BACTERIAL UTILIZATION OF CARBOHYDRATES IN THE SURFACE SEAWATER LAYERS OF THE GDAŃSK DEEP

ZBIGNIEW MUDRYK, PIOTR SKÓRCZEWSKI

Department of Experimental Biology, University of Education, ul. Arciszewskiego 22, 76-200 Słupsk, Poland

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Abstract

Studies on the utilization of various carbohydrates by marine neustonic and planktonic bacteria inhabiting surface seawater layers were carried out in the region of the Gdańsk Deep. Significant differences in the intensity of assimilation of carbohydrates by bacteria inhabiting various water layers were determined. Carbohydrates assimilation by microflora in the studied water layers was related by considerably diel cycle changes. Only in planktonic bacteria, utilization of various carbohydrates was related to their chemical structure.

INTRODUCTION

The dissolved organic matter (DOM) in lacustrine and marine environments is a mixture of thousands organic compounds of various structures and sizes, and of changing concentartions (Farrington 1992). In the surface layers of the open sea, DOM concentrations range from 0.5–3 mg $C \cdot dm^{-3}$ (Sepers 1977). Besides amino acids, particulate and dissolved carbohydrates constitute one of the major groups of monomeric substrates in DOM (Pakulski and Benner 1992, Mnster and Albrecht 1993). The concentration and chemical composition of carbohydrates in aquatic ecosystems is constantly changing, for the most part in relation to the intensity of microbial processes (Gahnström and Fleischer 1985). The total concentrations of carbohydrates in water basins

vary between 0.03–800 μg C·dm^{-3}; the concentartion of individual sugars is normaly lower than 50 μg C·dm^{-3} (Sepers 1977; Münster 1993).

In aquatic ecosystem carbohydrates are important sources of carbon and energy for all bacteria, and they are of utmost importance for the metabolism of aquatic heterotrophic bacteria (Hagström *et al.*, 1984; Münster and Chróst 1990) The assimilation of carbohydrates, particularly those in the form of monomeres, is not limited by transport processes or by the permeability of the bacterial cellular membranes (Bölter 1982; Wolter 1982). It is well known that bacteria utilize carbohydrates, both for their respiratory requiments and for the biosynthesis of new cell materials. Carbohydrates also act as precursors and subunits in the synthesis of biopolymers, and they participate in many pathways of microbial cell matabolism (Münster 1993).

Since individual carbohydrates are utilized by bacteria with various intensity, the aim of the present work was to determine the preferences of marine neustonic and planktonic bacteria for the utilization of those organic compounds as sources of carbon and energy. This seems to be essential for the better understanding of the microbial role in the conversion of organic matter in marine ecosystems.

MATERIALS AND METHODS

Bacteriological research was carried out in May 1994 at a research station P (φ = 55°1′ N, λ = 18°42′ E) in the region of the Gdańsk Deep (Fig. 1). The Gdańsk Deep is a central part of the Gdańsk Basin. The maximum depth equals 118 m. Seawater for microbial studies was taken on board of ORP „Kopernik" from three layers at eight-hour intervals in a diel cycle. Microlayer (ML) samples (thickness of 90±17 μm) were collected with a 30 x 30 cm glass plate (Harvey and Burzell 1972). Screen layer (SL) samples (thickness of 242±40 μm) were collected with a 40 x 50 cm Garrett net (24 mesh net of 2.54 cm length), (Garrett 1965). The glass plate and poliethylene screen were rinsed with ethyl alcohol and distilled water prior to sampling. Water from the subsurface layer (SUB) was taken directly into sterile glass bottles at the depth of about 15 cm.

In order to isolate the strains of neustonic (ML and SL layers) and planktonic bacteria (SUB layer), the collected samples were diluted with sterile sea water and inoculated by the spread method in five parallel replicates, on the ZoBell 2216 E agar medium (ZB) prepared according to Rheinheimer (1977). Incubation was carried out at 20°C for 10 days. After that, 60–79 bacterial colonies from each water layer were picked out and transferred to a semiliquid ZB medium. After a purity control, the bacteria were stored at 4°C and were subsequently used for fruther studies.

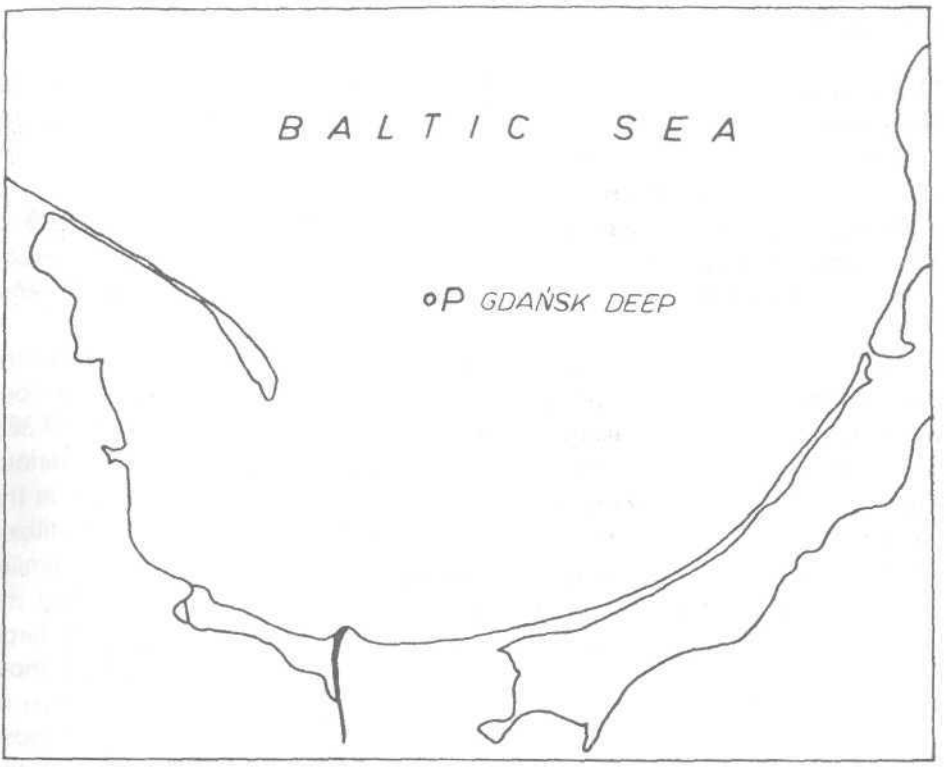

Fig. 1. Location of sampling station P in the Gdańsk Deep

The ability of the isolated neustonic and planktonic bacteria to utilize various carbohydrates was assayed in a modified medium B prepared according to Lochhead and Chase (1943), like in the experiments of Donderski and Mudryk (1996). Two-day-old bacterial cultures proliferated in liquid ZB medium were used as inoculum. The bacteria were incubated at 20°C for 6 days. The growth intensity of the bacteria in the presence of the investigated carbohydrates was determined by means of a spectrophotocolormeter SPECOL with the appendage ER –1 at 540 nm wavelenght.Turbidity level lower than 70% was assumed to indicate a good growth of bacteria in the presence of the used carbohydrate. The ability of bacteria to utilize carbohydrates occurring most commonly in water basins, such as: arabinose (A), fructose (F), fucose (FU), galactose (GA), glucose (G), lactose (L), maltose (MA), mannose (M), rhamnose (R), ribose (RI), saccharose (S) and xylose (K), was determined. The carbohydrates were divided into three groups according to their chemical structure (pentoses, hexoses, oligosaccharides). The results of the experiments were used to caluclate the utilization average index (UAI) for the bacteria, according to the formula proposed by Prieur (1989).

RESULTS

The results concerning the intensity of utilization of carbohydrates by sea water bacteria isolated from the site in the Gdańsk Deep region are presented in Table 1. These data indicate that the highest percentage (37%) of the isolated bacteria utilized mannose for their optimal growth. The majority of other sugars was assimilated with a comparable intensity by about 20–30% of all studied bacterial strains. Galactose was the least suitable source of carbon and energy for the bacteria (14%) inhabiting the studied region of the Gdańsk Deep.

The data presented in Table 1 indicate that significant differences in the level of intensity of carbohydrates assimilation by bacteria inhabiting various water layers occurred. Basing on the calculated value of the UAI (0.38), which is the measure of the ability of bacterial strains to utilize various carbohydrates, it was determined that assimilation was the most active in the bacteria inhabiting the microlayer where 30–50% of all studied strains utilized all sugars excluding saccharose as a source of carbon and energy. A similar intensity of sugar assimilation was observed in the microflora inhabiting the subsurface water layer (UAI = 0.34). In that layer, however, very large differences in the level of utilization of various sugars occurred. The most intensive growth of the planktonic bacteria was observed in the presence of mannose, saccharose and ksylose, whereas only a small percentage of those bacteria utilized ramnose, rybose, and galactose. Bacteria isolated from the screen layer assimilated sugars the least intensively, as indicated by a low value of UAI (0.15). Glucose and fructose were the optimal sources of carbon and energy for those bacteria, while the preference for galactose was the lowest.

The data presented in Figure 2 show that the intensity of carbohydrate utilization by bacteria isolated from various water levels was characterised by considerably to diel cycle changes. In the microlayer, all studied carbohydrates were assimilated the most actively by the bacteria isolated from the water samples collected in the morning (8:00). In the screen layer, the majority of sugars were utilizated the most intensively by the bacteria isolated from the water samples collected at midnight (0:00), while in the subsurface layer — from the water samples collected in the afternoon (16:00). In each of the studied water layers, significant changes in the level of assimilation of various carbohydrates was determined. In the microlayer, fucose, lactose, saccharose, and galactose, utilized by over a half of all the bacterial strains isolated from the water samples collected in the morning, were not utilized at all by the bacteria from the afternoon samples. Ramnose, which was not assimilated at all by the bacteria isolated from midnight samples, was preferred as a source of carbon and energy by over 95% of bacterial strains from the morning

Table 1

Utilization of different carbohydrates by bacteria isolated from surface seawater layers (in percentage)

Layers	Number of strains studied	Carbon and energy source												UAI
		A	R	K	M	G	RI	F	FU	MA	L	S	GA	
ML	60	37	43	35	40	40	33	42	35	50	42	25	32	0.38
SL	79	10	17	15	20	24	10	24	14	8	22	18	3	0.15
SUB	67	19	10	51	55	36	15	33	49	40	28	55	12	0.34
	Average	21	22	33	37	32	18	32	32	31	30	32	14	0.29

Explanations: A — arabinose, R — rhamnose, K — xylose, M — mannose, G — glucose, Ri — ribose, F — fructose, FU — fucose, MA — maltose, L — lactose, S — saccharose, GA — galactose

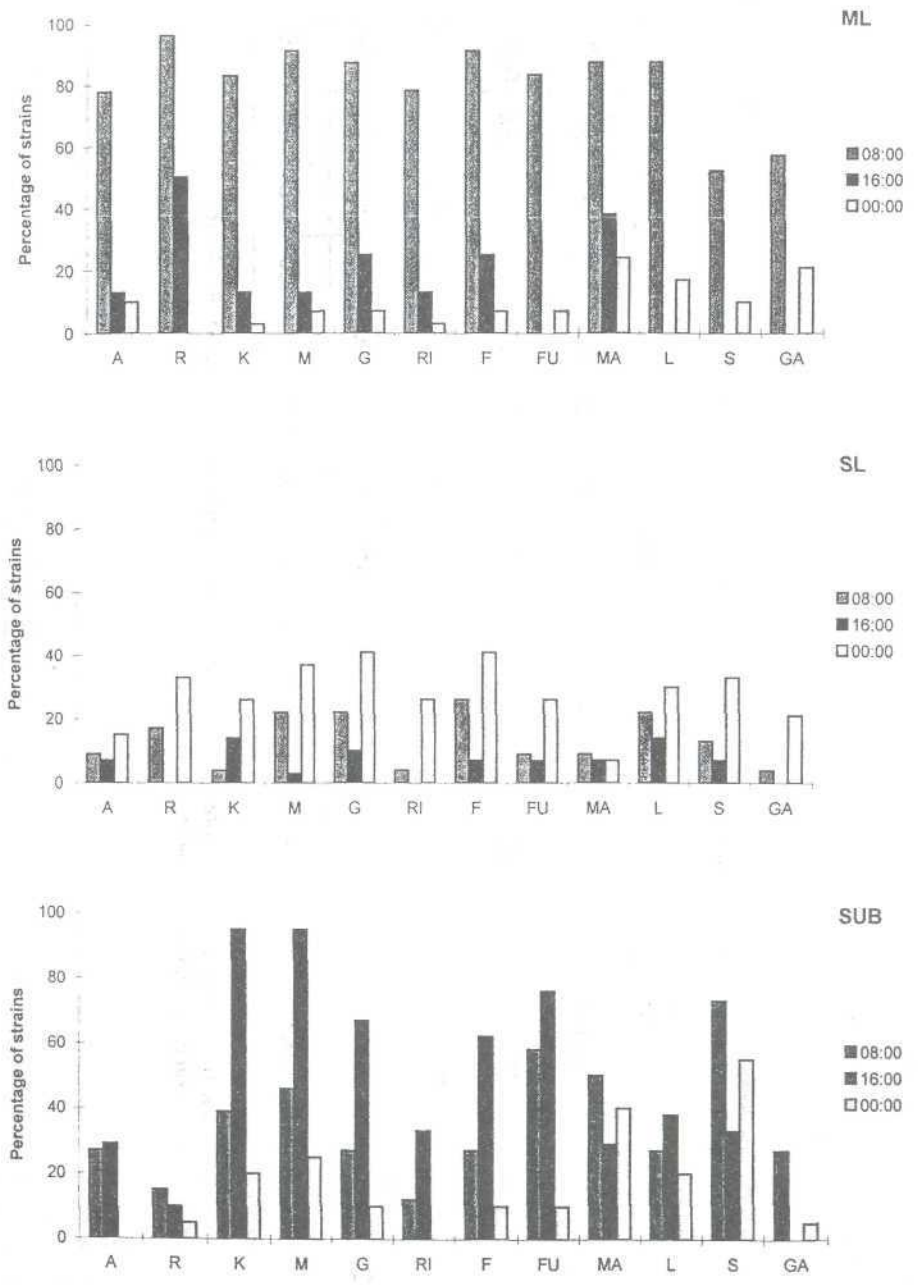

Fig. 2. Daily fluctuations in utilization carbohydrates by bacteria in different seawater layers

samples. Strong fluctuations in the intensity of utilization of various carbohydrates during the diel cycle was also observed in the bacteria isolated from the other two water layers.

Table 2 presents the results concerning bacterial utilization of carbohydrates in relation to their chemical structure. It has been determined that

Table 2

Assimilation by bacteria carbohydrates interdependence on the chemical structure (in percentage)

Layers	Sugars groups		
	P	H	O
ML	37	38	39
SM	13	17	16
SUB	24	37	41

Explanations: P— pentoses, H — hexoses, O — oligosaccharides

neustonic bacteria inhabiting the microlayer and the screen layer assimilated the three studied groups of carbohydrates with a similar intensity. On the other hand, planktonic bacteria isolated from the subsurface layer utilization oligosaccharides and hexoses most actively, whereas pentoses were the least preferred group of sugars.

DISCUSSION

Hetrotrophic bacteria play an important role in the transformation of organic matter in the sea, causing a significant loss of fixed energy and organic carbon from the system via respiration, mineralization and recycling of organically bound biologically important elements, and in themselves provide a source of nourishment for higher trophic levels (Azam and Hodson1977;MacKenzie and Gillespie 1984).

Dissolved free carbohydrates are an important exogenous organic carbon substrates for heterotrophic bacteria and have received a lot of attention in aquatic microbial ecology research (Münster 1993). According to Wiliams (1970), 51–77% of carbohydrates assimilated by bacteria are utilized in the cellular biosynthesis processes, whereas the remaining part constitutes a respiratory substrate for those organisms. a lot of natural sources of carbohydrates exist in water bodies. The largest quantities are provided by phytoplankton (Lochte 1985; Münster and Chróst 1990). They are produced as photosynthesate, reserve, extracellular excretions, cell wall materials, and may occur

as side chains on protein backbones in glycoproteins and mucopolysaccharides (Sieburth *et al.*, 1976). This is why the concentration and composition of carbohydrates in water bodies depends to a large degree on the numbers and composition of phytoplankton, as well as on its physiological state (Means and Wijayante 1984; Brown 1991). Also macrophytes (Burney 1986) and zooplankton (Brockmann *et al.*, 1979) release large quantities of carbohydrates into the water. Ittekot *et al.*, (1984); Pakulski and Benner (1992) draw attention to the fact that carbohydrates constitute 2–4% of the dry weight of the marine zooplankton, and as much as 20–70% of the dry weight of the marine algae, which explains the large quantities of carbohydrates released into the water during microbial destruction of the dead remains of those plants and animals.

There are a lot of data in the literature indicating active utilization of carbohydrates by bacteria in water bodies (Sieburth *et al.*, 1976; Münster and Albrecht 1993; Donderski and Mudryk 1996). Depending on the type of water basin, the rate of uptake of carbohydrates by bacteria range from 0.3 to 644 mg $C \cdot dm^{-3} \cdot h^{-1}$.

In the Baltic Sea, concentrations of dissolved free carbohydrates range from 0.05–97.6 $\mu g\ C \cdot dm^{-3}$ and they are actively assimilated by approximately 30% of bacterial populations inhabiting this water body (Mopper *et al.*, 1980; Bölter *et al.*, 1982). Also in the studied region of the Gdańsk Deep in May, i.e. in the time of the most intensive release of organic matter by phytoplankton (Renk 1993), most carbohydrates were actively assimilated by 20–30% of bacterial strains. Mannose was assimilated the most intensively by bacterial populations. Since — as it has been determined by MacKenzie and Gillespie (1984) — metabolic activity of bacteria depends on the concentration of substrates, it can be assumed that in the Gdańsk Deep mannose occurred in higher concentrations than other carbohydrates. This was most probably caused by an intensive growth of diatoms, whose bloom in the Gdańsk Deep reaches the highest biomass (0.2–0.5g $C \cdot m^{-3}$) at the end of April and beginning of May (Witek 1995), i.e. at the time when this study was conducted. Those algae occur in large numbers both in the screen layer and in the subsurface layers of water (De Souza Lima and Chretiennot-Dinet 1984). Diatoms can accumulate and release mannose into the water in much larger quantities than other algae (Means and Wijayante 1984; Brown1991). Mannose-rich polymeres are often associated with the siliceous frustules of diatom cell-walls, and mannose may constitute up to 50% of total cell polysacharides (Hecky *et al.*, 1973). At the same time during the hydrolysis of celluose, the main constituent of cell walls of all algae, mannose — apart from glucose — is released into the water (Burczyk and Śmietana 1991). Additionally, Ittekkot *et al.*, (1984) pointed out that mannose is present in the organic ecxretions of zooplankton.

The data obtained in the present study indicate that galactose was the least suitable carbohydrate substrate for the bacteria isolated from the Gdańsk Deep. Those data fully correspond with the results obtained by Bölter *et al.*, (1982) in Western Baltic Sea (Kiel Fjord). In this part of the Baltic Sea, the activity of bacterial assimilation of galactose was very low, while its concentration was lowest of all the sugars occurring there and did not exceed 10nM·dm^{-3}.

Previous bacteriological studies (Mudryk and Korzeniewski 1991, 1995; Mudryk and Donderski 1997) conducted in the region of the Gdańsk Deep determined very significant differences in the level of physiological and enzymatic activity as well as in the rate of destruction of organic matter between bacteria inhabiting the screen layer and those inhabiting the deeper layers of water. Also in the study presented here, differences in the level of utilization of carbohydrates by bacteria inhabiting various water layers has been shown. This can be explained by the differences in the concentration of carbohydrates, as indicated by Sieburth *et al.*, (1976); De Souza Lima and Chretiennot-Dinet (1984); Münster (1993).

Results of many studies indicate that concentrations of carbohydrates in water bodies and their utilization by bacteria fluctuate strongly in the diel cycle (Burney *et al.*, 1981; Burney 1986; Münster and Abrecht 1993). For example in lake Furceso, concentration of glucose varied between 9 nM (24:00) and 49 nM (18:00). At the same time bacterial gross assimilation (incorporation and respiration) of glucose varied between 0.4–3.1 nM·dm^{-3}·h^{-1} (Jorgensen and Jensen 1994). Even greater differences in diel changes of carbohydrates concentrations and their utilization by bacteria was determined by Cavari and Hadas (1979) in lake Kinnaret. This explains the results obtained in the present study, namely the large amplitude of changes in the level of intensity of assimilation of various carbohydrates by bacteria in the diel cycle.

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