

Original Research

The Role of Bacteria Growing on the Root System of the Common Reed (*Phragmites australis* [Cav.] Trin. ex Steudel) in the Metabolism of Organic Compounds

E. Lalke-Porczyk*, W. Donderski

Department of Environmental Microbiology and Biotechnology, Institute of Ecology and Environmental Protection, Nicolaus Copernicus University, Gagarina 9, 87-100 Toruń, Poland

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Abstract

Some physiological properties and a number of heterotrophic bacteria inhabiting the surface of the root system of the common reed (*Phragmites australis* [Cav.] Trin. ex Steudel) were studied in two different types of bottom sediments (sandy and silty), and the bacteria were identified.

For comparative purposes, samples of the bottom sediments from the sites where the reed grew were subjected to microbiological tests. It was found that the number of bacteria growing on the root system of the common reed and those inhabiting the types of bottom sediments tested increased from spring to summer and then decreased in autumn. On the surface of the rhizomes of the reed growing in silty sediments, a higher number of bacteria was always noted than on the surface of rhizomes growing in sandy sediments. Also, the number of heterotrophic bacteria in samples of bottom sediments was always higher in silty sediments than in sandy sediments. Lipo-, proteo- and amylolytic bacteria and bacteria capable of producing ammonia from organic compounds were dominant among all the isolated strains. The lowest numbers were found of chitinolytic bacteria and those that form hydrogen sulphide from organic compounds. Strains isolated in spring - both from the root system of the plant and from the samples of sediments - were potentially the most active physiologically. As a result of the identification of the isolated bacterial strains, it was found that bacteria from the *Arthrobacter-Corynebacterium* group and the *Achromobacter* genus are dominant in the root system of the common reed and in sandy and silty sediments.

Keywords: degradation of organic substances, epiphytic bacteria, heterotrophic bacteria, common reed

Introduction

There is a strong interaction between the root systems of plants and microorganisms. The zone in which this interaction is observed is called the rhizosphere. Within its scope we can distinguish the rhizoplane - the surface of the root together with the epiphytic microflora inhabiting it - the layer of soil closely connected with the root surface, and the soil that can easily be separated.

On the surface of roots and in their vicinity, the number of microorganisms found is usually from several to several hundred times higher than in the soil far from the root. The relationship between the number of microorganisms from the rhizosphere and the number of microorganisms inhabiting the soil beyond the reach of the influence of the roots is called the rhizosphere effect [1]. This phenomenon is explained by increased concentrations of organic substances secreted by the roots.

In addition, by secreting into the environment various compounds like aminoacids, organic acids, sugars,

*Corresponding author; e-mail: lalke@biol.uni.torun.pl

vitamins and plant growth regulators, the microorganisms influence the plants and the process of symbiosis. These compounds are secreted into the environment by saprophytic microorganisms, as well as potentially symbiotic and pathogenic microorganisms in relation to plants [2].

Information on the subject of microflora inhabiting the rhizosphere of hydromacrophytes is fragmentary. Hence the aim of the present paper was to determine the number of heterotrophic bacteria inhabiting the surface of the root system of the common reed (*Phragmites australis* [Cav.] Trin. ex Steudel), their enzymatic capabilities and systematic affiliation.

Materials and Methods

Study Area

Microbiological research was conducted within Moty Bay situated in the southwestern part of Lake Jeziorak. The lake, situated in northeastern Poland, belongs to the Hława Lake District and is part of the Drwęca-Vistula drainage basin. It is a tunnel-valley water body, orientated meridionally, which was formed during the last glaciation. The surface area of the water is 32.3 km², which makes it the fourth largest lake in Poland [3]. The maximum length of the lake is 27.45 km, and the width 2.35 km. The average depth of the lake is about 4.3 m. The shoreline of Lake Jeziorak (with a coefficient of 6.6) is well-developed, comprising numerous bays, including the strongly eutrophic Moty Bay. The western shore of Lake Jeziorak is surrounded by a mixed pine-beech and deciduous forest, while the eastern shore, over a 13 km stretch to the north of the town of Hława, borders on meadows and arable land, and, further away, a coniferous and mixed forest [4]. Lake Jeziorak is classified as a eutrophic water body. The water has a yellow-green colour, relatively poor transparency and an alkaline factor. In the littoral zone of Moty Bay, *Phragmites australis* [Cav.] Trin. ex Steudel is dominant among helophytes [5].

Sampling

15-centimetre sections of common reed rhizomes together with side roots growing in sandy and silty bottom sediments, and samples of these sediments were taken for microbiological tests. The plant material and deposit samples were collected in sterile glass jars. These were taken to the laboratory in containers filled with ice, where the temperature inside did not exceed +7°C. The time from the moment of taking the samples to performing the analyses did not exceed 6 hours. Material for tests was taken in the development cycle of the plant: in spring (20.05.2001) - during the period of intense growth and development of the plant; in summer (19.08.2001) - during the flowering of the plants; and in autumn (23.10.2001) - when the shoots were aging.

Estimating the Numbers of Heterotrophic Bacteria and Isolation of Bacterial Strains

In order to determine the number of heterotrophic bacteria inhabiting the root system of the common reed and that of benthic bacteria, 10 g of the epiderm was taken from the rhizome of the common reed growing in two different types of bottom sediments - in sandy and silty sediments - and 10 g of the bottom sediments from the site where the reed rhizome was taken. 90 cm³ of sterile buffer water [6] was added to these samples and then homogenized for 2 minutes in a homogenizer at 4000 rpm. The homogenates thus obtained were diluted 10 times with sterile buffer water. These dilutions were seeded on the surface of iron-peptone agar (IPA) after Ferrer et al. [7]. After 10 days of incubating the dishes at the temperature *in situ*, the bacterial colony that had grown was counted. Then, from the whole surface of the dishes or from specific sectors, about 120 colonies each of epiphytic and benthic bacteria were pricked out at random onto semi-liquid iron-peptone agar (5 g agar/l). These strains were multiplied for 6 days at a temperature of 20°C. After checking the purity of the cultures, each time 100 strains were used for further tests. These bacteria were transplanted every 3 months on to fresh semi-liquid IPA medium and stored at a temperature of +4°C for further tests.

Physiological Properties

The isolated epiphytic and benthic bacteria were seeded on a range of test media containing various organic compounds. The capability of the bacteria to decompose multi-particle compounds - starch, cellulose, pectin, chitin, fat and protein - was taken into consideration in the tests. Moreover, the ability of bacteria to ammonification, to produce H₂S and to acidify a medium with glucose was tested. The media were prepared after Donderski [8]. The coefficient of physiological activity (CA) of the bacteria was calculated according to the formula proposed by Dahlbäck et al. [9]:

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

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

Identification of Bacteria

The bacteria isolated from the rhizosphere and bottom sediments were identified on the basis of a pattern of diagnosis proposed by Shewan et al. [10], Allen et al. [11] and Bergey's Manual [12].

Results

The results of the study on the number of epiphytic bacteria inhabiting the surface of the rhizomes of the com-

Table 1. Number of heterotrophic epiphytic bacteria inhabiting the rhizosphere of the common reed and in bottom sediments.

Season	Habitat	Number of heterotrophic bacteria $\cdot 10^7 \cdot g^{-1} \cdot s.m.$
Spring 2001	R _{si}	85.30
	R _{sa}	40.50
	S _{si}	4.02
	S _{sa}	1.09
Summer 2001	R _{si}	116.24
	R _{sa}	58.11
	S _{si}	6.30
	S _{sa}	1.50
Autumn 2001	R _{si}	53.78
	R _{sa}	13.15
	S _{si}	3.82
	S _{sa}	0.28
Average	R _{si}	85.11
	R _{sa}	37.25
	S _{si}	4.71
	S _{sa}	0.96

Explanations: R_{si} - root system of common reed growing in silty sediments; R_{sa} - root system of common reed growing in sandy sediments; S_{si} - silty bottom sediments; S_{sa} - sandy bottom sediments

mon reed and silty and sandy sediments are presented in Table 1. It follows from these data that the number of bacteria growing on the root system of the common reed and inhabiting the bottom sediments increased from spring to summer and then decreased in autumn. A greater number of bacteria on the surface of the rhizomes of the common reed growing in silty sediments than on the surface of rhizomes growing in sandy sediments was noted every time. On average, the number of heterotrophic bacteria on the surface of the rhizomes of reeds growing in silty sediments during the testing period was $8.51 \cdot 10^8$ cells per g dry mass of rhizome, and on the surface of the rhizomes of reeds in sandy sediments, it was about twice as low. Also, in the silty sediments, the number of heterotrophic bacteria was always higher than in sandy sediments, on average $47.10 \cdot 10^6$ and $9.60 \cdot 10^6$ cells per g dry mass of sediments, respectively.

Among the isolated strains, lipo-, proteo- and amylolytic bacteria and those capable of producing ammonia from organic compounds were dominant (Table 2, Fig. 1). Lipolytic bacteria occurred in the highest numbers in sandy sediments in all of the studied seasons. This property was displayed by between 71 and 80% of bacteria strains isolated from sandy sediments, and from 61 to 68% of bacteria isolated from the root system of reeds growing in this type of deposit. Proteolytic properties were displayed on average by about 41 to 55% of strains isolated from the surface of rhizomes of the common reed and the types of deposit tested. The highest percentage (60%) of strains

capable of decomposing protein was found in summer among the strains isolated from sandy sediments. Bacteria capable of hydrolyzing starch and producing ammonia from organic compounds occurred in greater numbers on the surface of rhizomes growing in silty sediments and in silty sediments than in sandy sediments. This rule was observed particularly clearly in spring and summer. Bacteria capable of acidifying the environment as a consequence of decomposing glucose occurred in the greatest numbers in sandy sediments. In spring this property was displayed by 69% of the strains isolated from sandy sediments and 52% of the strains isolated from the surface of rhizomes growing in this deposit. At the same time, 34% of all the strains in silty sediments and 20% of the strains isolated from rhizomes growing in silty sediments displayed this property (Table 2).

In the samples studied, the bacteria capable of decomposing cellulose and pectin were less numerous. On average, from 8.7% to 12.7% of all the isolated strains displayed the ability to decompose these compounds. Pectinolytic bacteria occurred in the highest numbers in spring in both types of sediments, where they constituted 20-22% of all the isolated strains. A small percentage of strains displayed the ability to decompose chitin and to produce hydrogen sulphide from organic compounds.

Chitinolytic bacteria constituted on average from 1.7 to 6.7% of all the strains in all the studied seasons, and those producing hydrogen sulphide from only 0.7 to 3.7%. The highest number of bacteria capable of hydrolyzing chitin

Table 2. Physiological properties of epiphytic bacteria inhabiting the rhizosphere of the common reed and in bottom sediments.

Season	Habitat	Physiological groups bacteria in %								
		glucose acidifying	amylolytic	cellulolytic	chitinolytic	pectinolytic	lipolytic	proteolytic	ammonifying	H ₂ S producing
Spring 2001	R _{si}	20	62	13	1	11	51	40	59	2
	R _{sa}	52	32	8	2	9	68	58	28	4
	S _{si}	34	54	20	2	22	44	52	52	4
	S _{sa}	69	44	6	0	20	80	56	29	0
Summer 2001	R _{si}	47	44	9	2	11	52	44	45	0
	R _{sa}	33	38	10	2	8	68	39	34	7
	S _{si}	38	46	8	8	10	42	24	44	0
	S _{sa}	40	32	10	2	8	71	60	40	6
Autumn 2001	R _{si}	40	39	8	2	8	38	40	38	0
	R _{sa}	28	36	14	4	9	61	44	35	0
	S _{si}	16	20	10	10	8	50	30	50	0
	S _{sa}	32	28	10	6	4	71	48	24	4

Explanations: R_{si}, R_{sa}, S_{si}, S_{sa} - see Table 1

was observed in all the samples tested in autumn (from 2 to 10% of strains).

The physiological activity of the strains isolated from the root system of the common reed growing in both types of bottom deposit was the highest in spring, and then gradually decreased until autumn. The strains most active in decomposing all the compounds tested were strains isolated from sandy bottom sediments. The values of the coefficient of physiological activity (CA), which varied from 25.2 to 33.8% depending on the season, testify to this (Fig. 2). The strains isolated from the surface of rhizomes of reeds growing in both types of deposit were characterized by a slightly lower activity. In spring the values of CA

for bacteria isolated from the rhizosphere growing in both types of deposit were almost identical at 29%. In summer, strains isolated from the rhizosphere growing in silty sediments displayed a higher activity, while in autumn it was those from the rhizosphere growing in sandy sediments.

Among the isolated heterotrophic bacteria, the strains from the *Arthrobacter-Corynebacterium* group were dominant. On average, depending on the site, they constituted from 22 to 26% of all the strains. The bacteria from the *Achromobacter* genus occurred in particularly high numbers in silty sediments and on the surface of the rhizosphere growing in these sediments. A relatively high percentage of the bacteria isolated from both types

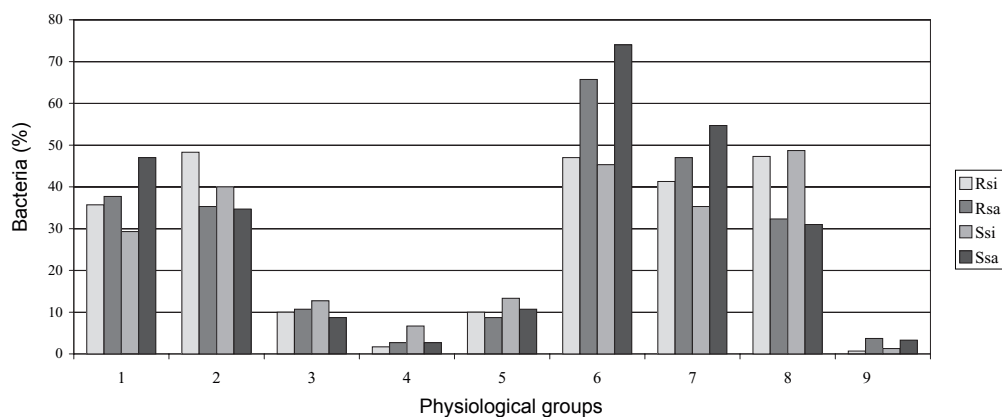


Fig. 1. Physiological properties of epiphytic bacteria inhabiting the rhizosphere of the common reed and in the bottom sediments (average).

Explanations: R_{si}, R_{sa}, S_{si}, S_{sa} - see Table 1; physiological groups of bacteria: 1 - glucose acidifying, 2 - amylolytic, 3 - cellulolytic, 4 - chitinolytic, 5 - pectinolytic, 6 - lipolytic, 7 - proteolytic, 8 - ammonifying, 9 - H₂S producing.

of sediments and from the surface of reed rhizomes was constituted by bacteria from the *Bacillus* genus. On average, depending on the site, they constituted from 11.3 to 12.7% of all the strains. They occurred in particularly high numbers in summer in silty sediments. On the other hand, bacteria from the *Flavobacterium-Cytophaga* group constituted on average from 9 to 11.7% of all strains in all the tested samples. Their highest percentage was found in spring on the surface of the rhizosphere growing in silty sediments and in silty sediments themselves (Table 3). The bacteria from the Enterobacteriaceae family and from the *Micrococcus* and *Pseudomonas* genera were slightly less numerous in all the samples. The bacteria from the *Pseudomonas* genus occurred in greater numbers on the surface of roots than in sediments (Fig. 3). The least numerous were the bacteria from the *Alcaligenes*, *Aeromonas* and *Chromobacterium* genera. Depending on the season and site, they constituted 0-14%, 1-14% and 0-4%, respectively, of all the strains.

Discussion

In the degradation of organic substances in water bodies, alongside planktonic and benthic bacteria, an important role is played by epiphytic bacteria, which grow on the surfaces of hydromacrophytes, their submerged parts and the parts trailing in the bottom sediments. As follows from the literature data, the surfaces of plants, both freshwater and marine, are abundantly covered in bacteria [13, 14, 15, 16, 17]. In comparison with planktonic or benthic bacteria, these bacteria are more metabolically active [18, 19].

From the data obtained in the present study, it follows that the number of heterotrophic epiphytic bacteria of the root system of the common reed and the number of ben-

thic bacteria were characterized by seasonal variability. The highest number of bacteria was found in summer, and the lowest in autumn. The high number of bacteria in the summer period is probably connected with the stimulating action of several different factors simultaneously. These include a rise in the temperature of the environment and an increased proportion of allochthonic contamination and plant secretion. Baker and Farr [20] write that duckweed (*Lemna minor*) can secrete into the environment from 1.1 to 2.6% of all the carbon connected with the process of photosynthesis. According to Libbert [21], macrophytes release organic substances not only through leaves and stems into the water, but also through the root system into the bottom sediments, which may explain the high number of bacteria found in this study on the surface of the root system of the common reed. Also, according to Coler and Gunner [22], the development of microorganisms on the surface of the roots of aquatic plants is connected with the secretion of organic substances by the roots, and the influence of the root system of aquatic plants can be compared to the rhizosphere effect caused by land plants.

In this paper, certain physiological properties of bacteria were studied, including the ability of epiphytic and benthic bacteria to decompose selected polymeric substrates occurring in natural waters. The results obtained indicate that the most numerous among epiphytic and benthic bacteria are lipolytic bacteria. Besides proteins, polysaccharides and polynucleotides as well as lignin and lignocellulose, lipids are one of the most important groups of biopolymers occurring in fresh waters [23]. They are produced by both plants and animals. In research on epiphytic microflora, Lalke-Porczyk [24] showed that lipolytic bacteria constituted the most numerous physiological group. Depending on the season, this property was displayed by between 70 and 98% of bacteria grow-

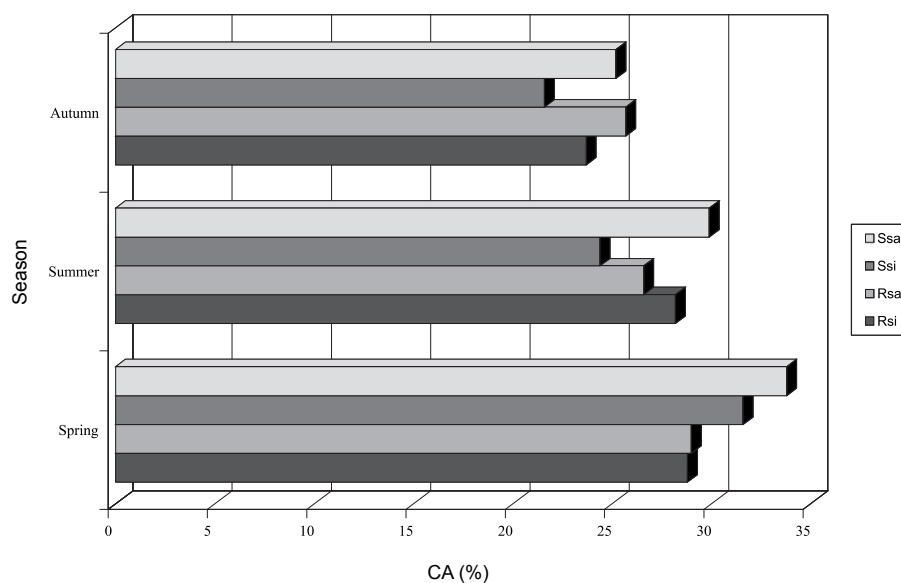


Fig. 2. Coefficient of physiological activity (CA) of bacteria inhabiting the rhizosphere of the common reed and in the bottom sediments. Explanations: R_{si} , R_{sa} , S_{si} , S_{sa} - see Table 1.

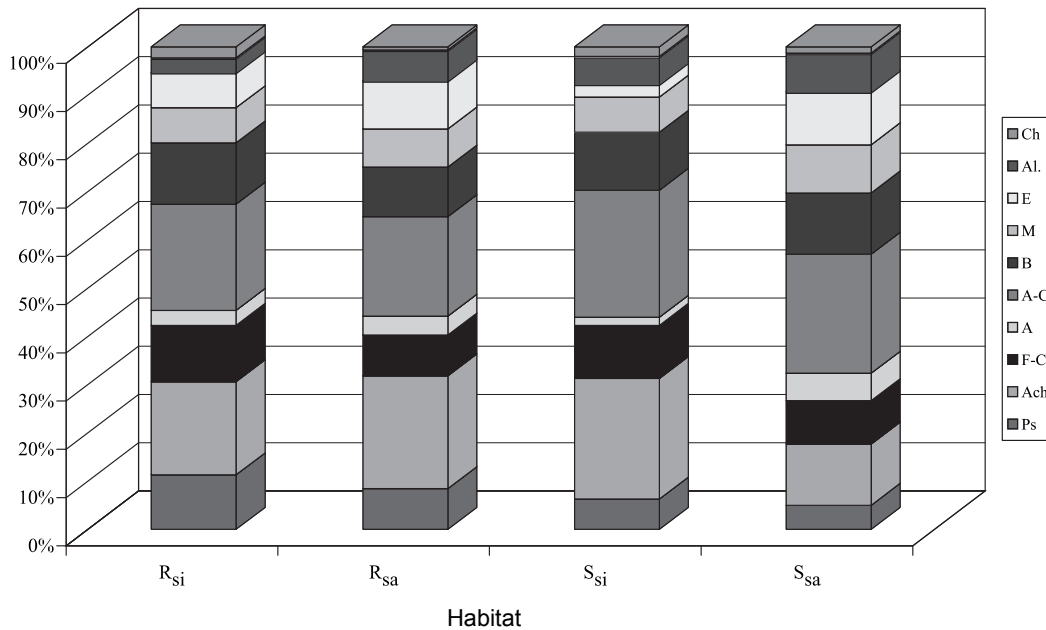


Fig. 3. Genera and groups of bacteria among all the tested strains (average).

Explanations: R_{si}, R_{sa}, S_{si}, S_{sa} - see Table 1; bacteria: Ch – *Chromobacterium*, Al – *Alcaligenes*, E – *Enterobacteriaceae*, M – *Micrococcus*, B – *Bacillus*, A-C – *Arthrobacter* – *Corynebacterium*, A – *Aeromonas*, F-C – *Flavobacterium* – *Cytophaga*, Ach – *Achromobacter*, Ps – *Pseudomonas*.

ing on the submerged surfaces of common reed stems. It follows from the study by Donderski and Strzelczyk [25] that lipolytic bacteria constituted one of the most numerous groups of bacteria occurring in the water and bottom sediments of Lake Jeziorak. A similarly high number of these organisms was found by Kjelleberg and Hakansson [26] in a marine environment.

Proteolytic bacteria constituted the second most numerous physiological group according to the results of this study. Paluch and Niewolak [27] drew attention to the high numbers of bacteria that decompose protein occurring in the water of Lake Jeziorak. The universality of the occurrence of proteolytic bacteria is a consequence of the widespread distribution of various kinds of proteins in water. According to Donderski and Strzelczyk [25], proteolytic strains occur more often in the bottom sediments of the lake than in the bulk water. In the water of the eutrophic Lake Jeziorak, they constituted from 54 to 74% of all heterotrophic bacteria, depending on the season, and from 70 to 94% in the bottom sediments.

As follows from the results of the present paper, the bacteria that are involved in the process of ammonification occurred in great numbers in the rhizosphere of the common reed and in bottom sediments. In the study on the microflora of lakes of different trophic, Donderski and Strzelczyk [25] write that ammonifying bacteria can constitute from 5 to 100% of all the strains inhabiting the bottom sediments.

One of the basic, and at the same time highly diverse, groups of natural compounds in nature is carbohydrates. They occur in particularly large quantities in plant organ-

isms. Besides mono- and disaccharides, multi-particle compounds like starch, cellulose, glycogen, chitin, pectin compounds, plant gums and mucus and many others are widespread in nature [28, 29].

The ability to hydrolyze starch was displayed, on average, by between about 35 to 48% of the bacteria we studied. It was most intensively decomposed by bacteria isolated from the surface of the root system growing in silty sediments. According to Donderski and Strzelczyk [25], amylolytic bacteria were less numerous in bottom sediments than in water, where they constituted from 18 to 48% of all heterotrophic strains. They occurred quite frequently in the water and bottom sediments of the lakes of Bory Tucholskie [25] and in the water and bottom sediments of estuary lakes [30].

Bacteria that acidify the substrate with glucose occurred in the greatest numbers in spring among strains isolated from sandy sediments and the rhizosphere of the common reed growing in sandy sediments. Donderski and Strzelczyk [25] also write that these bacteria occurred in the greatest numbers in the bottom sediments of Lakes Jasne and Jeziorak in spring (March-May), when they constituted 54-80% of strains and 52-70% of strains, respectively.

A commonly occurring polysaccharide in plants is cellulose. Apart from lignin and acidic polysaccharides, it constitutes the woody material of plant cell walls. It follows from the present paper that the ability to decompose cellulose was displayed by a relatively low percentage of epiphytic and benthic bacteria (on average 8.7-12.7% of all strains). As Lalke-Porczyk

Table 3. Generic composition (%) of epiphytic bacteria inhabiting the rhizosphere of the common reed and in the bottom sediments.

Group, family or genus of bacteria	Habitat															
	R _{si}				R _{sb}				S _{si}				S _{sb}			
	Spring	Summer	Autumn		Spring	Summer	Autumn		Spring	Summer	Autumn		Spring	Summer	Autumn	
<i>Pseudomonas</i>	15	15	4		12	9	7		11	4	4		4	6	5	
<i>Achromobacter</i>	20	21	17		16	17	14		32	18	25		12	11	15	
<i>Flavobacterium – Cytophaga</i>	19	5	11		6	10	12		15	14	4		11	11	5	
<i>Aeromonas</i>	3	3	3		6	5	2		1	2	2		14	2	1	
<i>Arthrobacter – Corynebacterium</i>	16	27	23		24	23	21		19	20	40		29	21	24	
<i>Bacillus</i>	2	14	22		9	8	17		3	29	4		10	13	15	
<i>Micrococcus</i>	6	10	6		6	9	11		6	6	10		11	4	15	
<i>Enterobacteriaceae</i>	10	5	7		14	11	8		3	3	2		5	23	5	
<i>Alcaligenes</i>	6	0	3		7	8	6		6	4	7		4	6	14	
<i>Chromobacterium</i>	3	0	4		0	0	2		4	0	2		0	3	1	

Explanations: R_{si}, R_{sb}, S_{si}, S_{sb} - see Table 1

writes [24], cellulolytic bacteria constituted on average from 20 to about 30% of the bacteria isolated from the submerged surfaces of common reed stems in the vegetative season.

Pectin was decomposed to a similar extent to cellulose. As follows from the study by Lalke-Porczyk [24] that pectinolytic bacteria constituted on average from 19 to 26% of all the strains on the surface of common reed stems. However, Donderski and Strzelczyk [25] write that pectin was decomposed by 4-12% of strains isolated from the water of Lake Jeziorak and 12-42% of bacteria isolated from the bottom sediments of that lake.

The most common aminopolysaccharide among natural polysaccharides occurring in the biosphere is chitin. According to the study by Donderski [8], the activity of chitinases synthesised by aquatic bacteria is the highest at pH 6-7. The study by Donderski and Strzelczyk [25] and Donderski and Trzebiatowska [31] imply that in the bottom sediments of lakes chitinolytic bacteria constituted on average from 0.6 to 28.0% of heterotrophic bacteria. A relatively large percentage of chitinolytic bacteria was found by Mudryk [30] in lakes near the Baltic Sea (from 4 to 43% in the water and 2 to 34% in bottom sediments). Epiphytic bacteria inhabiting the rhizosphere of the common reed in the littoral zone of Moty Bay in Lake Jeziorak constituted a small percentage of all the strains. They occurred in the greatest numbers in silty sediments, where they constituted on average 6.7% of strains.

The least numerous physiological group among those tested in the present study was the bacteria that produce hydrogen sulphide from organic compounds. A similarly low number of them was found by Donderski and Strzelczyk [25] in the water and bottom sediments of lakes situated in Bory Tucholskie.

Among the isolated heterotrophic bacteria inhabiting the rhizosphere of the common reed and the bottom sediments, the strains from the following groups were dominant: *Arthrobacter-Corynebacterium* and *Flavobacterium-Cytophaga* and from the following genera: *Achromobacter* and *Bacillus*. The above-mentioned groups and genera of bacteria were also noted by Donderski and Strzelczyk [25] as those occurring in the greatest numbers in the bottom sediments of Lake Jeziorak. However, according to the study by Lalke-Porczyk [24], the bacteria from the Enterobacteriaceae family were dominant during the whole vegetative period on the submerged shoots of the common reed. Depending on the season and the fragment of the stem tested, they constituted from 18 to 40% of the isolated strains. Moreover, as in the present paper, the bacteria from the genus *Achromobacter* and from the genus *Pseudomonas* and *Flavobacterium-Cytophaga*, considered to be typical bacteria inhabiting the water, were numerous on the surfaces of stems tested. Thus, the generic composition of bacteria growing on particular parts of plants is similar to the composition of bacteria inhabiting particular habitats, i.e. water and bottom sediments.

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