Polish Journal of Environmental Studies Vol. 7, No. 5 (1998), 289-293

Utilization of Dissolved Amino Acids by Marine Bacteria Isolated from the Surface and Subsurface Layers of the Gdańsk Deep

Z. Mudryk,[1,2], P. Skórczewski[1]

[1] Department of Experimental Biology, University of Education, Arciszewskiego 22, 76-200 Stupsk, Poland [2] Centre of Marine Biology, Polish Academy of Sciences, sw. Wojciecha 5, 81-347 Gdynia, Poland

Received 17 March,1998
Accepted 12 June, 1998

Abstract

Study of the utilization of various amino acids by bacteria inhabiting seawater in the region of the Gdansk Deep was carried out. The highest percentage of the neustonic and planktonic bacteria utilized glutamic acid, asparatic acid, histidine and cysteine for their optimal growth, whereas only a small percentage of bacterial strains utilized serine, phenylalanine, ornithine and glicyne. Significant differences in utilization of individual amino acids by bacteria inhabiting various water layers occurred.

Keywords: marine bacteria, utilization of amino acids, Gdansk Deep

Introduction

In aquatic ecosystems, the total mass of the dissolved organic matter (DOM) generally exceeds that of living organisms [37]. Heterotrophic microorganisms play a key role in modifying and decomposing DOM in marine ecosystems. The utilization of DOM by heterotrophic bacteria is an integral part of the circulation of organic and inorganic nutrients in the sea [12, 33].

The composition of DOM in seawater is certainly heterogeneous. Besides carbohydrates, dissolved amino acids constitute one of the major groups of monomeric compounds in DOM [36, 37].The concentration and chemical composition of amino acids in aquatic ecosystems constantly changes, mostly in relation to varying microbial activity. Therefore, the occurrence of dissolved amino acids in natural seawater reflects the balance between production and assimilation of those monomers [9]. In seawater, the concentrations of dissolved amino acids range from 300-1000 nM • dm^{-3} [17,24].

In aquatic ecosystems, amino acids and small peptides are the universal substrates preferably used by heterotrophic bacteria as important sources of carbon and nitrogen [14]. Assimilation of those low-molecular weight compounds is limited neither by transportation nor by permeability of the bacterial cellular membranes [21]. Amino acids are used by heterotrophic bacteria for the synthesis of biomelecules, predominately proteins, which comprise 60% of bacterial biomass, and are serve as a source of energy [29, 38]. In seawater, the first process appeares to dominate. About 80-90% of the amino acid carbon and nitrogen utilized from seawater enter biosynthesis pathways, while about 10-20% are used in respiration processes [12, 18, 36]. Bacterial amino acid utilization rates closely correspond to the rates at which amino acids are released. This suggests efficient regulatory mechanisms whereby bacteria use amino acids as rapidly as they become available [18, 35].

Considering that the intensity of the utilization of indvidual amino acids by bacteria is diversified, the aim of is the present study is to determine the preferences of marine bacteria for the utilization of amino acids which are likely to occur in the waters of the Gdansk Deep to provide information on the potential capability of the microflora to convert organic matter in marine ecosystems.

Materials and Methods

Study Area and Sampling

Bacteriological research was carried out on 9-10 May 1995 in the region of the Gdansk Deep, at research station P (φ = 55° 1 'N, λ = 18° 42'E) (Fig.l). Seawater samples were taken on board the ORP "Kopernik" from four layers. Microfilm layer (ML) samples (thickness of 11 ±5 μm)

were collected with a 30 x 30 cm teflon plate **[11]. Film** layer (FL) samples (thickness of 90 ± 17 μm) were taken with a 30 x 30 cm glass plate [16]. 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) [15]. The teflon plate, glass plate and poliethylene net 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 a depth of about 10-15 cm.

Experiments

Plating techniques were used in order to isolate neustonic (ML, FL, and SL layers) and planktonic bacteria (SUB layer). Seawater samples were diluted with sterile seawater and inoculated by the spread method in five parallel replicates on the ZoBell 2216 E agar medium (ZB) of 87‰ salinity [31]. The plates were incubated at 20°C for 10 days. After that, 34-40 bacterial colonies isolated from each water layer were picked out and transferred to a semi-solid ZB medium. After purity control, the bacteria were stored at 4°C and subsequently used for further studies.

All isolates were identified using morphological, physiological, and biochemical characteristics. For the identification of the isolates the following tests were taken into account: cell morphology and stainability with Gram's method, ability to move, pigment production, fermentation-oxidation reaction upon Hugh-Leifson's substrate, production of cytochrome oxidase, arginine hydrolysing ability and test API 20 (bio Merieux). Generic composition of the isolated bacteria was determined according to the scheme of Oliver [27] and Berge's key [19]

The ability of the isolated neustonic and planktonic bacteria to utilize various amino acids was assayed in a modified medium B prepared according to Donderski and Glazewska [10] and Mudryk et al. [25]. In medium B, potassium nitrate and glucose which are sources of nitrogen and carbon, were removed. To replace these compounds, an amount of amino acids equivalent to N and C was introduced. Two-day-old bacterial cultures proliferated in liquid ZB medium were used as inoculum. The results were obtained after 5 days incubation of the cultures at 20°C, the

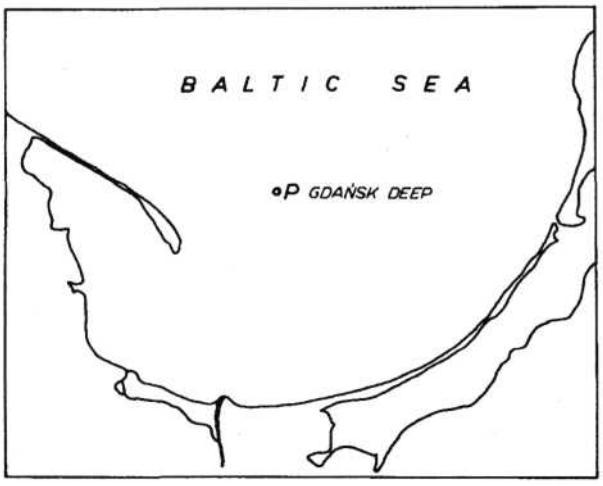

Fig. 1. Location of sampling station P in the Gdansk Deep.

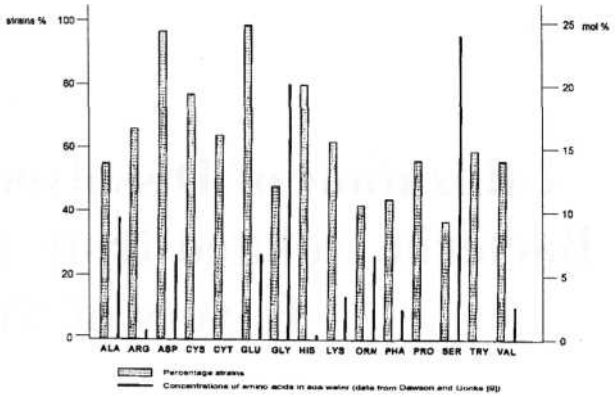

Fig. 2. Growth of bacteria isolated from Gdansk Deep area in the presence of different amino acids (mean values of all water layers).

intensity of their growth was measured on a spectrophotocolorimeter SPECOL with the appendage ER-1, at 540 nm. Light permeability lower than 70% was accepted as an indication of good growth of bacteria in the presence of a used amino acid. A medium without any bacteria was used as the blank. The light permeability of the blank was always 100%.

The capacity of bacterial isolates to utilize amino acids that occur most commonly in water basins, such as: alanine (ALA), arginine (ARG), asparatic acid (ASP), cystine (CYS), cysteine (CYT), glutamic acid (GLU), glycine (GLY), histidine (HIS), lysine (LYS), ornithine (ORN), phenylalanine (PHA), proline (PRO), serine (SER) tryptophan (TRY), and valine (VAL) was determined. The amino acids were divided into six groups (acidic, alkaline, polar, non-polar, aromatic, sulphuric) according to their chemical nature [20, 38].

The results of the above experiments were used to calculate the utilization average index (UAI) for the bacteria according to the formula proposed by Prieur [30] modified by the authors

$$UAI = \frac{\sum_{i=1}^{n} a_i}{100 \cdot n}$$

where:
ai - the percentage of utilization of individual amino acid,
n- the total number of tested amino acids.

Results

Figure 2 presents the results concerning the utilization of dissolved amino acids by marine bacteria isolated from the studied region of the Gdansk Deep. These results indicate that bacteria used different amino acids with various intensity. Among the 15 amino acids monitored, the highest percentage (77-99%) of the isolated bacteria utilized glutamic acid, asparatic acid, histidine and cysteine for their optimal growth.

Over half of all the bacterial strains inhabiting the waters of the Gdansk Deep most preferably used arginine, cystine, lysine, valine, alanine, tryptophan, and proline for their optimal growth, while serine, phenylalanine, ornithine, and glicyne were the least suitable sources of carbon, nitrogen and energy.

Table 1. Bacterial utilization of various amino acids in different water layers (percentage of strains)

Layers	Number of strains studied	Amino acids utilized (% of strains)														UAI
		ALA	ARG	ASP	CYS	CYT	GLU	GLY	HIS	LYS	ORN	PHA	PRO	SER	TRY	VAL	
ML	40	75	70	96	78	95	96	15	68	85	54	65	85	15	75	42	0.68
FL	40	75	95	100	86	100	100	30	95	72	52	95	90	75	78	50	0.80
SL	34	29	44	100	71	23	100	59	94	38	24	0	15	32	50	78	0.50
SUB	38	42	55	94	73	39	100	87	63	53	37	16	34	24	34	82	0.55

The data presented in Figure 2 show no relationship between the natural concentration of individual amino acids in the Gdansk Deep and their utilization by bacteria.

The results concerning of utilization of amino acids by bacteria isolated from various water levels are presented in Table 1 .These data indicate significant differences of utilization of amino acids by bacteria inhabiting various water layer. Based on the calculated value of the UAI, which is the measure of the ability of bacterial strains to utilize various amino acids, it was determined that the widest range of amino acids occurs among bacteria inhabiting the film layer (UAI = 0.80). In that water layer all studied strains utilized asparatic acid, glutamic acid and cysteine for their optimal growth. Bacteria isolated from the screen layer utilized amino acids least intensively, as indicated by the low value of UAI (0.50). Only a small percentage (15-38 %) of bacteria inhabiting that water level utilized proline, ornithine, alanine, serine and lysine as optimal sources of carbon, nitrogen and energy. Neustonic bacteria isolated from that water layer did not use phenylalanine at all.

On the basis of the data presented in Table 2 it has been determined that amino acid utilization by bacteria depends on their taxonomic position. Among bacterial strains isolated from Gdansk Deep bacteria of the *Pseudomonas* genus and *Enterobacteriaceae* family characterised by the highest capabilities of amino acid utilization (UAI = 0.74 - 0.88). On the other hand bacteria of the *Alcaligenes* genus showed the poorest growth in the presence of studied amino acids (UAI = 0.43).

Table 3 presents the results concerning bacterial utilization of amino acids depending on their chemical structure. It has been determined that in all the studied water layers, acidic amino acids were utilized most intensively. Polar amino acids were the group least preferably used by bacteria inhabiting microfilm and film layers, while aromatic amino acids were the group least preferably used by bacteria isolated from the screen layer and subsurface water.

Discussion

Natural marine and freshwater microlayers, or surface films, are ubiquitous phenomena in aquatic environments. The chemical composition of surface microlayers is very variable but consists, in significant part, of dissolved free and combined amino acids [5, 39]. Microlayer microbial communities can be highly active metabolically with respect to natural constituents such as amino acids [6]. The bacterial uptake and removal of those low molecular weight compounds from the seawater is an important factor controlling the concentration and distribution of organic carbon and nitrogen in the sea [22].

The main sources of amino acids in surface microlayers and subsurface water are extracellular exudates of the phytoneuston and phytoplankton [8, 23]. Considerable amounts of those compounds, mainly in the form of metabolites, are also secreted by zooplankton and flagellates [26, 32]. Moreover, proteins released from dead phyo- and zooplankton

Table 2. Amino acids uptake by different genera of bacteria (averages of four water layers)

Genera or groups of bacteria	Amino acids utilized (% of genus representatives)															UAI
	ALA	ARG	ASP	CYS	CYT	GLU	GLY	HIS	LYS	ORN	PHA	PRO	SER	TRY	VAL	
Flavobacterium--Cytophaga (64)[a]	73	80	92	66	89	97	61	66	42	30	45	64	33	61	39	0.62
Pseudomonas (22)	86	100	100	73	100	100	64	100	91	73	100	100	77	82	77	0.88
Aeromonas - Vibrio (17)	100	71	100	53	71	100	24	47	100	53	18	76	41	47	53	0.64
Alcaligenes (17)	88	65	94	53	0	88	0	29	35	41	24	59	0	41	29	0.43
Enterobacteriaceae (14)	100	100	100	64	93	100	50	64	100	57	21	100	86	43	29	0.74
Arthrobacter--Corynebacterium (6)	33	83	50	100	17	100	33	67	0	0	0	67	50	100	83	0.52
Other* (9) and unknown (3)	58	100	100	25	25	67	100	17	75	67	33	42	58	17	83	0.58

[a] – Indicates number of isolates represented

* – Includes - *Acinetobacter, Achromobacter, Bacillus, Micrococcus*

Table 3. Bacterial utilization of amino acids depending on their chemical structure and water layer (percentage of strains)

Amino acids groups	% of strains			
	Microfilm layer	Film layer	Screen layer	Subsurface layer
acidic (asparatic acid, glutamic acid)	96	100	100	97
alkaline (arginine, histidine, lysine, ornithine)	70	78	50	52
polar (glycine, serine)	15	53	46	56
non – polar (alanine, proline, valine)	67	72	41	53
aromatic (phenylalanine, tryptophan)	70	87	25	25
sulphuric (cysteine, cystine)	87	93	47	56

are hydrolyzed to free amino acids [2]. All those sources can supply water with considerable amounts of amino acids, particularly in basins with high primary production [28].

The data presented in this paper show that in the studied region of the Gdansk Deep, asparatic acid and glutamic acid were the most preferably used by bacteria. Results of many other studies are in consistency with these results [10, 34, 35]. Glutamic and asparatic acid are reported as extracellular products in many algae [3]. The decarboxylic amino acids such as glutamate and asparate, can readily enter the citric acid cycle by known metabolic pathways. According to Jorgensen [17] heterotrophic bacteria respirate 60-73% of the taken up glutamic and asparatic acid, the remaining part transforming into their own biomass. Decarboxylation of glutamic and asparatic acid by bacteria might also be the source of β-alanine. This amino acid is a precursor of the vitamin - pantothenic acid, and was found in the mureine complex of bacterial cell walls [7]. The data obtained in the present study have shown that a somewhat smaller number of bacteria inhabiting the waters of the Gdansk Deep utilized fairly intensively such amino acids as histidine and cysteine. This fact indicates that those substrates, along with glutamic and asparatic acid, are the main source of organic carbon and nitrogen for the studied bacteria. Tupas and Koike [38] draw attention to the fact that histidine composed about 22% of bacterial proteins.

Serine, phenylalanine, ornithine and glycine were the least suitable amino acids for the bacterial populations isolated from the studied region. Those data are consistent with the results of Burison and Morita [4], Crawford et al. [8] and Sepers [34].

Data presented in this paper show significant differences in the levels of intensity of amino acid utilization by bacteria inhabiting various water layers. Generally, in the studied seawater layers of the Gdansk Deep, bacteria isolated from deeper water layers were utilized less intensively than amino acids in the microfilm and film layers. These results are in agreement with earlier studies carried out in the Gdansk Deep by Dawson and Gocke [9]. A high percentage of bacteria utilizing amino acids in the surface microlayers is probably caused by higher concentrations of those monomers in the surface film compared to the subsurface water [5, 25, 39].

According to Amano et al. [1] utilization amino acids by bacteria depends on their taxonomic position. Different systematic groups of marine bacteria have different preferences for each amino acid occurring in seawater. In the study presented here it has also been determined that various genera of bacteria isolated from the Gdansk Deep showed different capabilities of amino acid utilization.

Results of numerous studies indicate that bacterial utilization of amino acid depends on their chemical structure because each bacterial strain has a special amino acid metabolic pathway [1, 20, 38]. This relationship is also confirmed by our studies, as neustonic and planktonic bacteria isolated from the waters of the Gdansk Deep utilized acidic amino acids most actively, whereas polar and aromatic ones were the least preferred groups.

References

1. AMANO M., HARA S., TAGA N. Utilization of dissolved amino acids in seawater by marine bacteria. Mar. Biol. **68,** 31, **1982.**
2. BILLEN G., FONTIGNY A. Dynamics of a pheaocystis- do minated spring bloom in Belgian coastal waters. II. Bacterioplankton dynamics. Mar. Ecol. Prog. Ser. **37,** 249, **1987.**
3. BROWN M. R. The amino-acid and sugar composition of 16 species of micro algae used in microculture. J. Exp. Mar. Biol. **145,** 79, **1991.**
4. BURISON B. K., MORITA R. Y. Heterotrophic potential for amino acid uptake in a naturally eutrophic lake. Appl. Mic robiol. **27,** 488, **1974.**
5. CARLUCII A. F., CRAVEN D. B., WOLGAST D. M. Mic robial populations in surface films and subsurface waters: ami no acids metabolism and growth. Mar. Biol. **108,** 329, **1991.**
6. CARLUCII A. F., WOLGAST D. M., CRAVEN D. B. Mic robial populations in surface flims: amino acid dynamics in nearshore and offshore waters off southern California. J. Geoph. Res. **97,** 5271, **1992.**
7. COLE J. J., LEE C. Rapid microbial metabolism of non-prote in amino acids in the sea. Biogeochemistry 2, 299, 1986.
8. CRAWFORD C. C, HOBBIE J. E., WEBB K. L. The utiliza tion of dissolved free amino acids by estuarine microorga nisms. Ecology **55,** 551, **1974.**
9. DAWSON R., GOCKE K. Heterotrophic activity in compar-

sion to the free amino acid concentrations in Baltic sea water samples. Oceanol. Acta **1,** 45, **1978.**
10. DONDERSKI W., GLAZEWSKA R. Utilization of amino acids by planktonic, benthic and epiphytic bacteria isolated from the lake Jeziorak. AUNC Torun Limnol. Papers **8,** 27, **1974.**
11. FALKOWSKA L. Mikrowarstwa powierzchnowa morza. Ed. Uniwersytet Gdanski 185pp. **1996.**
12. FERGUSON R.L., SUNDA W.G. Utilization of amino acids by planktonic marine bacteria: Importance of clean technique and low substrate additions. Limnol. Oceanogr. **29,** 258, **1984.**
13. FURHMAN J. FERGUSON R. L. Nanomolar concentrations and rapid turnover of dissolved free amino acids in seawater: agreement between chemical and microbiological measure ments. Mar. Ecol. Prog. Ser. **33,** 237, **1986.**
14. FURHMAN J. Dissolved free amino acid cycling in a estuarine outflow plume. Mar. Ecol. Prog. Ser. **66,** 197, **1990.**
15. GARRETT W. D. Collection of slick-forming materials from the sea surface. Limnol. Oceanogr. **10,** 602, **1965.**
16. HARVEY G. W., BURZELL L. A. A simple microlayer met hod for small samples. Limnol. Oceanogr. **17,** 156, **1972**
17. JORGENSEN N. O. Heterotrophic assimilation and occurren ce of dissolved free amino acids in a shallow estuary. Mar. Ecol. Prog. Ser. 8, 145, 1982.
18. JORGENSEN N. O. Free amino acids in lakes: Concentrations and assimilation rates in relation to phytoplankton and bac terial production. Limnol Ocenogr. 32, 97, **1987.**
19. KREIG N.R., HOLT J.G. Bergey's Mannual of Systematic Bacteriology. Ed. Wlliams and Wilkins Baltimore 964 pp.
20. LALKE E., DONDERSKI W., BLOTEVOGEL K.H. Role of epiphytic bacteria in the utilisation of organic matter. Oceanol. Stud. **4,** 19, **1996.**
21. LANCELOT C, BILLEN G. Activity of heterotrophic bac teria and its coupling to primary production during the spring phytoplankton bloom in the southern bight of the North Sea. Limnol. Oceanogr. **29,** 721, **1984.**
22. LEE C, JORGENSEN N. O. Seasonal cycling of putrescine and amino acids in relation to biological production in a strati fied coastal salt pond. Biogeochemistry **29,** 131, **1995.**
23. MAKI J. S., HERMANSSON M. The dynamics of surface microlayer in aquatic environments.In: The Biology of Particcles in Aquatic Systems. Ed. R. S. Wotton. Lewis Pub. Boca Roton Ann Arbor London, Tokyo, pp 161-182, **1994.**
24. MOPPER K., LINDROTH P. Diel and depth variations in dissolved free amino acids and ammonium in the Baltic Sea determined by shipboard HPLC analysis. Limnol. Oceanogr. 27, 336, **1982.**
25. MUDRYK Z., DONDERSKI W., MORKUNAS I. Amino acids as source of nitrogen and carbon for moderately halophilic bacteria isolated from the water of estuary lakes. AUNC Toruii Limnol. Papers **18,** 25, **1993.**
26. NAGATA T., KIRCHMAN D. L. Release of dissolved free and combined amino acids by bacteriovorous marine flagel lates. Limnol. Ocenogr. **36,** 433, **1991.**
27. OLIVER J.D. Taxonomic scheme for the identification of ma rine bacteria. Deep-Sea Res. **29,** 795, **1982.**
28. PALUMBO A. V., FERGUSON R. L., RUBLEE P. A. Ef ficient utilization of dissolved free amino acids by suspended marine bacteria. J. Exp. Mar. Biol. **69,** 257, 1983.
29. PANTOJA S., LEE C. Cell-surface oxidation of amino acids in seawater. Limnol. Oceanogr. 39, 1718, **1994.**
30. PRIEUR D. Preliminary study of heterotrophic bacterial com munities in water. An intervertebrates from deep sea hydrothermal vents. Proc. 21st EMBS Gdansk 393, **1989.**
31. RHEINHAIMER G. Microbial ecology of a brackish water environment. Ecological Studies 25 Springer-Verlag Berlin 291 pp. **1977.**
32. RIEMAN B., JORGENSEN N. O. LAMPERT W., FUHRMAN J. A. Zooplankton induced changes in dissolved free amino acids and in production rates of freshwater bacteria. Microbiol. Ecol. 12, 247, **1986.**
33. RITZRAU W., THOMSEN L. Spatial distribution of particu late composition and microbial boundary layer (BBL) of the Northeast water Polynya. J. Mar. Syst. **10,** 455, **1997.**
34. SEPERS A. B. The aerobic mineralization of amino acids in the saline Lake Grevelingen and freshwater Haringvliet basin (The Netherlands). Arch. Hydrobiol. **92,** 114, **1981.**
35. SIMON M. Isotope dilution of intracellular amino acids as a tracer of carbon and nitrogen sources of marine planktonic bacteria. Mar. Ecol. Prog. Ser. **74,** 295, **1991.**
36. SIMON M., ROSENSTOCK B. Carbon and nitrogen sources of planktonic bacteria in lake Constance studied by the com position and isotope dilution of intracellular amino acids. Lim nol. Oceanogr. 37, 1496, **1992.**
37. THOMAS J.D., EATON P. The spatio-temporal patterns and ecological significance of free amino acids and humic substan ces in contrasting oligotrophic and eutrophic freshwater eco systems. Hydrobiologia **332,** 183, **1996.**
38. TUPAS L., KOIKE I. Amino acid and ammonium utilization by heterotrophic marine bacteria grown in enriched seawater. Limnol. Oceanogr. 35, 1145, **1990.**
39. WILLIAMS P. M., CARLUCCI A. F., HENRICHS S. M. HORRIGAN S. G..REID F. M., ROBERSTON K. J. Chemical and microbiological studies of surface films in the southern Gulf of California and off the west coast of Baja California. Mar. Chem. **19,** 17, **1986.**