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Metabolic Activity of Epiphytic Bacteria Inhabiting the Common Reed *(Phragmites australis (Cav.) Trin. ex Steudel)* in Moty Bay of Jeziorak Lake

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Abstract

This paper presents research on the metabolic activity of bacteria isolated from the surface of stems of the common reed and from the water in the pelagic zone of Moty Bay in Lake Jeziorak, in the presence of the following exogenous substrates: casein hydrolyzate, glucose, sodium succinate and sodium acetate, and in the presence of plant extracts obtained from the stems of the common reed and the leaves of floating pond-weed. It results from the research that epiphytic bacteria inhabiting the upper (near-surface) and lower (near-bottom) sections of the stems of the common reed were more metabolically active than planktonic bacteria. The best utilised substrate was casein hydrolyzate, and the most poorly was sodium acetate. In the presence of plant extracts, the metabolic activity of epiphytic bacteria was up to 13 times higher than the activity of planktonic bacteria.

Keywords: epiphytic bacteria, heterotrophic bacteria, metabolic activity, common reed, decomposition of plant extracts.

Introduction

Aquatic plants are the perfect place for many plant and animal organisms to develop. The groups of organisms, also called periphytic groups, which grow on macrophytes and underwater objects include algae [4, 14, 19, 33, and others], fungi [11], protozoa [2] and small fauna like the larvae of some insects and oligochaetes [12], snails [12, 18] and nematodes [21]. Periphyton is also a site for bacteria to exist. They can be found among other periphytic organisms, on all underwater objects, stones, plants and also on the outer coat of aquatic animals. Moss [16] defined the accumulation of algae, fungi and invertebrates occurring on the submerged surfaces of plants as **epiphyton,** by analogy with periphyton. Baker [1] defined microorganisms and small invertebrates which grow on the surface of living plants as **epiphytes.** Periphytic accumulations are generally regarded as being very active in the production and also in the decomposition of organic material. In recent years, interest in periphytic organisms has grown considerably among researchers. The impact of periphyton on the cleaning processes of water bodies has been researched by Szlauer [28, 29] and others. Szlauer and Swierczynska [32], Piesik [22], Szlauer and Szlauer-Lukaszewska [31] and Szlauer and Szlauer [30] indicate the possibility of making practical use of various types of artificial substrate, e.g. nylon nets or used car tyres, for breeding epiphytic organisms which constitute food for fish, or groups of organisms serving to clean and purify natural waters and the sewage often drained into them. Epiphytic bacteria growing on

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the surface of aquatic macrophytes in the littoral zone definitely have a far-reaching significance in the protection of water bodies against allochthonous pollution and in the self-cleaning processes of water bodies. There is, however, a lack of information on this subject in literature. The aim of the present paper is to define the metabolic activity of epiphytic bacteria in the presence of easily assimilated organic substrates.

Material and Methods

Study Area

Macrobiological research was carried out in Moty Bay in the southwest part of Lake Jeziorak. Situated in northeastern Poland within the Hawa Lake District, this lake lies in the Vistula-Drweca catchment area. It is a channel-type drainage lake, orientated meridionally, which was formed during the last glaciation. Its 32.3 km^2 surface area makes it the sixth largest lake in Poland, with a maximum length of 27.45 km, a width of 2.35 km, and an average depth of about 4.3 m. Lake Jeziorak's shoreline is well-developed (with a factor of 6.6), with many bays including the strongly eutrophic Moty Bay. The western shore is surrounded by a mixed pine-beech and deciduous forest, while the eastern shore, along the section lying 13 km to the north of the town of Hawa, borders on meadows and cultivated fields, and further away on a coniferous and mixed forest [3]. Lake Jeziorak is counted as being a eutrophic water body. Its water is yellowish green, its transparency poor, and its pH alkaline.

Sampling

Epiphytic bacteria inhabiting the surface of the common reed *(Phragmites australis (Cav.) Trin. ex Steudel)* were used for the study. This is a plant species which predominates among helophytes in the littoral zone of this part of the lake. Plants for study were collected from a site located on the east coast of Moty Bay. For comparative purposes, research was carried out on planktonic bacteria isolated from water in the pelagic zone, from a position opposite the studied plant community.

Fifteen-centimetre-long sections from shoots of the common reed (measuring from the water level) and from near-bottom water (measuring from the rhizome) were collected for microbiological study. Water samples were collected in the pelagic zone from depths of about 20 and 100 cm using a tube sampler made by researchers. The plant material and water samples were transferred to sterile glass jars and transported to the laboratory, packed in ice to keep the temperature below +7°C. The time between collecting the samples and conducting the analyses did not exceed 6 hours.

Study material was collected in spring, during the period of intensive growth of the young shoots (20.05.1995); in summer, during flowering (24.07.1995); and in autumn, when the plants were dying (20.10.1995).

Isolating Bacterial Strains

In order to isolate epiphytic bacteria from the surface of the common reed, 10.0 g of fresh mass was taken from the epiderm of the common reed from the upper and lower sections of the stem. This was covered in 90 cm^3 of sterile buffer water [6] and blended for 2 minutes in a Unipan homogenizer type 392 at 4000 revolutions per minute. Plant homogenates prepared in such a way and lake water samples in 10 cm^3 quantities were diluted ten times with sterile buffer water (most often at a ratio of $l:10^6$ and $l:10^3$ respectively). These dilutions were inoculated on the surface of iron-peptone agar (IPA) according to Ferrer et al. [10] using the spread plate method. The plates with the inoculates were incubated at a temperature of 20°C for 10 days. After incubation, 50 epiphytic and planktonic bacterial colonies were each isolated randomly from the whole surface of the plates or from particular sectors, and transferred to semi-solid iron-peptone medium containing 5.0 g of agar • dm^{-3}. These strains were grown for 6 days at a temperature of 20°C. After checking the purity of the culture, 25 strains were then used for each further experiment.

Determining the Metabolic Activity of Bacteria Using the Respirometric Method

Before being used for research, the bacterial strains were multiplied in liquid IPA medium, poured into Erlenmayer flasks in 25 cm^3 quantities and bred in a shaker for 48-72 hours at a temperature of 20-22°C. After incubation, the bacterial cultures were centrifuged for 20 minutes at 13,000 revolutions per minute, and after decan tation the bacterial sediment was rinsed twice in 0.01 M phosphate buffer with a pH of 7.2; each time the sample was centrifuged as above. The rinsed bacterial sediment was resuspended in 0.01 M phosphate buffer, and the optical density of the suspension was increased to an absorbancy value of about 0.7 (which is equivalent to a value of about 10^9 cells per cm^3). The absorbancy of the bacterial suspension was measured using a Marcel PRO s330 spectrophotometer at a wavelength of 560 nm, as against 0.01 M phosphate buffer as a blank test.

The metabolic activity of the bacteria, based on the measurement of oxygen use, was tested in a Warburg apparatus at a temperature of 22°C for 3 hours. The following substrates were used for the investigation: D-glucose (Sigma), casein hydrolyzate without vitamins (casamino acids vitamin free, Difco), sodium succinate and sodium acetate (Merck), which were dissolved in 0.01 M phosphate buffer with a pH of 7.0-7.2. The concentration of D-glucose, sodium succinate and acetate in the solutions was 10 µM.dm^{-3}, while that of casein hydrolyzate was 5.0 mg • cm^{-3} of buffer. All of the measurements were conducted in 3 parallel replicates. The results of the experiment on the metabolic activity of the bacteria were expressed in ml of oxygen used • mg^{-1} of bacterial protein • h^{-1}. The amount of bacterial protein in the suspension used for the experiment was determined according to Bradford's method [5].

Determining the Rate of Sodium Acetate Uptake Using the Gas Chromatograph Method

As above, 25 strains of epiphytic bacteria growing on the surface of the common reed, and 25 planktonic strains were examined. 2 cm^3 of aqueous solution of sodium acetate with a concentration of 0.04 M was added to 20 cm^3 of bacterial suspension prepared as above. This substrate, as followed from the previous experiment, was least well taken up by the bacteria from among the compounds used for the investigation. The strains were incubated in the presence of sodium acetate for 72 hours at a temperature of 20°C, with constant shaking of the samples. Readings of the concentration of sodium acetate in the samples were taken at the beginning of the experiment at time (t_o) and then after 5, 10, 24, 48, and 72 hours using a gas chromatograph (Varian 3400 GC). For this purpose, 0.5 cm^3 sub-samples of the culture were taken and transferred to Eppendorf tubes, centrifuged for 10 minutes at 13,000 revolutions per minute, and then 100 µl of post-culture liquid from above the bacterial sediment was taken from them and this volume was mixed thoroughly with 100 µl of 1% phosphoric acid, the samples being kept cool all the time in a water-bath with ice. Then 0.5 µl of samples prepared in this way were transferred to a gas chromatograph column, as against a solution of standard sodium acetate, and readings were taken using an integrator (Varian 2470). The results obtained were expressed as nM of used sodium acetate • g^{-1} of bacterial protein • h^{-1}. All the determinations were conducted in three parallel replicates.

Determining the Metabolic Activity of Heterogenic Bacterial Mixture

From among the most frequently occurring strains of epiphytic bacteria of the common reed and planktonic bacteria, 4 strains each were taken in each of the seasons being investigated. These were bacteria belonging to the following groups or genus: *Flavobacterium-Cytophaga, Enterobacter, Achromobacter* and *Pseudomonas.* The systematic site of the strains was defined on the basis of "Biotest" tests: ID-Standard and ID-GNI (Pharma). Each of the strains was multiplied in 300 ml of liquid IPA medium at a temperature of 20°C for 48-72 hours, after which it was centrifuged 3 times as above. The rinsed bacterial sediments were resuspended in 0.01 M phosphate buffer, their optical density was increased to an absorbancy value of 0.7 and they were mixed with each other in equal proportions as regards volume. The mixture of bacteria prepared in such a way was used for research on metabolic activity.

Plant extracts taken from the stems of the common reed and the leaves of floating pond-weed were used as substrates for research in this experiment. Fresh fragments of plants in 100 g quantities were blended with 900 ml of 0.01 M phosphate buffer, pH 7.2, in a homogenizer (Unipan type 392) at 4000 revolutions per minute. Next this was filtered successively through 200 µm and 60 µm diameter nylon mesh, a glass fibre filter GF/D (Whatman) and a membrane filter (Sartorius) with a pore diameter of 0.2 µm. Plant extracts prepared in such a way were frozen until the moment of testing.

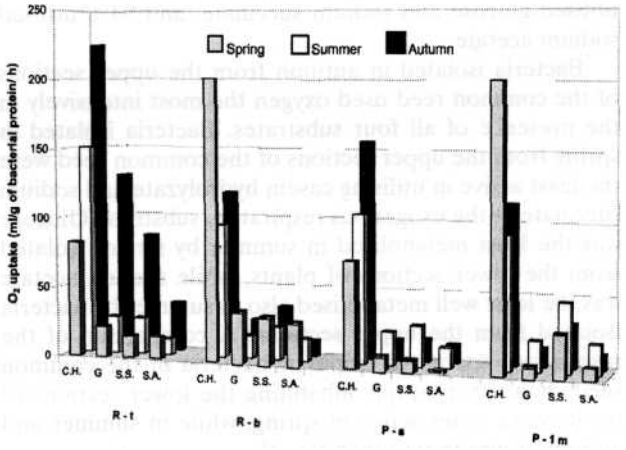

Fig. 1. Use of oxygen by epiphytic and planktonic bacteria in the presence of various substrates.
Explanations: C.H. - casein hydrolyzate; G - glucose; S.S. - sodium succinate; S.A. - sodium acetate; R-t - upper sections of the common reed; R-b - lower sections of the common reed; P-s - surface layer of water in the pelagic zone; P-l m - water from a depth of 1 m in the pelagic zone.

Measurements of oxygen use by the bacterial mixtures were conducted in a sterile fermentation chamber "Biostat" (Braun) with a total capacity of 1500 cm^3, to which 1200 cm^3 of 0.01 M phosphate buffer with a pH of 7.2, 100 cm^3 of the appropriate substrate, and 30 ml of bacterial suspension were added each time. The experiments were conducted for 48-72 hours at a temperature of 22°C, with constant mixing of the contents of the chamber (500 revolutions/min). As the concentration of oxygen in the solution fell, its content was replenished to 100% saturation of the solution (with the rate of oxygen flow at 1.33 dm^3 • min^{-1}). The oxygen content in the solution was recorded constantly using an electrode for measuring dissolved oxygen (DO_2 - Ingold). The electrode was calibrated before use each time by marking the "0%" point at 100% saturation of the phosphate buffer with nitrogen, and the 100% point at 100% saturation of the phosphate buffer with oxygen.

The results obtained are presented as mg of oxygen used • g^{-1} of bacterial protein • h^{-1}.

Results

The results of the investigation on the respiratory activity of epiphytic bacteria isolated from the surface of the common reed in the presence of various substrates are presented in Table 1 and Figure 1. It follows from them that the greatest metabolic activity of the bacteria was displayed in the presence of casein hydrolyzate, and the lowest in the presence of sodium acetate. Oxygen use in the presence of casein hydrolyzate was on average about 2-6 times higher than in the presence of glucose and sodium succinate, and about 5-13 times higher than in the presence of sodium acetate. 100% of the strains used for the experiments utilised casein hydrolyzate, 98%

utilised glucose and sodium succinate, and 94% utilised sodium acetate.

Bacteria isolated in autumn from the upper sections of the common reed used oxygen the most intensively in the presence of all four substrates. Bacteria isolated in spring from the upper sections of the common reed were the least active in utilising casein hydrolyzate and sodium succinate as the exogenous respiratory substrate. Glucose was the least metabolised in summer by strains isolated from the lower sections of plants, while sodium acetate was the least well metabolised also in summer by bacteria isolated from the upper sections. A comparison of the respiratory activity of epiphytic bacteria of the common reed shows that strains inhabiting the lower sections of plants were more active in spring, while in summer and autumn it was those inhabiting the upper sections.

The results of the research on the respiratory activity of planktonic bacteria in the pelagic zone of Moty Bay are presented in Table 2 and Figure 1. It follows from them that casein hydrolyzate was the best utilised substrate. Planktonic bacteria, similarly to epiphytic bacteria, displayed the highest metabolic activity in the presence of casein hydrolyzate, lower activity in the presence of glucose and sodium succinate, and the lowest activity in the presence of sodium acetate. Glucose and sodium succinate were utilised about 2-26 times less, and sodium acetate 4-38 times less than casein hydrolyzate. Casein hydrolyzate was also most actively metabolised by bacteria isolated in spring from a depth of 1 m, glucose in summer by bacteria isolated from the surface layer of water, and sodium succinate and acetate also in summer by bacteria isolated from a depth of 1 metre. In general, the least respiratory activity in the presence of all the exogenous substrates, with the exception of sodium caseinate, was noted in spring. Sodium succinate and acetate were more actively utilised by bacteria isolated from a depth of 1 metre in the pelagic zone, while glucose and casein hydrolyzate were more utilised in most cases by bacteria isolated from surface waters from a depth of 20 cm.

The results of research on the rate of sodium acetate uptake, denoted using gas chromatography, are presented in Table 3 and Figure 2. It follows from them that, during the 72 hours while the measurements were taken, epiphytic bacteria isolated from the surface of the common reed utilised sodium acetate on average in quantities from 1.193 to 9.292 nM calculated per gram of bacterial protein in an hour. Strains isolated in the summer and autumn from the upper sections of the common reed

Table 1. Respiratory activity of epiphytic bacteria in the presence of various substrates.

Time of sampling	Source of epiphytic bacteria	Respiratory substrate	N/N_s	Amount of oxygen taken up (ml $O_2 \cdot g^{-1}$ of bacterial protein $\cdot h^{-1}$) range of values *
Spring	R-t	Casein hydrolyzate	25/25	19.089 – 196.170
		Glucose	25/25	2.808 – 56.336
		Sodium succinate	25/25	2.859 – 43.822
		Sodium acetate	25/24	0.000 – 58.933
	R-b	Casein hydrolyzate	25/25	67.319 – 359.066
		Glucose	25/25	9.094 – 78.830
		Sodium succinate	25/25	18.772 – 57.083
		Sodium acetate	25/24	0.000 – 72.822
Summer	R-t	Casein hydrolyzate	25/25	17.447 – 256.112
		Glucose	25/25	10.834 – 43.005
		Sodium succinate	25/24	0.000 – 47.328
		Sodium acetate	25/22	0.000 – 35.053
	R-b	Casein hydrolyzate	25/25	15.045 – 377.541
		Glucose	25/23	0.000 – 40.892
		Sodium succinate	25/24	0.000 – 37.020
		Sodium acetate	25/23	0.000 – 43.234
Autumn	R-t	Casein hydrolyzate	25/25	41.323 – 631.847
		Glucose	25/24	0.000 – 482.938
		Sodium succinate	25/24	0.000 – 264.942
		Sodium acetate	25/24	0.000 – 97.372
	R-b	Casein hydrolyzate	25/25	19.577 – 249.673
		Glucose	25/25	2.900 – 53.375
		Sodium succinate	25/25	7.406 – 71.958
		Sodium acetate	25/24	0.000 – 26.422

Explanations: R-t - upper sections of the common reed, R-b - lower sections of the common reed, N - number of strains tested, N_s - number of strains using a given substrate, * - values of endogenic respiration subtracted.

utilised this substrate faster than bacteria coming from the lower fragments of the plant. In spring, the opposite phenomenon was observed.

Planktonic bacteria occurring in waters of the pelagic zone on average utilised from 0.392 to 2.007 nM of sodium acetate per gram of bacterial protein in an hour, and the highest utilisation of the substrate was noted in summer. Bacteria occurring in the surface layer of water to a depth of 20 cm utilised sodium acetate more actively in summer and autumn, while at a depth of 1 metre it was in spring.

It was discovered that metabolic activity was distinctly higher in cultures of epiphytic bacteria than in those of planktonic bacteria (Figures 3 a-c) based on research on the metabolic activity of four types of culture composed of dominating genus of bacteria isolated from the surface of the common reed and planktonic bacteria isolated from surface water in the pelagic zone in the presence of organic material composed of an extract of the common reed and floating pond-weed. In all the variants of the experiment, the highest metabolic activity was displayed by bacteria isolated in the autumn, and the lowest by those isolated in the spring. The most intensive processes

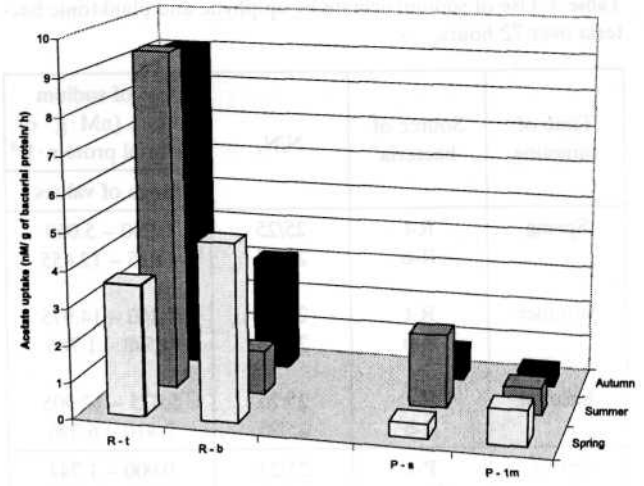

Fig. 2. Use of oxygen by epiphytic and planktonic bacteria in the presence of sodium acetate.
Explanations: R-t - upper sections of the common reed; R-b - lower sections of the common reed; P-s - surface layer of water in the pelagic zone; P-1 m - water from a depth of 1 m in the pelagic zone.

Table 2. Respiratory activity of planktonic bacteria from the pelagic zone of Moty Bay in the presence of various substrates.

Time of sampling	Source of epiphytic bacteria	Respiratory substrate	N/N_s	Amount of oxygen taken up (ml $O_2 \cdot g^{-1}$ of bacterial protein $\cdot h^{-1}$)
				range of values *
Spring	P-s	Casein hydrolyzate	25/25	34.364 – 179.984
		Glucose	25/24	0.000 – 18.817
		Sodium succinate	25/23	0.000 – 23.569
		Sodium acetate	25/22	0.000 – 6.616
	P-1m	Casein hydrolyzate	25/25	96.756 – 453.141
		Glucose	25/24	0.000 – 18.750
		Sodium succinate	25/23	0.000 – 136.825
		Sodium acetate	25/24	0.000 – 25.309
Summer	P-s	Casein hydrolyzate	25/25	16.634 – 179.345
		Glucose	25/23	0.000 – 71.741
		Sodium succinate	25/23	0.000 – 81.022
		Sodium acetate	25/23	0.000 – 36.089
	P-1m	Casein hydrolyzate	25/25	62.500 – 150.912
		Glucose	25/23	0.000 – 93.158
		Sodium succinate	25/24	0.000 – 93.158
		Sodium acetate	25/23	0.000 – 88.525
Autumn	P-s	Casein hydrolyzate	25/25	51.909 – 260.919
		Glucose	25/25	2.989 – 27.081
		Sodium succinate	25/25	3.159 – 32.812
		Sodium acetate	25/25	1.584 – 23.488
	P-1m	Casein hydrolyzate	25/25	22.684 – 364.344
		Glucose	25/24	0.000 – 28.197
		Sodium succinate	25/24	0.000 – 72.181
		Sodium acetate	25/24	0.000 – 24.898

Explanations: P-s - surface layer of water in the pelagic zone; P-1m - water from a depth of 1 m in the pelagic zone; N - number of strains tested; N_s - number of strains using a given substrate; * - values of endogenic respiration subtracted.

Table 3. Use of sodium acetate by epiphytic and planktonic bacteria over 72 hours.

Time of sampling	Source of bacteria	N/N_s	Use of sodium acetate ($nM \cdot g^{-1}$ of bacterial protein $\cdot h^{-1}$) range of values
Spring	R-t	25/25	0.940 – 5.060
	R-b	25/25	1.025 – 12.455
Summer	R-t	25/25	4.260 – 14.975
	R-b	25/25	0.598 – 1.786
Autumn	R-t	25/25	5.475 – 12.905
	R-b	25/25	0.410 – 6.396
Spring	P-s	25/23	0.000 – 1.744
	P-1m	25/25	0.043 – 2.572
Summer	P-s	25/25	0.158 – 7.197
	P-1m	25/24	0.000 – 1.594
Autumn	P-s	25/25	0.342 – 1.402
	P-1m	25/24	0.000 – 0.624

Explanations: R-t - upper sections of the common reed; R-b - lower sections of the common reed; P-s - surface layer of water in the pelagic zone; P-lm - water from a depth of 1 m in the pelagic zone.

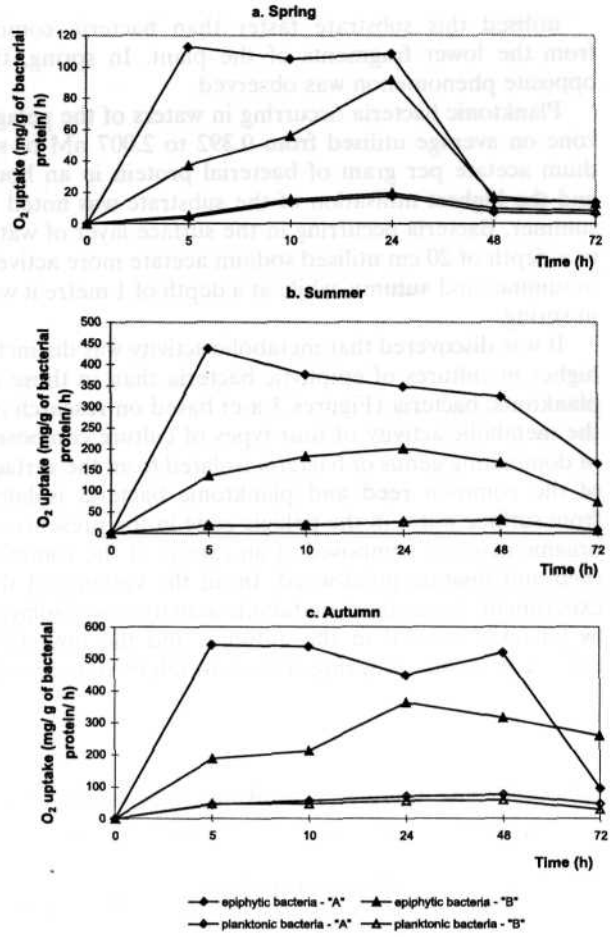

Fig. 3. Use of oxygen by epiphytic and planktonic bacteria in the presence of plant extracts.
Explanations: A - extract of the common reed, B - extract of floating pond-weed.

of decomposition of organic plant material were found each time in the presence of bacteria coming from the common reed and from the extract obtained from that plant. In the presence of the extract from the common reed, these bacteria took up oxygen most intensively during the first 5 hours of the experiment in spring. During the next nineteen hours of the experiment, their activity decreased somewhat, and after 24 hours a dramatic drop in respiratory activity was noted. A mixture of bacteria made up of cultures isolated in the summer displayed the most intensive oxygen uptake also during the first 5 hours of culture, but then a slow, steady fall in the rate of decomposition of organic material was observed until 48 hours of the experiment, after which a dramatic drop in activity occurred. In autumn, the situation was similar, except that the metabolic activity of the strains was greater.

On average, these mixtures of bacterial strains displayed from 1.3-2 times less metabolic activity in the presence of the extract from the pond-weed. In spring, the rate of respiratory processes increased during the first 24 hours, then fell quickly and remained at a low but steady level until the end of the experiment. In summer and autumn, in the presence of the extract from the floating pond-weed, the metabolic activity of the mixtures being tested also increased during the first 24 hours of the experiment, but then steadily decreased until the end of the experiment.

The metabolic activity of planktonic bacteria in the presence of both plant extracts was similar, except that, in comparison to the metabolic activity of bacterial mixtures composed of epiphytic bacteria from the common reed, it was 3-13 times less. The mixtures of planktonic bacteria inhabiting the depths of water in the pelagic zone in summer and autumn displayed oxygen use in the presence of the tested plant extracts during the first 5 hours of the experiment, after which a further small increase in activity was noted, lasting until the 48th hour of the experiment, after which a distinct drop in activity of the bacterial mixture took place. However, the culture composed of strains isolated in spring displayed a distinctly lesser rise in respiratory activity during the first 5 hours of the experiment. This rise lasted until the 24th hour, after which it began to decrease.

Discussion

The rate of uptake of organic, easily assimilated substances is a good gauge of the activity of bacteria [23]. In water bodies it depends on the quantity and quality of nutritive substances or trophy, the time of year (temperature), and also the place or zone in which the micro-organisms live. Isotopic methods used in research on meta-

bolic activity involve measuring the rate at which compounds containing certain marked elements are built into bacterial cells. The most commonly used substrates are those containing marked carbon [^{14}C]. Another of the methods is based on measuring the level of ATP in cells. The metabolic activity of heterotrophic bacteria can also be evaluated on the basis of the rate of oxygen uptake by bacterial mixtures and pure cultures in the presence of a certain substrate. Measurements of oxygen uptake are most often taken in a Warburg apparatus (respirometric method), and the marking of dissolved oxygen is conducted using the Winkler method.

In order to evaluate the metabolic activity of pure cultures of epiphytic and planktonic bacteria in this paper, the oxygen method was applied using a Warburg respirometer. Epiphytic bacteria from the common reed and planktonic bacteria metabolised casein hydrolyzate the most actively, and sodium acetate the most weakly. In spring, bacteria growing on the lower sections of the common reed were more active, while in summer and autumn it was those growing on the upper fragments of the plants. Planktonic bacteria displayed a greater metabolic activity in spring than in the other seasons. In general, epiphytic bacteria were more metabolically active in the presence of all the tested substrates than planktonic bacteria. High metabolic activity of bacteria in the presence of casein hydrolyzate was also found by Strzelczyk and Donderski [24], Strzelczyk et al. [25, 27], Ossowska-Cypryk [20] and Donderski and Strzelczyk [8]. Glucose was less actively used than casein hydrolyzate. This weak use of glucose by bacteria in water bodies was earlier observed by Strzelczyk and Mielczark [26], Donderski and Trzilowa [9], Meyer-Reil [15] and Donderski and Strzelczyk [8].

Changes in the concentration of the most weakly used respiratory substrate - sodium acetate - were also investigated in this paper using a gas chromatograph. Extending the length of the experiment to 72 hours made it possible to discover that almost all the strains (including some of those that did not use sodium acetate within 3 hours) can use this compound as a respiratory substrate. This research confirmed the fact that epiphytic bacteria were decidedly more metabolically active than planktonic bacteria. The low activity of bacteria in the presence of sodium acetate as respiratory substrate is probably a result of the fact that this compound considerably lowers the pH of the environment [13].

The environment from which the bacterial strains were isolated undoubtedly affects the metabolic activity of the microflora. Strzelczyk and Mielczarek [26] found that epiphytic bacteria were characterised by having the highest activity, planktonic bacteria displayed a weaker one, and strains isolated from bottom sediments had the weakest. Donderski and Strzelczyk [8] also found a higher metabolic activity of planktonic strains in comparison with benthonic strains. According to Donderski [7] this is linked with the content of easily absorbed substances in surface waters and their greater variety. In bottom sediments, on the other hand, there are mostly large particled substances, which are difficult to break down, and the generally lower pH than in the water depths and the frequent lack of oxygen are factors that impede the metabolic activity of bacteria.

In this paper, the metabolic activity of heterogenic mixtures of epiphytic bacteria from the common reed and planktonic bacteria were also studied. Bacteria isolated from the common reed in the presence of an extract obtained from this plant displayed the most intensive oxygen use. A slightly lower activity was observed in the presence of an extract from floating pond-weed. This fact indicates the adaptation of epiphytic bacteria to the substrates on which they have developed. A particular intensification of the respiratory processes was observed in strains isolated in autumn, which is probably connected with the necrosis and decay of macrophytes in the water at that time and the better adaptation of the bacteria to the substrate. The least oxygen in the presence of both substrates was used by planktonic bacteria. This may testify to the fact that plant extracts were not the most appropriate alimentary and energy substrate for them. The use of a mixed population of bacteria and of natural plant substrates brings us closer to the processes taking place in natural environments. It follows from research by Mudryk [17] on the metabolic activity of mixtures of bacteria that oxygen uptake by combined cultures is usually less than the total oxygen taken up by the individual pure cultures that make up those mixtures. This may be a result of competition between the individual strains for the substrate, or the inhibitory effect of bacteriocides secreted by some strains on the metabolic activity of other strains in the mixture.

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