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Short research contribution

Zbigniew Jan MUDRYK¹, Beata PODGÓRSKA²

¹ Department of Experimental Biology, Pedagogical University,
Arciszewskiego 22, 76-200 Słupsk, Poland,
e-mail: mudryk@pap.edu.pl (corresponding author)

² Department of Genetic and Marine Biotechnology, Institute of Oceanology,
św. Wojciecha 5, 81-347 Gdynia, Poland

GENERIC COMPOSITION AND RESPIRATORY ACTIVITY OF HETEROTROPHIC BACTERIA OF MARINE SANDY BEACH (SOUTHERN BALTIC SEA)

ABSTRACT: In the bacterial community occurring in a sandy marine beach (region of the Gdańsk Gulf, Southern Baltic Sea), bacteria of the genera *Acinetobacter* and *Micrococcus* predominated among 230 isolated strains. Bacteria strains of the genera *Alteromonas*, *Bacillus*, *Cytophaga*, *Erwinia* and *Prosthecomicrobium* contributed in a small percent. The measurements of respiratory activity revealed that casein hydrolyzate was the most actively metabolised respiratory substrate while sodium pyruvate and cellobiose were oxidised less actively. The intensity of utilization of respiratory substrates by bacteria in the whole perpendicular profile of the beach was alike. They were more intensive in the surface (0–1 cm) than in the subsurface (5–10 cm) sand layers.

KEY WORDS: Baltic Sea, marine beach, bacteria, taxonomy, respiratory activity

Marine sandy beaches can be regarded as buffer zones between the sea and the land. They form extremely dynamic environments shaped by the action of the wind, sand, and water (Brown and McLachlan 1990), and therefore they are morphodynamically and climatically strongly differentiated. Very often they are subject to considerable anthropopressure due to their recreational role (Węclawski *et al.* 2000).

Numerous microorganisms occur in psammon of marine beaches. According to Novitsky and MacSween (1989), bacteria constitute over 90% of those microorganisms. Bacteria play a key role in the functioning of sandy beach ecosystems, as it carries out the processes of decomposition and transformation of organic matter (Koop *et al.* 1982, Olańczuk-Neyman and Jankowska 1998, Mudryk *et al.* 2001).

The rate of respiratory activity of bacteria together with the level of their enzymatic activity, is a major factor affecting the rate of mineralization of organic matter (Goossens *et al.* 1984, Griffiths *et al.* 1984). A multifunctional endoenzyme complex responsible for the electron flux and rate of oxygen diffusion across the cell membranes controls respiratory activity of bacterial cells (Martinez and Estrada 1992). The rate of oxidation of respiratory substrates should be related to the taxonomic composition of the bacterial microflora (Mudryk 1997).

The purpose of the present study was the taxonomic analysis of bacteria community inhabiting a marine sandy beach, and to analyse their metabolic behaviour in the presence of naturally occurring respiratory substrates.

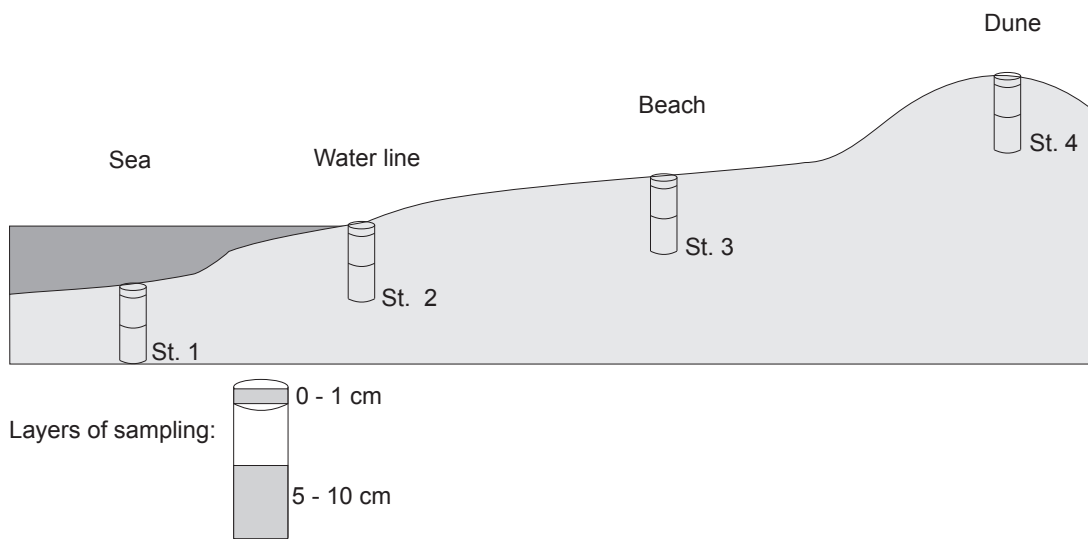


Fig. 1. Location of sampling stations on the sandy beach under study

Table 1. Taxonomic composition of bacteria strains isolated from the marine beach on four stations (value derived from the pooled data for two layers) (see Fig.1).

Species name	Station				Total strains
	1	2	3	4	
<i>Acinetobacter calcoaceticus</i>	3	1	0	0	4
<i>Acinetobacter coryneform 1</i>	14	17	17	13	61
<i>Acinetobacter coryneform 2</i>	3	6	1	7	17
<i>Acinetobacter coryneform 3</i>	1	4	0	0	5
<i>Alcaligenes faecalis</i>	0	1	1	0	2
<i>Alteromonas haloplanktis 1</i>	0	0	1	0	1
<i>Alteromonas haloplanktis 2</i>	0	0	1	0	1
<i>Bacillus cereus</i>	1	0	1	1	3
<i>Cytophaga flexibacter</i>	0	0	0	1	1
<i>Erwinia herbicola</i>	1	0	0	1	2
<i>Escherichia coli</i>	2	0	4	5	11
<i>Micrococcus roseus</i>	16	12	20	21	69
<i>Micrococcus varians</i>	3	2	6	3	14
<i>Photobacterium logei</i>	5	2	2	0	9
<i>Photobacterium phosphoreum</i>	0	1	0	0	1
<i>Prosthecomicrobium sp.</i>	1	1	0	0	2
<i>Pseudomonas marina</i>	0	1	0	0	1
<i>Pseudomonas sp.</i>	2	1	0	0	3
<i>Serratia liquefaciens</i>	0	0	0	1	1
<i>Serratia marnorubra</i>	2	1	2	3	8
<i>Vibrio costicola</i>	2	5	2	2	11
<i>Vibrio fischeri</i>	1	0	0	0	1
<i>Vibrio harveyi</i>	0	1	0	0	1
<i>Vibrio sp.</i>	0	0	1	0	1
Total number of strains	57	56	59	58	230
Total number of species	15	15	13	11	24

The study was carried out on a sandy beach near Sopot, (southern Baltic Sea coast) (54°27'N, 18°33'E). The beach has a slope of 7° and is 46 m wide. It represents a dissipative beach type with longshore bars and troughs, composed of medium grain size quartz sand. The salinity of the overlying water ranges from 0.8 to 3.6‰. Organic content of the sand varied from 0.20 to 0.57%; lower values were recorded in the middle part of the beach, higher ones towards both the dune and the waterline (Jędrzejczak 1999). The Sopot beach is a suitable and very popular recreational area. It is frequented by holidaymakers, whose density in summer reaches 30 persons per 100 m²; about 3,000 people can pass there daily (Węcławski *et al.* 2000).

Sand samples were taken in July 2001. A transect was marked along a profile formed perpendicularly to the shoreline. Four sampling sites (Fig. 1) were located as follows:

1. in the sea, approximately 1 to 1.5 m from the waterline into the water (about 1 m of the water depth),
2. at the waterline,
3. on the beach, at a 30 m distance from the waterline,
4. in the dune, about 60 m away from the waterline.

Core samples were taken with a 30 × 15 cm core sampler. The sand cores were divided into two sections: 0–1 cm (surface layer) and 5–10 cm (subsurface layer), and placed in sterile glass boxes. The samples were placed on ice and immediately transported

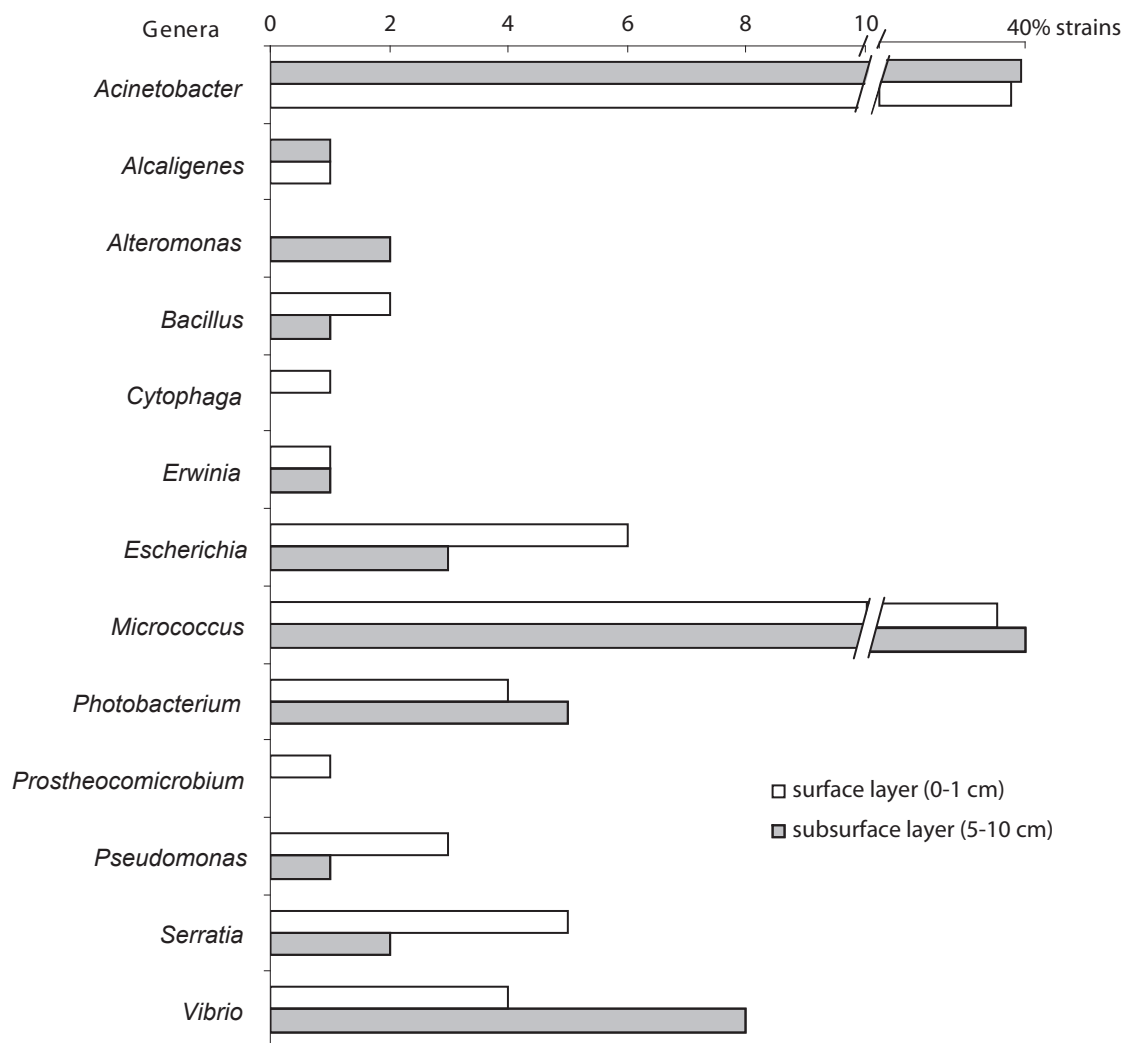


Fig. 2. Percent contribution of the genera of bacteria strains isolated from surface (0–1 cm) and subsurface (5–10 cm) sand layers (data for all stations, Fig. 1)

to the laboratory; the analysis commenced within 2–3 h.

Plate techniques were used to isolate bacterial strains inhabiting the sand. Aseptically weighted 10.0 g sand samples were transferred into 100 cm³ of sterile seawater and subsequently homogenised (NPW120 homogeniser, 5 minutes at 23.000 rpm) in order to desorb bacteria from the sand grains. The supernatant was diluted with sterile seawater and plated by the spread method onto ZoBell 2216 agar medium (ZB) (Rheinheimer 1977). Triplicate plates from each tenfold dilution were incubated for 14 days at 20°C. Afterwards, *ca.* 25 bacterial colonies from each sampling site and each core section were collected at random and transferred to semi-solid (5.0 g agar per dm³) ZB medium. After purity control, bacteria were kept at 4°C and used for the analysis of their taxonomic composition and respiratory activity.

All isolated strains of bacteria were identified using morphological features as well as physiological and biochemical properties. Identification was based on the following determinants: cell morphology, pigment production, sodium chloride impact on the growth, oxidising-fermentation test (Hugh-Leifson), arginine hydrolysis, production of cytochrome oxidase, catalase, indol out of tryptophan, ability of bacteria to hydrolyse casein, chitin, DNA, lipid and dezamination

of phenylalanine. Analysis of the obtained data and determination of taxonomic position were carried out according to Austin (1988) procedures.

In order to determine the rate of bacterial respiratory activity, oxygen uptake was measured with Clark's electrode (Rank Brothers Ltd. Model 10) (Konopka and Zakharova 1999). Respiratory activity of 20 bacterial strains from surface and subsurface sand layers was determined. Pure cultures of bacteria were multiplied on ZB agar slants for 48–72 h at 20°C. Subsequently, they were washed off from the slants with phosphate buffer (0.01 M, pH 7.0), centrifuged at 15.000 rpm for 15 min and washed twice with the buffer. The washed bacteria were re-suspended in the same buffer and adjusted to the turbidity of 4 in Mac Farland standard. Typically, 1 cm³ of such a suspension contained 10⁹ bacteria. Casein hydrolyzate (Casamino acids vitamin-free Difco), glucose, sodium pyruvate and cellobiose were used as a respiratory substrates. Before measurements, the respiratory chamber of Clark's electrode was calibrated with sodium dithionate at the polarizing voltage of 6.0 V. After calibration, 1.5 cm³ of the bacterial suspension and 30 µl of respiratory substrate were put into the respiratory chamber. Changes in voltage on the electrode were recorded by an analogue recorder XY Line Record TZ 5000

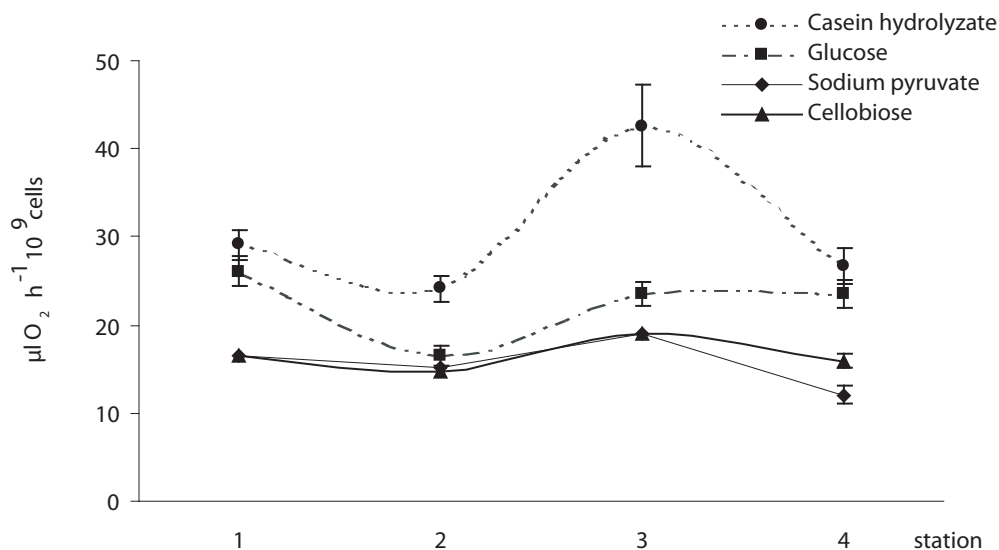


Fig. 3. The respiratory activity of bacteria isolated from different sites in marine sandy beach (average value for two layers). Vertical bars represent standard deviation.

and stored in a computer program BS81x-BS51x Data Recording System Ver. 3.3.05. The number of measurements was set at 30, taken every 6 seconds. During the measurements, the Clark's electrode was connected to a flow stabilizer of temperature, which ensured thermal stability in the respiratory chamber. Data were corrected for endogenous respiration and the oxygen uptake was converted into $\mu\text{l O}_2 \cdot \text{h}^{-1}$ per 10^9 cells.

The procedure of identification carried out in this study revealed that 24 species of heterotrophic bacteria inhabited both sand layers of the marine beach under study (Table 1). Bacteria of the genera *Acinetobacter* and *Micrococcus* predominated. Genus *Acinetobacter* constituted 38% of total number of 230 strains of the studied microflora, with species *Acinetobacter coryneform 1* being the most numerous among studied strains. Bacteria of the genus *Micrococcus* constituted 37% of total number of studies strains, with the predominance of species *Micrococcus roseus*. Bacteria of the genera *Escherichia*, *Vibrio* and *Photobacterium* were much less numerous, accounting for only 4–6% of all identified strains of bacteria. Bacteria of the genera: *Alteromonas*, *Bacillus*, *Cytophaga*, *Erwinia* and *Prosthecomicrobium* occurred only sporadically.

Data presented in table 1 show the differences in taxonomic structure between the

studied sites. In the sea and at the waterline, most numerous among isolated strains were bacteria of the genus *Acinetobacter*, while in the middle part of the beach and in the dune the genus *Micrococcus* predominated, with *Acinetobacter* also relatively abundant. Strikingly, bacteria of the genus *Escherichia* accounted for as much as 6.5–8.5% of all studied strains isolated from the middle part of the beach and from the dune.

Data referring to taxonomic composition of bacteria inhabiting surface (0–1 cm) and subsurface (5–10 cm) sand layers indicate that some bacterial genera preferred the one or other sand layer (Fig. 2). Higher percent of bacteria strains of the genera *Bacillus*, *Escherichia*, *Pseudomonas*, and *Serratia* inhabited the surface sand layer, while those of *Micrococcus*, and *Vibrio* preferred the deeper layers. The distribution of the bacteria strains of the genera *Alcaligenes* and *Erwinia* were nearly uniform in both sand layers.

Casein hydrolyzate was the most actively utilized respiratory substrate, while sodium pyruvate and cellobiose were utilized less actively by bacteria isolated from the sand (Fig. 3). The intensity of utilization of respiratory substrates was similar in the whole perpendicular profile of the beach. Rate of casein hydrolyzate oxidation was the highest in all studied sampling sites, but bacteria isolated from the middle part of the beach (st. 3)

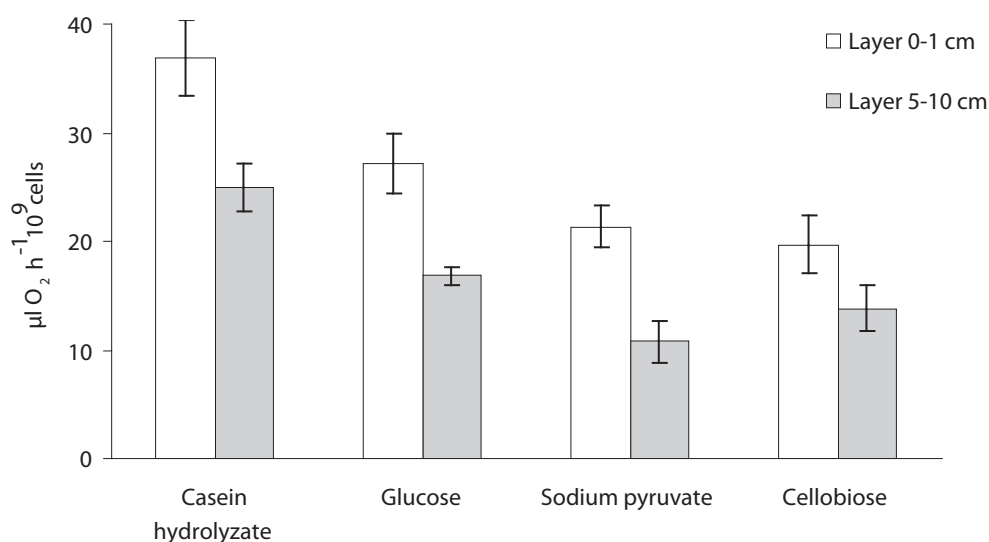


Fig. 4. Oxygen uptake by bacteria isolated from surface (0–1 cm) and subsurface (5–10 cm) sand layers (average for all measurements and 4 stations, Fig. 1). Vertical bars indicate standard deviation.

most actively oxidized this mixture of amino acids. As a rule, bacteria from all sampling sites also actively oxidized glucose.

In the presence of all tested substrates, respiratory processes were more intensive in the surface (0–1 cm) than in the subsurface (5–10 cm) sand layers. In both layers, casein hydrolyzate was the most actively utilized respiratory substrate (Fig. 4).

The study of the taxonomic differentiation of bacterial microflora inhabiting the sand of Baltic beach showed that bacteria of the genera *Acinetobacter* and *Micrococcus* were the most abundant among the isolated strains. A high frequency of the genus *Acinetobacter* among bacteria inhabiting marine bottom sediments was confirmed by results of other studies. In the Osaka Bay, they constituted 9–47% of all isolated strains (Ishida and Kadota 1974). They were even more numerous (55%) in the coastal sediments of the Sagami Bay (Shieh and Simidu 1986), and were found to predominate in the taxonomic composition of the sediment bacteria of the Indian Ocean (Nair and Bharathi 1982). Many researchers (Kobori *et al.* 1979, Hashimoto *et al.* 1983, Hidaka and Shimazu 1984) draw attention to the fact that those bacteria are especially abundant in the high pollution zones of water bodies. This can explain the abundance of those bacteria in the sand of the Sopot beach situated in strongly polluted Gulf of Gdansk.

Bacteria of the genus *Micrococcus* were also numerous among 230 isolated strains in the sand of the Sopot beach. They are of soil origin and can be introduced into the marine environment with the surface land flow. Therefore, they are not abundant in the open sea (Gunn *et al.* 1982, Simidu *et al.* 1982), whereas in the coastal zone they can constitute a considerable fraction of the microflora. This is confirmed by the present study, as well as by the results obtained by Boye *et al.* (1975).

Strikingly, about 10% of bacteria isolated from the middle part of the beach and from the dune belong to the genus *Escherichia*. Those bacteria are of faecal origin and their presence is one of the basic indicators of sanitary pollution. Most probably, surface pollution carried from the inland into the Gulf of Gdansk is a source of those bacteria on

the Sopot beach. Additionally, the increase in the abundance of the genus *Escherichia* on a beach can be attributed to the presence of holidaymakers, as was determined by Rheinheimer and Kulleman (1972) for a summer season on a Baltic beach Falckenstein in the Kiel Bight.

The bacteria inhabiting the sand of the Sopot beach oxidized various respiratory substrates with various intensity. Casein hydrolyzate was an optimal respiratory substrate for the studied bacterial strains. Active utilization of this mixture of aminoacids was also confirmed in the studies of bacteriocenoses carried out in lakes (Donderski and Strzelczyk, 1980, Mudryk, 1997), and coastal marine waters (Pomeroy *et al.* 1994, Mudryk 1998). The ability to utilize actively the aminoacids as sources of energy is a universal characteristic of heterotrophic bacteria (Simon 1991).

According to Munaro *et al.* (1978) and Urban-Malinga and Opaliński (1999) the highest biotic oxygen consumption of a sandy beach was recorded in upper (1–6 cm) sediment layers. The results of the study presented here also indicate clearly that bacterial respiratory activity was higher in the surface than in the subsurface sand layer. McLachlan *et al.* (1981) has suggested that bacteria assimilate easily-removable components of organic matter in the top few centimeters of the sand, which stimulates rapid oxygen uptake. In deeper layers organic matter is less easily removed and is thus broken down much more slowly.

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