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Original Research

Utilization of Low Molecular Weight Organic Compounds by Marine Neustonic and Planktonic Bacteria

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Abstract

The efficiency of utilization of low molecular weight organic molecules by neustonic and planktonic bacteria inhabiting the waters of the Gdansk Deep region has been determined. The most intensive growth was observed in the presence of amino acids, while carbohydrates and organic acids were utilized less actively. Glutamic acid, asparatic acid, histidine, glycine, cysteine and calcium lactate were the most suitable sources of carbon and energy for the bacteria. Significant differences in the level of intensity of assimilation of low molecular weight organic molecules by bacteria inhabiting various water layers occurred.

Keywords: marine bacteria, utilization, amino acids, carbohydrates, organic acids.

Introduction

In aquatic ecosystems, the dissolved organic carbon (DOC) is an important substrate for the growth of heterotrophic bacteria. In pelagic environments they are the predominant group of organisms that assimilate DOC, transform it into biomass, and respire as a source of energy, making it available to higher trophic levels [1, 2, 3]. Lancelot and Billen [4] showed that DOC contains a large amount of organic molecules of low and high molecular weight. Results of several studies suggest that - as substrates for heterotrophic bacteria - low molecular weight components are quantitatively more important than components of high molecular weight [1, 5]. Organic acids, carbohydrates, and amino acids constitute major classes of dissolved low molecular weight organic compounds in water basins [5, 6, 7]. Those substrates are important potential sources of carbon and energy for marine bacteria [8, 9, 10]. Only organic monomers such as amino acids, carbohydrates, and organic acids can be taken up directly by bacteria because assimilation of those compounds is not limited by transport processes or by the premeability of cellular membranes [4, 11]. Heterotrophic bacteria take up and oxidize those monomers as fast as they are formed [2, 12]. Extracellular exudates of phytoplankton, extractes of zooplankton, and decomposition products of dead organisms are the main autochthonous sources of amino acids, carbohydrates, and organic acids in seawater [3, 13].

Since utilization of dissolved organic substrates by heterotrophic bacteria is an integral part of the circulation of organic and inorganic nutrients in the sea, the aim of the present study was to determine the preferences of marine neustonic and planktonic bacteria for the utilization of amino acids, carbohydrates and organic acids as sources of carbon and energy. It was hoped that such a study might provide information on the potential capability of bacteria to decompose organic matter and to control the distribution of organic carbon in the marine ecosystem.

Materials and Methods

Study Area and Sampling

Bacteriological investigations were carried out in the region of the Gdansk Deep (Fig. 1), at a research station

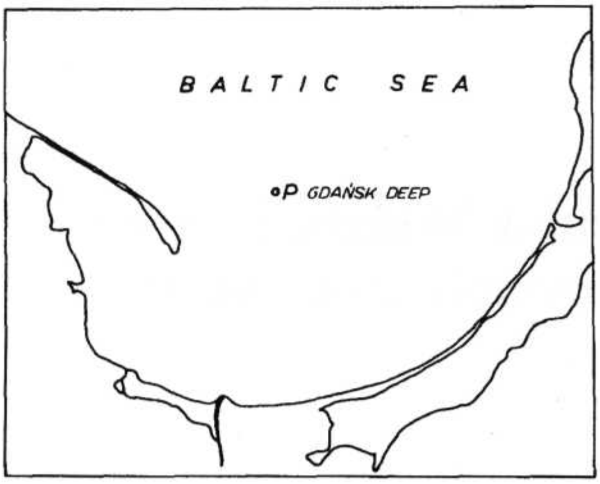

Fig. 1. Location of sampling station P in the Gdansk Deep.

P (φ = 55°1' N, λ = 18°42' E). Seawater samples were taken on board the ORP "Kopernik" from two layers. Surface microlayer (SM) samples (thickness of 242 + 40μm) were collected with a 40 x 50 cm Garrett net (24 mesh net of 2.54 cm length) [14]. The polietylene screen was rinsed with ethyl alcohol and distilled water prior to sampling. Water from the subsurface layer (SUB) was taken directly into sterile glass bottles at a depth of about 10-15 cm.

Isolation of Bacterial Strains

Plating techniques were used to isolate neustonic (SM) and planktonic bacteria (SUB). Seawater samples were diluted with sterile sea water and inoculated by the spread method in five parallel replications on the ZoBell 2216E agar medium (ZB) of 8‰ salinity [15]. The plates were incubated at 2O°C for 10 days. After that, ca. 40 bacterial colonies isolated from both water layers were picked out and transferred to a semisolid ZB medium. After purity control, the bacteria were stored at 4°C and were subsequently used for further studies.

Utilization of Low Molecular Weight Organic Compounds

In this study, aerobic utilization of 38 different low molecular weight organic compounds as sole sources of carbon and energy for the growth of neustonic and planktonic bacteria was tested. The concentrations of organic substrates added corresponded to 1.0 g of carbon per 1 dm^{-3} of the medium [16]. The capability of individual bacterial isolates to utilize 14 amino acids, 12 carbohydrates and 12 organic acids occurring most commonly in water basins was determined.

The ability of the isolated neustonic and planktonic bacteria to utilize single amino acids and carbohydrates was assayed in a modified medium B prepared as in the experiments of Mudryk et al. [17], Donderski and Mudryk [18]. 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 intensity of bacterial growth in the presence of the investigated amino acids and

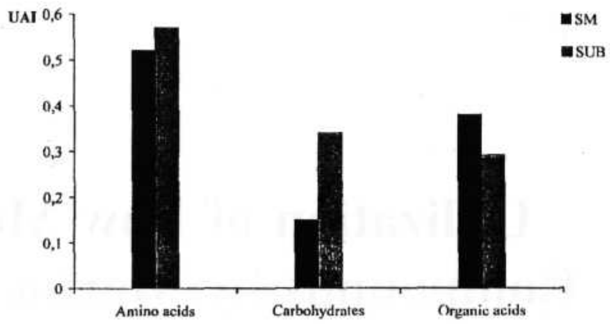

Fig. 2. Level of intensity of assimilation of organic compounds by bacteria isolated from the Gdansk Deep.
Explanation: UAI - utilization average index. SM - surface microlayer, SUB - subsurface layer.

carbohydrates was determined by means of a SPECOL spectrophotocolorimeter with the appendage ER-1, at 540 nm wavelength. Light permeability lower than 70% was accepted to indicate good growth of bacteria in the presence of the used amino acids and carbohydrates. The ability of bacteria to utilize amino acids such as: alanine, arginine, asparatic acid, cysteine, cystine, phenylalanine, glycine, glutamic acid, histidine, lysine, ornithine, proline, serine and tryptophan was determined. The following carbohydrates were tested: arabinose, fructose, fucose, galactose, glucose, lactose, maltose, mannose, rhamnose, ribose, saccharose, and xylose.

Utilization of organic acids was tested in a medium prepared according to Kobel-Boelke et al. [19]. It was detected by the changes of colour in the medium from green to blue. The following organic acids or their salts were tested: glycollic acid, lactic acid, malic acid, malonic acid, succinic acid, stearic acid, uric acid, sodium pyruvate, sodium citrate, sodium succinate, sodium tartrate, and calcium lactate.

The results of the above experiments were used to calculate the utilization average index (UAI) for bacteria, according to the formula proposed by Prieur [20].

Results

Table 1 presents the percentage of bacterial strains which can utilize the tested low molecular organic substrates as their sole carbon and energy source. More than 50% of the strains demonstrated good growth on asparatic acid, glutamic acid, histidine, glycine, cysteine and calcium lactate. Those organic compounds may be appropriate test substrates for determining activity of heterotrophic bacteria populations in natural waters. Only a small percentage of all the tested organic substrates was not utilized actively by the bacteria. Thus, phenylalanine and galactose were utilized by only 8% of the tested organisms, the other percentages being: glycollic acid 10%, ribose 11%, rhamnose 13%, and arabinose 15%. Marine bacteria isolated from the studied region of the Gdansk Deep used neither sodium tartrate nor stearic acid for their growth.

The data presented in Figure 2 show differences in the level of intensity of assimilation of low molecular weight organic molecules by bacteria isolated from the study site of the Gdansk Deep region. Based on the calculated value of the UAI, which is the measure of the ability of individual bacterial strains to utilize various organic compounds, it was determined that neustonic and planktonic bacteria utilized amino acids most intensively (UAI 0.52 - 0.57), while carbohydrates and organic acids were used less actively (UAI 0.15 - 0.38).

Data presented in Figures 3, 4, and 5 indicate the occurrence of significant differences in the level of intensity of assimilation of amino acids, carbohydrates, and organic acids by bacteria inhabiting the microlayer and subsurface water. It follows from the data presented in Figure 3 that bacteria inhabiting subsurface water utilized amino acids more intensively than microflora isolated from the surface microlayer. The amino acids assimilated most actively by bacterioneuston and bacterioplankton were: glutamic acid, asparatic acid, cysteine, glycine, histidine, and tryptophan. They were utilized by 58-94% of the studied strains. Alanine, arginine, cysteine, and lysine were utilized to a lesser degree. Only a small percentage of neustonic and planktonic bacteria used ornithine, proline, and serine for optimal growth. Phenylalanine was the least actively utilized amino acid. Neustonic bacteria did not use it at all.

It appears from data shown in Figure 4 that carbohydrates used in this study stimulated the development of marine bacteria. It may be worth noting that significant differences in the assimilation of particular carbohydrates between neustonic and planktonic bacteria have been found. As many as 11 out of 12 sugars were utilized more actively by bacteria inhabiting subsurface water than by bacteria isolated from the surface microlayer. Mannose, saccharose, xylose, and fucose as sources of organic carbon and energy were most preferred by planktonic bacteria. They were actively utilized by 48-55% of the total number of the examined strains. The strongest growth of bacterioneuston

Fig. 3. Uptake of various amino acids by neustonic and planktonic bacteria.

was observed in the presence of glucose, mannose, fructose, and lactose. Only very few neustonic and planktonic bacterial strains preferred such carbohydrates as galactose, arabionose, rhamnose, or ribose.

Table 1. Percentage of bacterial strains utilization of different low molecular weight organic compounds

Carbon and energy source					
Amino acids		Carbohydrates		Organic acids	
Alanine	36	Arabinose	15	Calcium lactate	51
Arginine	50	Fructose	24	Glycollic acid	10
Asparatic acid	97	Fucose	28	Lactate acid	43
Cysteine	31	Galactose	8	Malic acid	43
Cystine	72	Glucose	28	Malonate acid	41
Glutamic acid	100	Lactose	22	Sodium citrate	43
Glycine	73	Maltose	21	Sodium pyruvate	44
Histidine	79	Mannose	38	Sodium succinate	45
Lysine	46	Rhamnose	13	Sodium tartrate	0
Ornithine	28	Ribose	11	Stearine acid	0
Phenylalanine	8	Saccharose	33	Succinate acid	46
Proline	25	Xylose	33	Uric acid	42
Serine	28				
Tryptophan	42				

Fig. 4. Utilization of different carbohydrates by marine bacteria.

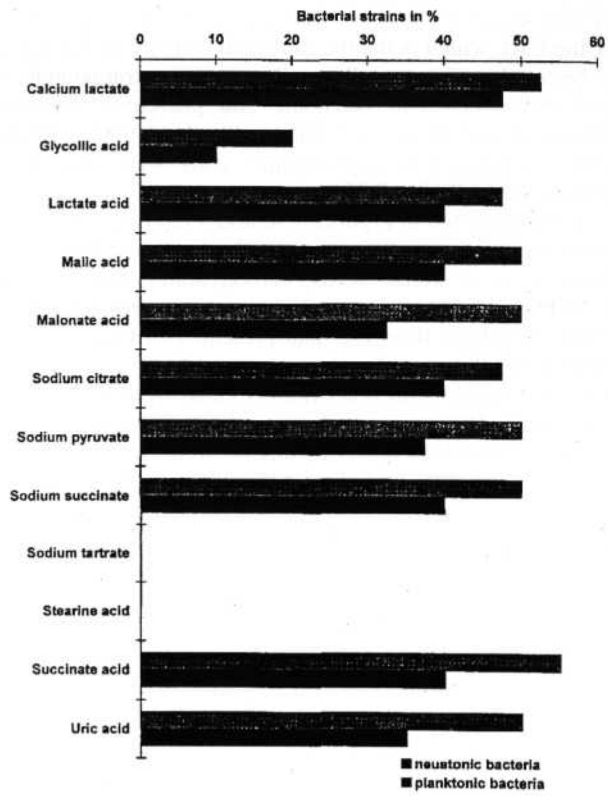

Fig. 5. Assimilation of organic acids by bacteria isolated from surface microlayer and subsurface layer.

Figure 5 illustrates the capability of marine bacteria to utilize various organic acids or their salts. It has been established that all the tested organic acids were utilized less actively by planktonic than by neustonic bacteria. Bacteria isolated from the surface microlayer and subsurface water required different organic acids for optimal growth. Of the organic acids or their salts used in this study, the most suitable were sodium pyruvate, sodium succinate, calcium lactate, malic acid, and succinic acid. Sodium tartrate and stearic acid were not utilized at all by neustonic or planktonic bacteria.

Discussion

Populations of heterotrophic bacteria which are characterised by high diversity can utilize a wide variety of dissolved organic carbon compounds for their growth. The uptake and removal of those compounds from seawater by marine bacteria is thought to be important in controlling the distribution and concentration of organic carbon in the sea [21]. The capability to actively utilize amino acids, carbohydrates and organic acids as the sole carbon and energy source is apparently a common property among the bacterial populations in natural waters [8, 9, 22].

Data presented in this paper show that in the studied region of the Gdansk Deep low molecular weight organic compounds such as amino acids, carbohydrates, and organic acids were utilized intensively by bacterial populations. The majority of those substrates could be used by more than 30% of the bacterial strains. Among the 38 monitored compounds glutamic acid, asparatic acid, histidine, glycine, cysteine and calcium lactate were utilized most actively. Phenylalanine and ribose were suitable for a smaller number of the tested organisms, while none of them was capable of using sodium tartrate or stearic acid. Generally, the gathered data are essentially the same as the results reported in previous studies [2, 18, 22, 23]. The intensity of utilization of amino acids, carbohydrates, and organic acids by bacteria inhabiting the Gdansk Deep is most probably due to the very high primary production (155 - 253 g $C{\bullet}m^{-2}\ yr^{-1}$) in this part of the Baltic Sea [24]. According to Chrost and Faust [25], 33% of the organic carbon released by phytoplankton can have molecular weight lower than 500 daltons. Monomers such as sugars, amino acids, and organic acids may constitute a large fraction of this material.

The results of the present study indicate that bacterial populations inhabiting the waters of the Gdansk Deep utilized different low molecular organic molecules with different intensity. Amino acids were assimilated most actively by the studied bacterial strains. There are a lot of data in literature [3, 9, 12, 22] indicating active utilization of amino acids by bacterial populations. Those low molecular organic compounds are immediate precursors in the synthesis of proteins and they participate in many path-ways of microbial cell metabolism [2, 28].

Carbohydrates and organic acids were less suitable than amino acids for the bacteria isolated from the Gdansk Deep. These data agree with the results obtained by Gillespie et al. [23] who determined that marine bacteria have much higher capability to utilize amino acids than sugars and organic acids. This is most probably due to the fact that carbohydrates and organic acids constitute only a source of carbon and energy, while amino acids are additionally a source of nitrogen [12].

The results of this study indicate the occurrence of differences in the intensity of assimilation of low molecular weight organic molecules between bacteria inhabiting the surface microlayer and subsurface water. Also, previous bacteriological studies [28, 29] conducted in the region of the Gdansk Deep show very significant differences in the utilization of those monomers between neustonic and planktonic bacteria.

The data obtained in the present study indicate that bacteria inhabiting the studied region of the Gdansk Deep by actively participating in the energy flux and biological conversions of various organic monomeric compounds are an important component of marine biocenosis.

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