mudryk [26] in the littoral lakes of the Baltic (from 4 to 43% in the water and from 2 to 34% in the bottom deposits).

According to Baker and Farr [27] and Hossel and Baker [28] the generic composition of epiphytic bacteria is similar to the composition of the bacterial population in the surrounding water. As follows from research by Donderski and Kalwasińska [22] on the generic composition of planktonic bacteria in Chełmżyńskie Lake, the most commonly occurring genera were *Flavobacterium*, *Alcaligenes* and *Aeromonas*. In the present paper, apart from the genera of bacteria mentioned above, a high percentage of bacteria from the group *Arthrobacter-Corynebacterium*, the genus *Bacillus* and the Enterobacteriaceae family was observed on the surfaces of plants (depending on the season and the species of plant).

Research by Lalke-Porczyk [18] on the generic composition of epiphytic bacteria growing on six different species of macrophytes in the littoral zone of Lake Jeziorak demonstrates that bacteria from the group *Flavobacterium-Cytophaga* occurred most often among epiphytic bacteria (on average during the year they constituted 18% of the total number of heterotrophic bacteria). These bacteria belong to the most commonly occurring in waters [20]. The high proportion of this group of bacteria among the total number of heterotrophic planktonic and epiphytic bacteria probably results from their resistance to sunlight. These bacteria display a great ability to produce pigments which protect them from the lethal effect of sunlight, particularly UV [29].

The numerous occurrence among epiphytic bacteria of representatives of the Enterobacteriaceae family may indicate contamination of the water by faeces, probably coming from waterfowl nesting in this area. The genus *Bacillus*, well-represented among epiphytic bacteria, deserves attention. Donderski and Strzelczyk [20] noticed the high proportion of these bacteria in the water depths of lakes. These bacilli occur abundantly in soil and the rhizosphere of plants. Their occurrence among epiphytic bacteria should be linked with precipitation and run-off waters from the drainage area, which, when they fall into the water body, remain in the strip of vegetation in the littoral zone. Certain species of bacteria from the genus *Bacillus* take part in the utilization of compounds found in industrial waste [30]. These bacilli easily move from the soil and water onto plants. According to Święcicka and Hauschild [31] these bacteria can provide plants with nitrogen assimilated from the atmosphere; they also impede the development of pathogenic bacteria, producing antibiotic substances, including some with fungicidal properties. They display an ability to decompose dead plant tissue containing aromatic compounds, pectin, starch and to a lesser degree cellulose.

Similarly, bacteria from the group *Arthrobacter-Corynebacterium* can be supposed to originate from the soil. Representatives of the genus *Arthrobacter* belong to autochtonic soil microflora. Many strains from this group inhabit the surfaces of land plants, often causing disease [32].

Conclusions

1. Number of bacteria growing on helophytes, i.e. common reed and cattail increased from spring to summer, and then decreased in autumn.
2. On the surface of hornwort the number of heterotrophic bacteria increased throughout the whole vegetative season.
3. Among all of the isolated strains lipolytic, cellulolytic and proteolytic bacteria were dominant.
4. Bacteria isolated from the surface of cattail were characterized by the potentially highest physiological activity.
5. Generic composition of the isolated strains changed with the development of the macrophytes and depended on the species of plant.
6. Among strains inhabiting surface of the hydromacrophytes dominated bacteria from the genera *Bacillus, Alcaligenes*, *Flavobacterium*, *Aeromonas*, the group *Arthrobacter- Corynebacterium* and the family Enterobacteriaceae.

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