

Baltic Coastal Zone No. 9	
(29-41) 2005	Institute of Biology and Environmental Protection Pomeranian Pedagogical University Słupsk

BACTERIAL UTILIZATION OF AMINO ACIDS AND CARBOHYDRATES IN A MARINE BEACH

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Abstract

Utilization of various amino acids and carbohydrates by heterotrophic bacteria isolated from a sandy beach in Sopot, Poland, southern Baltic Sea coast, was determined. The most intensive growth of bacteria was observed in the presence of amino acids, while carbohydrates were utilized less actively. Differences in the utilization of individual amino acids and carbohydrates by bacteria have been determined. The highest capability to assimilate amino acids and carbohydrates was observed in bacterial strains isolated from the middle part of the studied beach. No major differences were determined in the intensity of assimilation of the tested compounds by bacteria isolated from the surface and subsurface sand layers. Bacterial utilization of amino acids and carbohydrates depended on the chemical structure of those compounds.

Key words: marine beach, bacteria, amino acids, carbohydrates

INTRODUCTION

Dissolved organic matter (DOM) in seas comprises a similar mass of carbon as atmospheric CO₂ or the global biomass (Hedges et al. 1997, Dittmar and Kattner 2003). Populations of heterotrophic bacteria inhabiting marine ecosystems play a key role in the transformation and mineralization of DOM, making it available for higher trophic levels (Cividanes et al. 2002, Lemée et al. 2002). Utilization of DOM by bacteria is an integral part of the circulation of organic and inorganic nutrients in the sea (Fuhrman and Ferguson 1986, Ritzrau and Thomsen 1997).

Biochemical composition of DOM in water basins results from the dynamic equilibrium between external inputs, autochthonous production, and heterotrophic utilization (Incera et al. 2003). The composition of DOM is certainly heterogeneous; it is a mixture of thousands organic molecules of low and high molecular weight (Far

rington 1992). Amino acids and carbohydrates constitute two major classes of low molecular weight organic compounds in water basins (Kiel and Kirchman 1993, Rich et al. 1996, Yamashita and Tanoue 2003). Extracellular exudates of phytoplankton and macrophytes, extracts of zooplankton and decomposition products of dead organisms are main autochthonous sources of amino acids and carbohydrates in aquatic basins (Simon and Rosenstock 1992, Dittmar et al. 2001). All those sources can produce considerable amounts of amino acids and carbohydrates, particularly in high trophy basins (Münster 1993).

It is well known that bacteria actively utilize amino acids and carbohydrates in the processes of respiration and biosynthesis, and can take them up and oxidize as fast as they are formed (Simon 1991, Pantoja and Lee 1994). Those compounds can be taken up directly because their assimilation is not limited by transport processes or by the permeability of bacterial cellular membranes (Tupas and Koike 1990, Korner et al. 1994). The bacterial uptake of amino acids and carbohydrates is an important factor controlling the concentration and distribution of organic carbon and nitrogen in the sea (Lee and Jørgensen 1995, Yamashita and Tanoue 2003).

Since the intensity of bacterial utilization of individual amino acids and carbohydrates is diversified, the aim of the present study was to determine the preferences for those organic compounds in bacteria inhabiting the dynamic ecosystem of a marine beach. It was hoped that such study might help understand the role of heterotrophic bacteria in the conversion of organic matter in marine coastal ecosystems.

MATERIAL AND METHODS

The studies were carried out on the sandy beach near Sopot, Poland, at the Southern Baltic 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. The organic content of the sand varied from 0.20 to 0.57% with lower values recorded in the middle of the beach, and 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 (Węclawski et al. 2000).

Sand samples were taken once, in July 2001. A transect was marked along a profile formed perpendicularly to the shoreline and four sampling sites were located along this transect (Fig. 1). Site 1 was located approximately 1-1.5 m from the waterline into the water, at a depth of about 1 m; site 2 was situated at the waterline, site 3 lay halfway up the beach, at a 30 m distance from the shore, and site 4 lay in a sheltered place among the dunes, 60 m away from the shore.

Core samples were taken with a 30×15 cm Morduchaj-Boltowski core scoop. Immediately after recovery 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

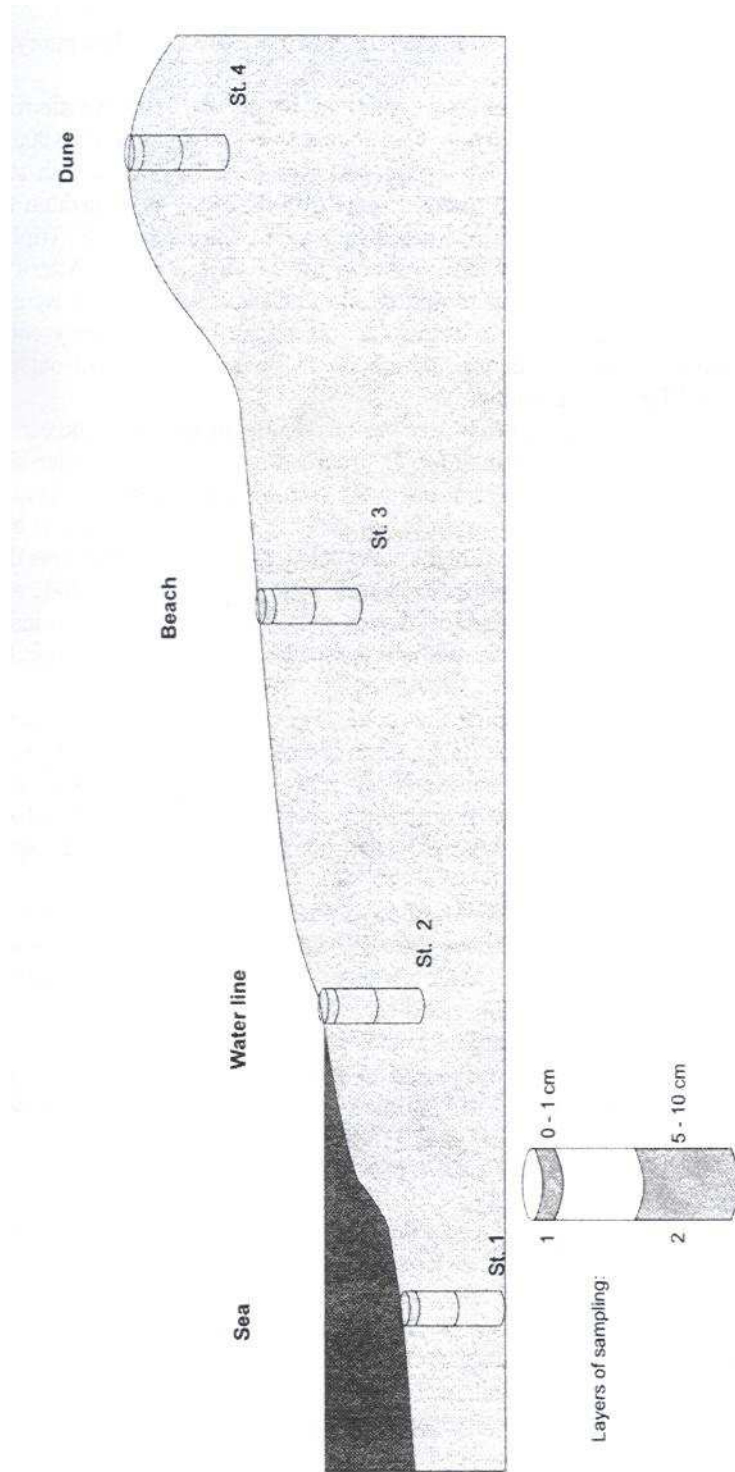


Fig. 1. Location of the sampling sites on the sandy beach in Sopot

samples were placed on ice and immediately transported to the laboratory; the analysis commenced within 2-3 h.

Each of the 10.0 g sand samples was weighed aseptically and transferred to 100 cm³ of sterile seawater for subsequent homogenisation (5 minutes at 23 000 rpm in the NPW 120 homogeniser). The supernatant was serially diluted with sterile seawater and plated by the spread method onto ZoBell 2 216 agar medium (ZB) (Rheinheimer, 1977) prepared with old brackish water, of the salinity 8. Triplicate plates from each tenfold dilution were incubated for 14 days at 20°C. Afterwards, ca. 30 bacterial colonies from each sampling site and each core section were collected at random and transferred to semisolid ZB medium. After purity control, bacteria were stored at 4°C, with inoculation on fresh medium carried out every 3 months, and used for further studies.

The ability of the isolated bacteria to utilize various single amino acids and carbohydrates was assayed in a modified medium B prepared according to Donderski and Mudryk (1996). As inoculum, 48-72 h bacterial cultures proliferated in liquid ZB medium were used. Bacteria were incubated at 20°C for 6 days. Intensity of bacterial growth in the presence of the studied amino acids and carbohydrates was determined with a SPECOL spectrophotometer with an appendage ER-1, at the wavelength of 540 nm. Light permeability lower than 70% was accepted to indicate a good growth of bacteria. A medium without any bacteria was used as blank. Light permeability of the blank was always 100%.

The study included those amino acids that occur most commonly in water ecosystems, such as alanine (Ala), arginine (Arg), aspartic acid (Asp), cysteine (Cys), glycine (Gli), glutamic acid (Glu), histidine (His), proline (Pro), serine (Ser), tryptophan (Trp). They were divided into five groups, according to their chemical structure: (acidic (AC), alkaline (AL), polar (P), non-polar (NP), aromatic and sulphuric (AS)).

Similarly, bacterial utilization of those carbohydrates that occur most commonly in water basins was studied, i.e. arabinose (Ara), fructose (Fru), galactose (Gal), glucose (Glu), lactose (Lac), maltose (Mal), mannose (Man), ribose (Rib), saccharose (Sac), and xylose (Xyl). They were divided into three groups (pentoses (P), hexoses (H), and oligosaccharides (O)) according to their chemical structure.

The results of those experiments were used to calculate the metabolic profile and utilization average index (UAI) for the studied bacteria, according to the formula proposed by Prieur (1989) and Prieur et al. (1989).

RESULTS

Metabolic profile presented in Figure 2 shows that bacteria inhabiting the Sopot beach utilized amino acids more intensively than carbohydrates. The ability to utilize at least one amino acid was shown by 35% of bacteria and 5% had the ability to utilize all 10 tested amino acids, while only 16% of all the studied bacteria was able to assimilate just one of the 10 studied carbohydrates and none of the strains was able to utilize the whole studied carbohydrate spectrum.

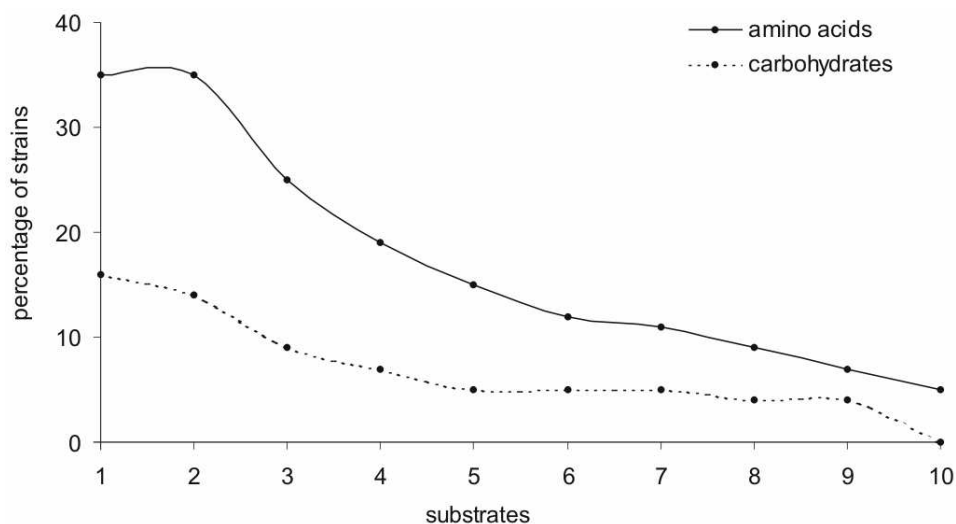


Fig. 2. Metabolic profiles of bacterial strains isolated from studied beach
 Y-axes: percentages of utilization of amino acids and carbohydrates by bacteria
 X-axes: amino acids and carbohydrates (number 1 to 10) were arranged as a function of the decreasing utilization percentages

Figure 3 presents the results concerning the utilization of individual amino acids and carbohydrates by the studied bacteria. The intensity of utilization of amino acids and carbohydrates varied. The highest percentage of the isolated bacteria utilized aspartic acid and histidine, while proline was the least suitable of the tested amino acids. Of the carbohydrates, the most intensive bacterial growth was observed in the presence of mannose and ribose. Only very few studied bacteria preferred such carbohydrates as glucose, lactose, maltose or saccharose; none utilized xylose.

Data presented in Figure 3 indicate that there were no major differences in the intensity of assimilation of amino acids and carbohydrates between bacteria isolated from the surface and subsurface sand layers. The only differences concerned utilization of cysteine, tryptophane, fructose, galactose and saccharose which were assimilated by a higher percentage of the surface layer bacteria, and utilization of proline, lactose, maltose, and ribose which were assimilated by a higher percentage of bacteria from the subsurface sand layer.

Data presented in Table 1 and 2 show that bacteria inhabiting the whole perpendicular profile of the Sopot beach were able to utilize various amino acids and carbohydrates, although their number varied depending on the site. Calculated values of the utilization average index (UAI) which is a measure of the ability of individual bacterial strains to utilize low molecular weight compounds indicate that bacterial strains isolated from the middle part of the studied beach were characterized by the highest level of assimilation of amino acids (UAI = 0.25) and carbohydrates (UAI = 0.11). Bacteria isolated from the other parts of the beach showed lower val-

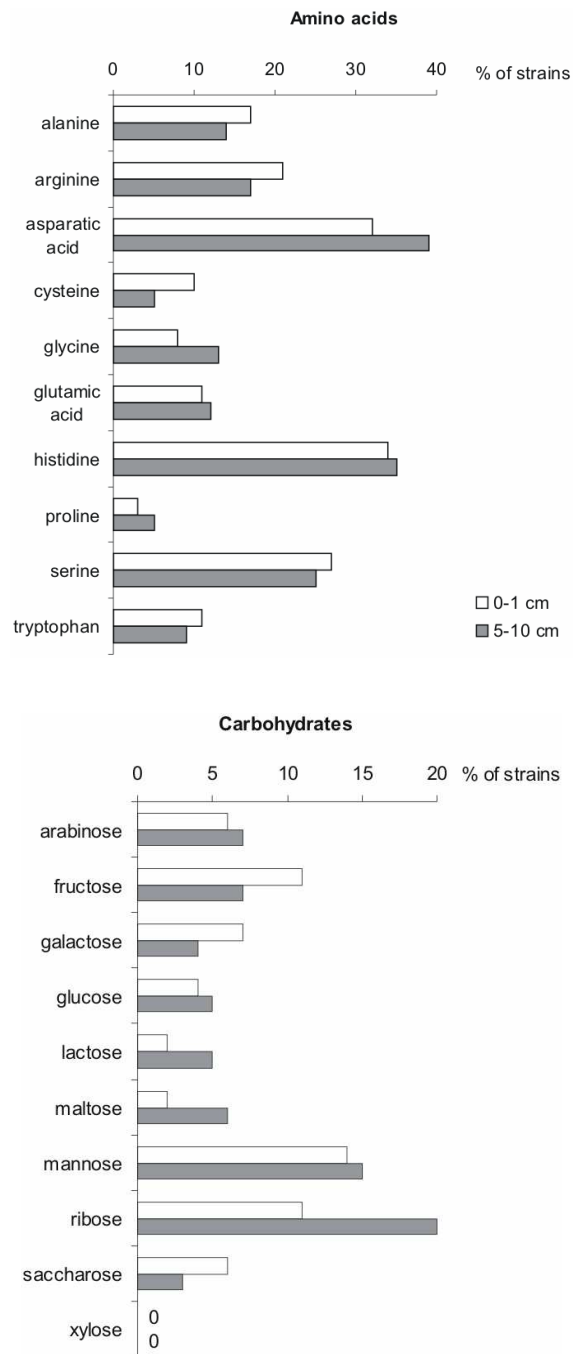


Fig. 3. Utilization of different amino acids and carbohydrates by bacteria isolated from surface and subsurface sand layer

Table 1
Uptake of amino acids by bacteria isolated from different sites (bacteria in percentage)

Sites	Number of studies strains	Amino acids											UAI
		Ala	Arg	Asp	Cys	Gli	Glu	His	Pro	Ser	Trp		
1	61	8	21	23	5	11	11	44	3	20	15	0.16	
2	60	5	15	33	5	10	11	35	3	20	5	0.18	
3	64	28	20	58	6	10	13	45	13	46	9	0.25	
4	61	20	20	26	13	12	10	10	2	15	5	0.13	

Table 2
Uptake of carbohydrates by bacteria isolated from different sites (bacteria in percentage)

Sites	Number of studies strains	Carbohydrates											UAI
		Ara	Fru	Gal	Glu	Sac	Lac	Mal	Man	Rib	Xyl		
1	61	5	3	11	0	2	2	3	18	2	0	0.05	
2	61	8	2	3	0	0	2	7	16	21	0	0.06	
3	63	8	21	2	8	11	6	3	16	32	0	0.11	
4	61	5	10	5	5	5	5	2	7	7	0	0.05	

ues of UAI, ranging from 0.13 to 0.16 for amino acids and from 0.05 to 0.06 for carbohydrates.

Figure 4 presents the results concerning bacterial utilization of amino acids and carbohydrates in relation to their chemical structure. The highest percentage of the isolated bacteria utilized aromatic and sulphuric aminoacids (AAS), while non-polar amino acids (ANP) were least preferred. Of the three studied groups of carbohydrates, bacteria utilized hexoses most intensively, while oligosaccharides were the least preferred group of sugars. Generally, there were no major differences in the intensity of assimilation of amino acids and sugars in relation to their chemical

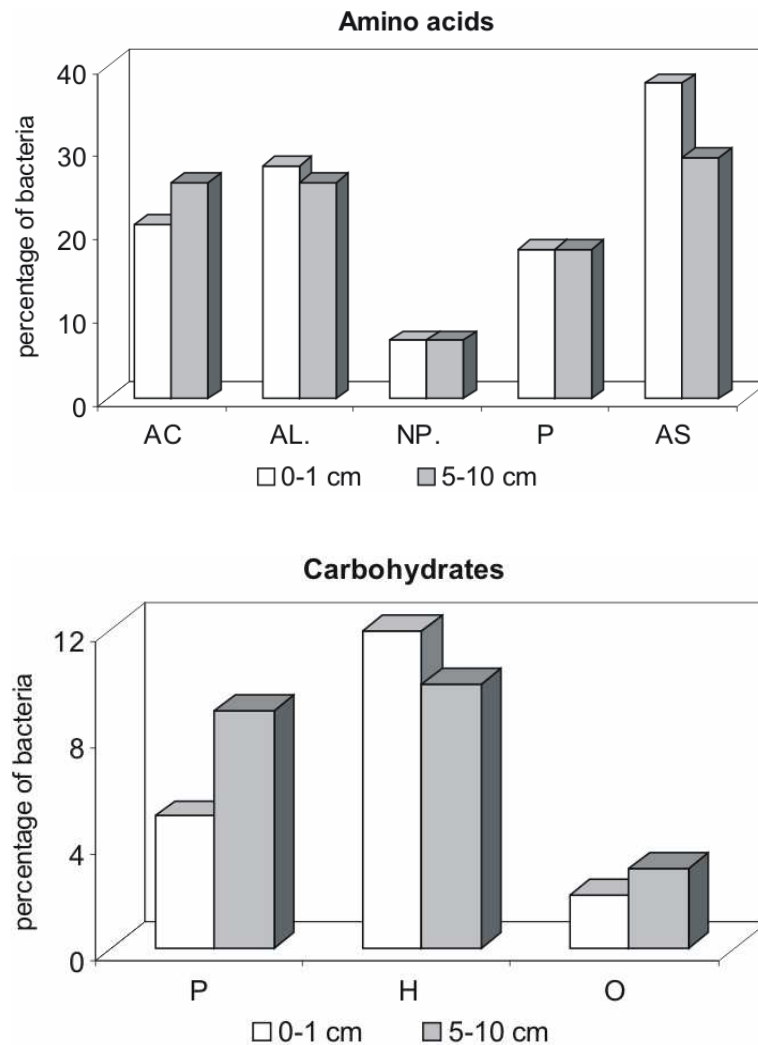


Fig. 4. Bacterial utilization of amino acids and carbohydrates depending on their chemical structure

structure between bacteria inhabiting surface and subsurface sand layers. Only aromatic and sulphuric amino acids were assimilated more actively by bacteria inhabiting the surface sand layers, while pentoses were assimilated more actively by bacteria from the deeper sand layers.

DISCUSSION

High energy marine sandy beaches are the most dynamic of soft bottom habitats (McLachlan et al. 1996, Rodriguez et al. 2003). Those extreme environments are provided with considerable amounts of organic matter by the sea water, phytobentos assimilants, products washed and leached out from the seaweeds, animal faeces, and dead remains of plants and animals (Koop and Griffiths 1982, Koop et al. 1982). This organic matter consists mainly of proteins and carbohydrates. In a study carried out on ten beach localities on the Northwest coast of the Iberian Peninsula (Incera et al. 2003), protein concentrations ranged from 37.4 to 4165.0 $\mu\text{g} \cdot \text{g}^{-1}$ sed.d.w. and carbohydrates from 3.6 to 1783.0 $\mu\text{g} \cdot \text{g}^{-1}$ sed.d.w.

The capability to actively utilize carbohydrates and amino acids is apparently common among bacterial populations in marine environments (Tupas and Koike 1990, Cividans et al. 2002). Also in the marine beach in Sopot both those groups of low molecular weight compounds were utilized by bacterial populations, however with different intensities as carbohydrates were less suitable than amino acids. This is in agreement with the early studies realised on this beach by Podgórska (2002) and results obtained by Newell and Field (1983) and Donderski et al. (1998) who also determined that marine bacteria have much higher capability to utilise amino acids than sugars. That is most probably due to the fact that carbohydrates constitute only a source of carbon and energy, while amino acids are also a source of nitrogen (Furhman 1990, Rich et al. 1996). Additionally, as has been shown by Incera et al. (2003), in marine beaches proteins constitute 71-84% of total organic matter, while carbohydrates only 12-23%.

There is a lot of data (Simon and Rosenstock 1992, Lalke et al. 1996, Yamashita and Tanoue 2003) indicating very active utilization of amino acids by bacterial populations. Those low molecular weight organic compounds are immediate precursors in the synthesis of proteins and participate in many pathways of microbial cell metabolism (Pantoja and Lee 1994, Simon 1998).

In the present study, aspartic acid apart from histidine was the most preferred amino acid by studied bacterial strains. Also in the Gdańsk Deep (southern Baltic Sea), of various amino acids tested, aspartic acid was most preferred by bacteria (Mudryk and Skórczewski 1998). Studies carried out by Grover and Chrzanowski (2000) in Canadian lakes determined that of 20 amino acids tested, aspartic acid was also best metabolized. Such intensive utilization of that amino acid by bacteria can result from the fact that aspartic acid is an optimal respiratory substrate (Jørgensen 1982). Additionally, decarboxylation of aspartic acid can also be a source of β -alanine, a precursor of pantothenic acid, one of the components of the mureine complex of the bacterial cell wall (Cole and Lee 1986).

Bacterial strains isolated from the sand of the Sopot beach were also relatively active at utilizing histidine which indicates that this amino acid, along with asparatic acid, is a main source of organic carbon and nitrogen for the studied bacteria. These data correspond with the results of the studies concerning active utilization of histidine by neustonic and planktonic bacteria inhabiting the Gdańsk Deep (southern Baltic Sea) (Mudryk and Skórczewski 1998). According to Tupas and Koike (1990), active assimilation of that amino acid by bacteria results from the fact that it constitutes 22% of bacterial proteins.

Among carbohydrates tested in this study, mannose and ribose were assimilated most intensively. Mannose was also actively utilized by bacterial strains inhabiting the Gdańsk Deep (Mudryk and Skórczewski 1997). According to Rich et al. (1996), active utilization of mannose is due to the fact that 60-90% of this monosaccharide is utilized in respiratory processes. Active assimilation of ribose by bacteria isolated from the sand of the studied beach is doubtlessly related to the fact that this sugar is a component of RNA, DNA, ATP and many co-factors, as well as a potential source of energy. Before entering the metabolism, ribose is phosphorylated to D-ribose-5-phosphate, a precursor for nucleotide, histidine and tryptophan (Lager et al. 2003).

It is noteworthy that only a small percentage of bacteria in this study assimilated glucose. This does not correspond with results of studies carried out in different water bodies (Bölter 1982, Mudryk and Skórczewski 1997, Donderski et al. 1998), which indicated active bacterial utilization of this sugar. The small percentage of bacteria assimilating glucose in the present study is presumably an effect of competition between bacteria and meiofauna inhabiting the studied beach. As has been shown by Rowe and Deming (1985), *Nematoda*, *Polycheta* and *Oligocheta* can be very efficient at absorbing large quantities of glucose through epidermis.

Results of numerous studies (Amano et al. 1982, Lalke et al. 1996, Mudryk and Skórczewski 1997, 1998) indicate that bacterial utilization of amino acids and carbohydrates depends on their chemical structure, as each bacterial strain has specific metabolic pathways for those compounds. This relationship is confirmed also in the present study. Bacteria isolated from the sandy beach in Sopot utilized aromatic and sulphuric amino acids and hexoses most intensively, whereas non-polar amino acids and oligosaccharides were the least preferred groups.

Data obtained in the present study indicate that bacteria inhabiting marine beaches actively participate in the biological conversion of various organic low molecular weight compounds and are therefore an important component of marine biocoenoses.

CONCLUSIONS

1. Bacteria inhabiting the Sopot beach utilized amino acids more intensively than carbohydrates.
2. The intensity of utilization of amino acids and carbohydrates by the studied bacteria varied. The highest percentage of isolated bacteria utilized asparatic acid,

- histidine, mannose and ribose, while very few bacteria preferred proline glucose, lactose, maltose or sacharose; none utilized xylose.
3. There are no major differences in the intensity of assimilation of amino acids and carbohydrates between bacteria isolated from surface and subsurface sand layers.
 4. Bacterial strains isolated from the middle part of the studied beach were characterized by the highest level of assimilation of amino acids and carbohydrates.
 5. Bacterial utilization of amino acids and carbohydrates depended on the chemical structure of those compounds.

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WYKORZYSTYWANIE AMINOKWASÓW I CUKRÓW PRZEZ BAKTERIE WYZIŁOWANE Z PLAŻY MORSKIEJ

Streszczenie

W pracy przedstawiono wyniki badań dotyczących wykorzystania w procesach metabolicznych aminokwasów i cukrów przez bakterie heterotroficzne wyizolowane z plaży morskiej południowego Bałtyku. Stwierdzono, że bakterie wykazywały znacznie lepszy wzrost w obecności aminokwasów niż cukrów. Najbardziej preferowanymi przez bakterie aminokwasami były kwas asparaginowy i histydyna, a cukrami mannoza i ryboza. Nie stwierdzono różnic w intensywności asymilacji aminokwasów i cukrów przez bakterie wyizolowane z powierzchniowych i podpowierzchniowych warstw piasku. Natomiast wykazano, że poziom przyswajania przez bakterie obu badanych grup organicznych związków niskocząsteczkowych zmienił się istotnie w płaszczyźnie horyzontalnej plaży morskiej. Szczepy bakterii wyizolowane ze środkowej części plaży charakteryzowały się największą aktywnością metaboliczną wobec testowanych aminokwasów i cukrów. Poziom bakteryjnego wykorzystania aminokwasów i cukrów zależał od ich budowy chemicznej. Najwyższy procent testowanych szczepów bakterii preferował aminokwasy aromatyczne i siarkowe, a spośród cukrów heksozy.