

**CHARACTERISTIC OF HETROTROPHIC BACTERIA INHABITING  
THE GULF OF GDAŃSK**

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**Abstract**

The number maximum of heterotrophic bacteria in the Gulf of Gdańsk was recorded in the vicinity of the Vistula mouth. The taxonomic composition of heterotrophic bacteria was dominated by organisms of *Flavobacterium - Cytophaga* group. Among the bacterial microflora proteolytic, amylolytic and lipolytic bacteria were very numerous. The bacterioplankton occurring in the Gulf of Gdańsk was very active in assimilated amino acids among other low-molecule compounds. For the respiratory activities, the bacteria used casein hydrolyzate very intensely.

**Key words:** Gulf of Gdańsk, heterotrophic bacteria, number, taxonomic composition, organic matter transformation.

**INTRODUCTION**

Bacterial microflora plays the key role in the process of biological balance in the marine ecosystem (Heinänen 1992, Berman *et al.* 1994). Those organisms, heterotrophic bacteria in particular, are very active in the process of destruction and transformation of the auto- and allochthonic organic matter in water basins (Goosen *et al.* 1995). Through a dynamic induction of their enzymatic systems, the bacteria response very quickly in terms of metabolism to every new organic compound introduced into the water ecosystem (Wright and Coffin 1983). As a result, the bacteriocenosis actively assimilates the organic matter transforming it into its own biomass or uses it for the respiratory purposes as a source of energy (Donderski *et al.* 1998, Mudryk and Skórczewski 1998). Hence the key role of the bacteria in the process of organic matter decomposition and its flow through the microbiological loop which makes the necessary condition of homeostasis keeping in every water ecosystem.

The aim of the present paper was to define the number and taxonomic composition of heterotrophic bacteria that inhabit the Gulf of Gdańsk and their contribution to the organic matter transformation processes.

## MATERIALS AND METHODS

Between 1994-1997 microbiological research in the Gulf of Gdańsk was conducted at three stations, the locations of which are presented in Fig. 1. The samples of water were collected by means of the Ruttner's sampler of 0.5 l capacity and transferred to sterile glass and stored in an ice-box, where the temperature did not exceed 7°C until they were taken for analysis. The time between sample collection and performance of the analyses usually did not exceed 6-8 h. Water samples were serially diluted with sterile seawater and plated by the spread method onto ZoBell 2216 agar medium (ZB) (Rheinheimer 1977) prepared of old 8‰ salinity brackish water. Triplicate plates from each tenfold dilution were incubated for 10 days at 20° C. Afterwards, ca. 30 bacterial colonies from each sampling site were collected at random and transferred to semisolid ZB medium. After purity control, the bacteria were stored at 4°C, with inoculation on fresh medium carried out every 3 months, and used for further studies.

The taxonomic classification of the isolated bacteria strains was defined basing on the morphologic features, physiological and biochemical properties. The following determination made the grounds for identification: bacteria morphology, mobility, pigment production, sodium chloride impact on the bacteria growth, oxidation-fermenting test (Hugh-Leifson), arginin hydrolysis production of cytochrome oxidase and catalase, indol production from tryptophan, ability to casein, chitin, DNA, Tween 20 hydrolysis, fenylalanine desamination. The analysis of the obtained data and selecting for taxonomic classification were done according to Austin (1988) scheme and Bergey's key (Krieg and Holt 1984).

In order to define their ability to decompose organic macromolecules of the isolated strains the bacteria were inoculated on different test media (Mudryk and Donderski 1997). The following decomposition abilities were tested: proteins, lipids, starch, DNA, RNA, cellulose, pectin and chitin.

The ability of the isolated planktonic bacteria to utilize various amino acids, organic acids and carbohydrates were assayed according to Donderski *et al.* (1998).

Respiratory activity was measured as oxygen uptake in a Warburg apparatus according to experiments by Mudryk (1998)

## RESULTS AND DISCUSSION

The results displayed in Table 1 proved that heterotrophic bacteria number in the Gulf of Gdańsk, was dramatically different in various parts of this Baltic sea water body. The greatest number of heterotrophic bacteria ( $6.6 \cdot 10^3 \cdot \text{cm}^{-3}$ ) and their biomass ( $10.4 \text{ mg} \cdot \text{m}^{-3}$ ) were recorded at station 1 located in the Vistula river mouth into the Baltic Sea. Whereas, the minimum number of heterotrophic bacteria ( $2.4 \cdot 10^3 \cdot \text{cm}^{-3}$ ) and their biomass ( $3.9 \text{ mg} \cdot \text{m}^{-3}$ ) were recorded in the open areas (st.3) of the Gulf of Gdańsk. Earlier investigations conducted in the Baltic Sea and its gulfs (Gocke *et al.* 1990, Pollehne *et al.* 1995, Ameryk *et al.* 1999) indicated to a rule that maximum numbers of bacteria were recorded at rivers mouths into the Baltic Sea.

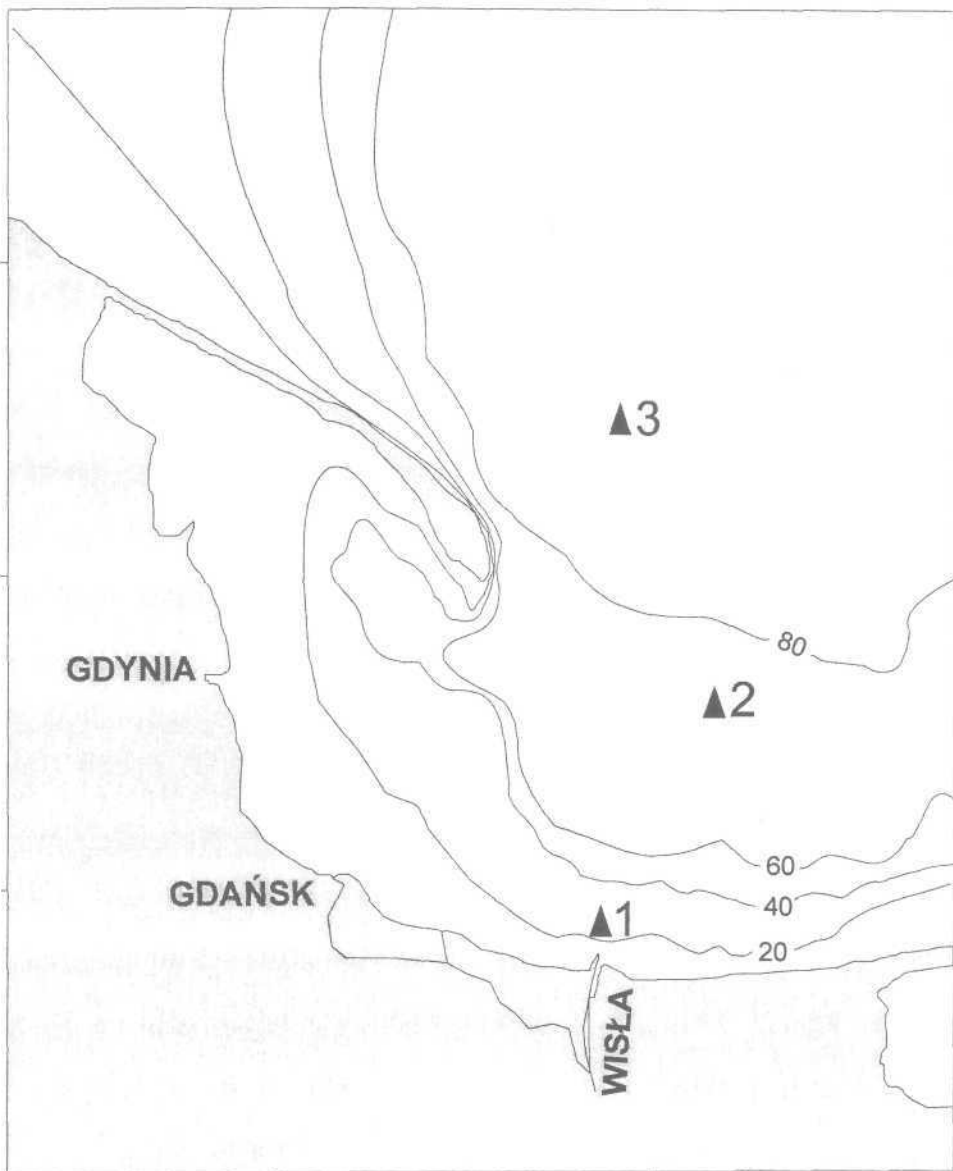


Fig. 1 Location of sampling stations in the Gulf of Gdansk

Table 1

Number and biomass of heterotrophic bacteria isolated from water Gulf of Gdańsk

Station	Number of bacteria	Bacterial biomass			
	$10^3 \cdot \text{cm}^{-5}$	$\text{mg} \cdot \text{m}^{-3}$	$\mu\text{gC} \cdot \text{m}^{-3}$	$\mu\text{gN} \cdot \text{m}^{-3}$	$\mu\text{gP} \cdot \text{m}^{-3}$
1	6.6	1.4	15.7	3.9	0.78
2	5.1	8.2	12.0	3.1	0.60
3	2.4	3.9	5.7	1.4	0.29

This must have resulted from the fact that those areas of the Baltic Sea are usually richer in allochthonic organic matter, which is brought in by the rivers. Results of still earlier research (Goosen *et al.* 1995) showed that the incoming and high concentration of organic matter imply an increased access to food substrate which may highly affect the increase in number, biomass and production of heterotrophic bacteria. According to Heinänen (1992) great quantities of allochthonic matter continuously brought into the marine water body, may enhance the forming of detritus derivative food network that would completely be independent primary production.

Fig. 2 presents data concerning taxonomic variability of the heterotrophic bacteria that inhabits the Gulf of Gdańsk. What we can gather from the results, bacteria of the *Flavobacterium-Cytophaga* group were the dominating group of microorganisms and made over 40% of the bacterial microflora. Some earlier research by Rheinheimer (1981) and Mudryk (1998) also confirm the existence of the domination of those chromogenic bacteria in the Baltic Sea. The great amounts of that taxonomic group bacteria in the pelagic zones must be related to their being very resistant to the bacteriostatic and often lethal sun rays, the UV radiation in particular. Those bacteria are able to actively synthesise great amounts of yellow-orange carotenoid pigmentation, which effectively protect those organisms from photosensitization caused by exposure porphyrin compounds to sun radiation, cytochroms in particular (Maki 1993). At the same time Rieper-Krichner (1990) points to the fact, that bacteria of the *Flavobacterium-Cytophaga* group are very active in the processes of mineralising the organic matter in water basins. Hence such abundant occurrence of that taxonomic group in the Gulf of Gdańsk which is heavily eutrophicated, seems very logical. The genera of *Pseudomonas*, *Alcaligenes* and also those of *Aeromonas-Vibrio* group were fairly abundant (10-19%) in the water. Research conducted in other marine water bodies (Sieburth 1971, Fehon and Oliver 1979) revealed that bacteria falling into the said taxonomic groups occur fairly frequently among bacterial microflora. On the other hand, quite small numbers (1-4%) of the total heterotrophic bacteria isolated from the waters of the Gulf of Gdańsk were bacteria of the *Achromobacter* and *Bacillus* genera. Also results obtained by Suguhara *et al.* (1988) confirmed the scarce occurrence of those taxonomic groups among the overall microflora.

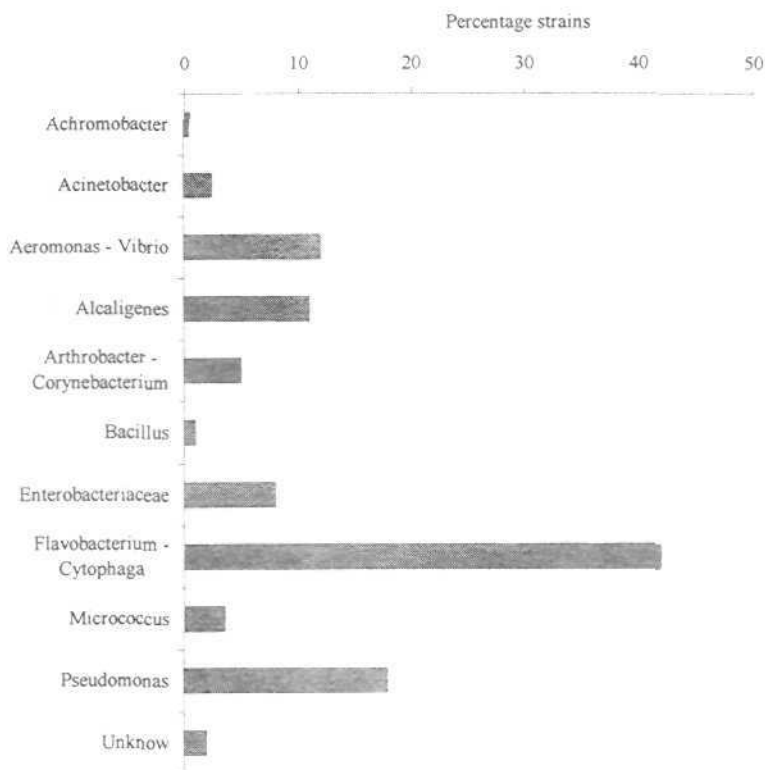


Fig. 2 Taxonomy composition of heterotrophic bacteria inhabiting Gulf of Gdańsk

The bacterial microflora occurring in the Gulf of Gdańsk, was found able to decompose many different organic weight molecular compounds (Fig.3). Among the heterotrophic bacteria inhabiting this part of the Baltic Sea, the proteolytic, amylolytic, lipolytic ones were most frequently recorded. Krstulović and Solić (1988) consider those three physiological groups of bacteria to be organisms that play the crucial role in the process of destruction and transformation of the organic matter in water bodies. The research done so far (Hashimoto *et al.* 1983, Mudryk 1991) have shown that in a marine environment, the most abundant bacteria group are those organisms that are able to hydrolyse protein and may be as numerous as 70-100% of the total water bacterial microflora. Also in the Gulf of Gdańsk, over 60 % of the bacteria inhabiting this area of the Baltic Sea were characterised by the ability to perform an enzymatic process of protein depolymerizing. Those results match the ones that were recorded in the Central Baltic, which showed that about 75% of the bacteria were able to hydrolyse protein (Bölter and Rheinheimer 1987). According to Lit-

tle *et al.* (1979) such abundant occurrence of proteolytic bacteria result from the fact that in water basins proteins, polipeptides and amino acids are major components of the organic matter whose main source are the phyto and zooplankton excretions and their dead bodies.

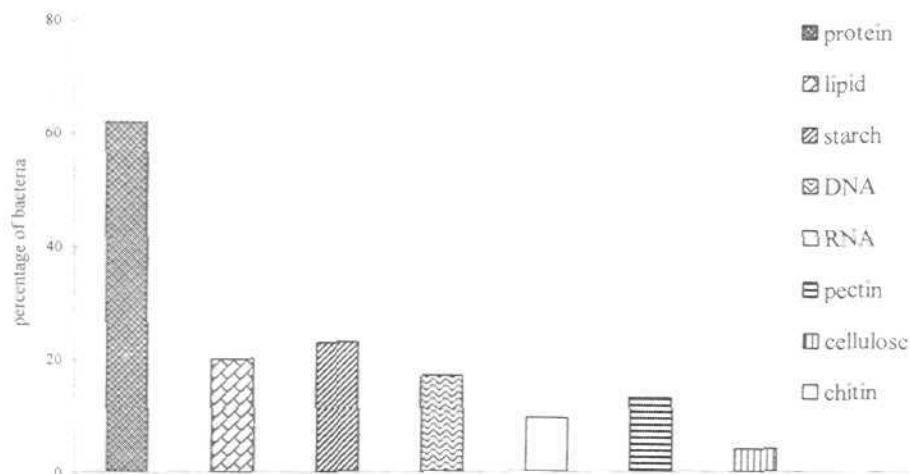


Fig. 3. Occurrence of heterotrophic bacteria able to decompose macromolecules

Researches done to the starch decomposition have revealed that amylolytic bacteria in water bodies make fairly abundant physiological group (Helemke and Weyland 1991). Also in the Gulf of Gdańsk, those bacteria which were able to synthesise amylases made over 20% of the total bacterial microflora (Fig.3). Being a homopolymer, starch made of glucose and, beside lipids makes major reserve and energetic material accumulated in algae and macrophyte cells (Howling 1989). Therefore starch always occurs in water ecosystems and is actively used by bacteria at as little concentration as  $10 \mu\text{g} \cdot \text{Cdm}^{-3}$  (Kooji and Hijnen 1985). Bacterial amylolytic enzymes are able to decompose starch at a rate of  $0.2-0.7 \text{ g} \cdot \text{m}^{-2} \cdot 24 \cdot \text{h}^{-1}$  and the thus obtained simple compounds such as: glucose, maltose, maltotetrose or maltohexose are actively absorbed by bacteria as food substrate (Cotta 1988).

Lipids, making 3-55% of the total organic matter in water ecosystems (Gajewski *et al.* 1993) are actively used up by heterotrophic bacteria as substantial source of carbon and energy and also serve as mechanic and thermal insulators (Arts *et al.* 1992). In the Gulf of Gdańsk, about 20% of all the planktonic bacterial microflora were composed of lipolytic bacteria (Fig.3). The fairly abundant occurrence of bacteria hydrolysing lipids speaks for the research results by Bölter and Rheinheimer (1987). The main source of lipid compounds for bacteria is alive and decayed phytoplankton and zooplankton (Arts *et al.* 1992).

A fairly large number of heterotrophic bacteria in the Gulf of Gdańsk was

made of organisms hydrolysing deoxyribonucleic acid (DNA) (Fig. 3). The mineralising process of that organic matter is a very important function of heterotrophic bacteria. The DNA acid, being the basic inter-cellular structure of all living organisms is released from decayed plants and animals and for that reason it occurs at a fairly high concentration ( $1.8-50.0 \text{ mg}\cdot\text{dm}^{-3}$ ) in water bodies (Paul *et al.* 1988). The process of the DNA hydrolysis is done in the presence of deoxyribonuclease synthesised mainly by bacteria. That enzyme decomposes DNA into sugars, pyrimidins, purins and inorganic phosphates that are actively used by bacteria and phytoplankton as growth agents.

Cellulolytic bacteria made low percentage of the bacterial microflora in the Gulf of Gdańsk and not a single strain of bacteria hydrolysing mucopolysaccharide, i.e. chitin, were isolated from the water. Perhaps, it is owing to the fact that the bacteria having an easy access to easier metabolised organic matter (protein, lipids, and polysaccharides) did not use chitin, the decomposition of which is more energy consuming.

Results of some earlier research have revealed that low molecular compounds of monomer type were much better food substrate for heterotrophic bacteria than large organic macromolecules (Sundh 1992). Organic acids, carbohydrates and amino acids make a most abundant group of low molecular compounds in water basins and are a potential source of carbon or nitrogen for water bacteria (Kiel and Kirchman 1993). It is only those organic monomers that may be absorbed directly by bacteria because assimilation of those compounds is not limited in any way by transport processes or the permeation of the cytoplasmic membrane of those organisms (Furhman 1990). Results presented in Fig. 4 display those heterotrophic bacteria populations inhabiting the Gulf of Gdańsk, used the organic low-molecular compounds with varied intensity. The said bacteria strains assimilated most actively amino acids. The ability to actively absorb amino acids is a common property among bacterial populations inhabiting marine water bodies (Furhman 1990). Those low molecular organic compounds make a universal food substrate preferred by bacteria as a source of carbon and nitrogen. The amino acids taken by bacteria were used in 80 - 90% for the processes of protein biosynthesis whereas 10 - 20% is used for respiratory processes (Ferguson and Sunda 1984). A largest percentage (96 - 100%) of heterotrophic bacteria isolated from waters of the Gulf of Gdańsk incorporated glutamic acid and aspartic acid. Simon (1991) pointed to a very active use of those two amino acids by marine bacteria, which are extracellular excretion of numerous algae (Brown 1991). Over 50% bacterial strains under examination, in their metabolic processes used also arginine, cysteine, glycine and histidine. Those data indicate that those amino acids, beside glutamic acid and aspartic acid, make a very substantial source for carbon and nitrogen in the bacteriocenosis of the Gulf of Gdańsk. Heterotrophic bacteria inhabiting the Gulf of Gdańsk, beside amino acids, in their life processes, used organic acids fairly actively (Fig. 4). Over 40% of bacterial microflora inhabiting the said part of the Baltic Sea, assimilated such organic acids or their salts as: lactic, malic, malonate, succinic, uric acids and citrate, pyruvate, succinate sodium and also calcium lactate. On the other hand, no bacteria strain isolated from the water of the Gulf of Gdańsk, incorporated stearic acid or sodium tartrate. The least actively



actively used group of low molecule compounds by hetrotrophic bacteria isolated from the Gulf of Gdańsk were carbohydrates (Fig. 4). It was only slightly over 30% of the examined bacteria that used mannose, saccharose and xylose for their metabolic cycles. The remaining carbohydrates under investigation were used only by 10 – 20 % of all the bacterial microflora. Those data were in conformity with the results of research by Gillespie *at al.* (1976), that revealed those marine bacteria than organic acids and sugars much more actively used amino acids. Following Furhman (1990) it was owing to the fact that sugar and organic acids make merely a source carbon for the bacteria whereas amino acids are a source of carbon and nitrogen at the same time.

Amino acids, organic acids and carbohydrates make not only a source of carbon or nitrogen for the bacteria, but they also are a substantial source of energy. Oxidising those low molecular compounds, the bacteria gain the energy that is necessary for all life processes. The determination of oxygen consumption rate against various respiratory substrates is a widely used method for the measurement of metabolic activity of the bacteria in water ecosystems (Parkinson and Coleman 1991, Mudryk 1997). Data presented in Fig. 5 show that bacteria populations inhabiting the Gulf of Gdańsk used the respiratory substrates with varied intensity. The most active respiratory processes among bacteria were performed in the presence of casein hydrolysate, which is a mixture of amino acids. That respiratory substrate was used by all bacteria strains under examination (Fig.5A). Wirsén and Jannasch (1974) point to the fact that an active use of casein hydrolysate as a respiratory substrate is a common property among type populations of marine bacteria. According to Novitsky (1983) also marine bacteria as a respiratory substrate actively use glucose. However, the bacterial microflora inhabiting the Gulf of Gdańsk used glucose as energetic material much less actively than casein hydrolysate (Fig.5B). Those data are in conformity with research results by Mudryk (1989), that revealed that respiratory activity in bacteria in the presence of amino acids was much greater than in the case of carbohydrates. Sodium acetate was less attractive for the bacteriocenosis of the Gulf of Gdańsk as exogenous respiratory substrate and was oxidised only by the half of the tested bacterial strains (Fig.5 C).

Summing up, it must be said that the presented results show that the heterotrophic bacteria in the Gulf of Gdańsk are very active in the biological conversion of various organic compounds while staying a major link in the marine biocenosis.



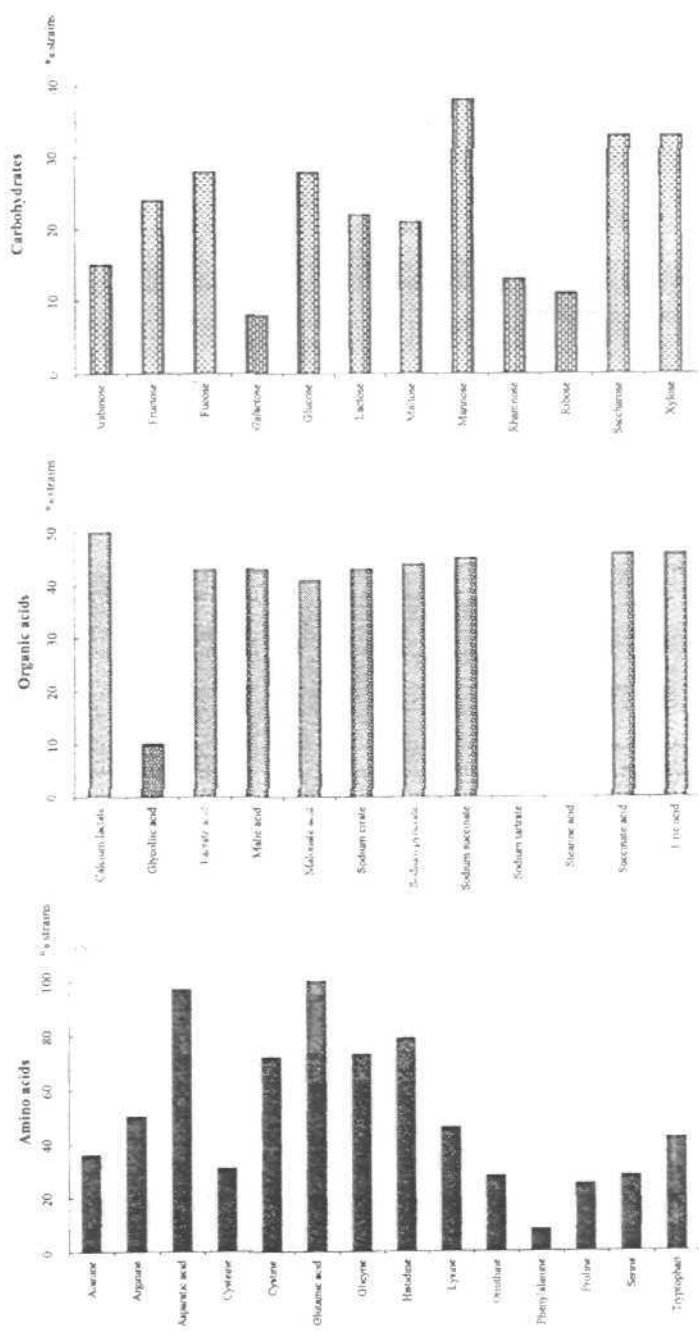


Fig. 4 Utilization by marine bacteria different low molecular weight organic compounds

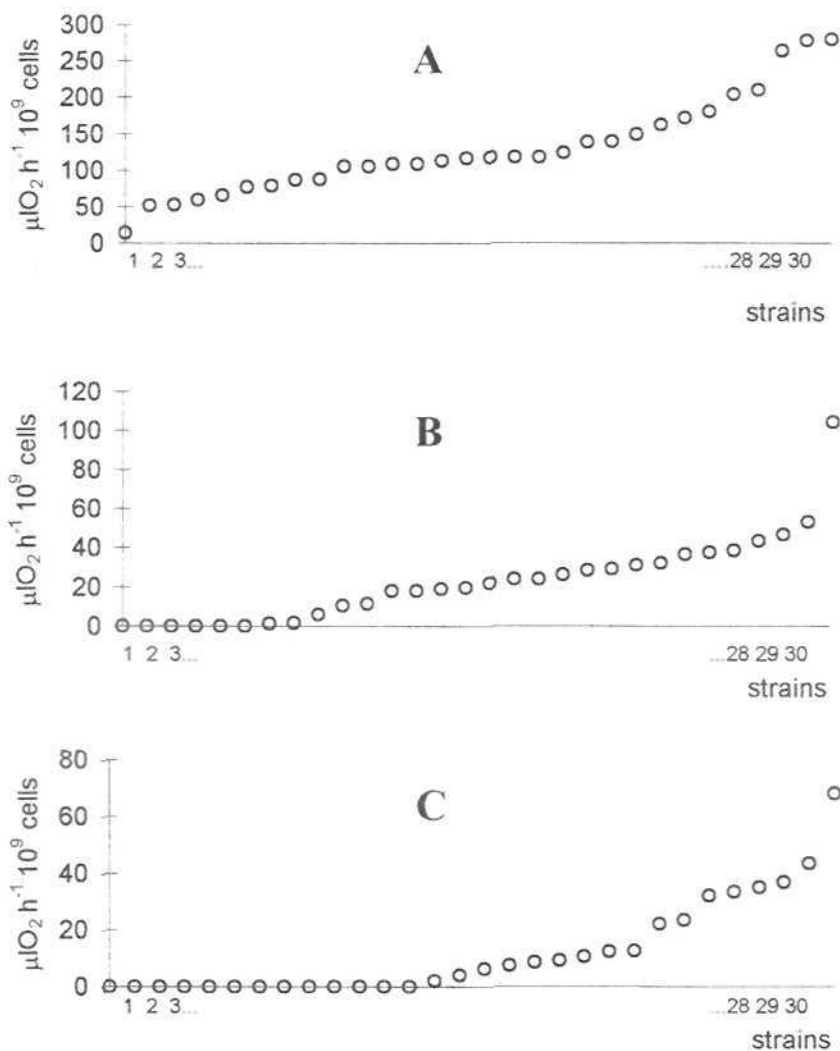


Fig. 5. Respiration of bacteria in the presence of casein hydrolyzate (A) glucose (B) and sodium acetate (C)

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## CHARAKTERYSTYKA BAKTERIOPLANKTONU ZASIEDLAJĄCEGO ZATOKĘ GDAŃSKĄ

### Streszczenie

Badania bakterii heterotroficznych zasiedlającej Zatokę Gdańską wykazały, że maksima ich liczebności notowano w rejonie ujścia Wisły do zatoki, a minima w otwartej części tego akwenu Morza Bałtyckiego. W składzie taksonomicznym bakterii dominowały organizmy z grupy *Flavobacterium-Cytophaga* a znikomy procent ogółu wyizolowanej mikroflory stanowiły bakterie z rodzaju *Achromobacter* i *Bacillus*. Wśród bakterii heterotroficznych zasiedlających Zatokę Gdańską bardzo licznie występowały bakterie proteolityczne, amylolityczne i lipolityczne natomiast nie stwierdzono obecności bakterii chitynolitycznych. Badana mikroflora bakteryjna bardzo aktywnie w swoich procesach metabolicznych wykorzystywała organiczne związki niskocząsteczkowe w szczególności aminokwasy. Również mieszaninę tych związków w formie hydrolizatu kazeiny bakterie wyizolowane z Zatoki Gdańskiej bardzo aktywnie wykorzystywały w procesach oddechowych. Natomiast stosunkowo mało aktywnie bakterie te jako substrat oddechowy wykorzystywały cukry i kwasy organiczne.