PRODUCTION OF B-VITAMINS BY PLANKTONIC BACTERIA ISOLATED FROM THE MESOTROPHIC LAKE JASNE

WOJCIECH DONDERSKI*
BARBARA NOWACKA*

SUMMARY : Studies were carried out on the occurrence of organisms producing vitamins of group B among the heterotrophic planktonic bacteria of the littoral and pelagic zone of the mesotrophic lake Jasne. In the littoral zone the most numerous group were bacteria producing nicotinic acid, pantothenic acid and riboflavin. In the pelagic zone the number of B-vitamins producers was much smaller, and the predominant group were strains synthesizing folic acid, biotin and thiamin. The percentage of B-vitamins producers in the pelagic zone was higher in spring than in autumn. In the littoral zone the reverse situation was stated. The majority of planktonic bacteria of both zones were able to produce 2-4 vitamins. Only a few strains synthesized only one vitamin.

Key words : Planktonic bacteria, heterotrophic bacteria, littoral zone, pelagic zone, B-vitamins.

INTRODUCTION

Heterotrophic bacteria living in water environment transform matter and energy cycling in it. This is so, among other things, because of their abilities, including the ability to synthesize vitamins of group B, contained in many enzymes participating in the processes of transformation and mineralization of various organic compounds (1,13,19, 21).

The presence of vitamins in water environment is one of the factors conditioning the growth and development of many groups of organisms, such as phytoplankton, zooplankton, macrophytes and bacteria themselves. According to Ohwada and Taga (20) and Nishijima and Hata (17), many algal species need for their development vitamins of group B, separately or in various combinations. After Hagedorn (11), Gillespie and Morita (9) and Kurata *et al.* (14), quantitative and qualitative variations in the vitamins in water environment may not only affect growth and productivity of phytoplankton and macrophytes, but also the seasonal successions of various groups of algae as well.

The main source of vitamins in water basins is microbiological synthesis, claim Burkholder (3), Hagedorn (10), Rheinheimer (22) and Hishijima and Hata (18). The aim of the present work was to detect among the heterotrophic planktonic bacteria inhabiting various zones and layers of water in the mesotrophic lake Jasne strains synthesizing some vitamins of group B and to estimate the amounts of vitamins produced by individual strains depending on the place and time of their isolation.

* From Department of Water Microbiology and Biotechnology, Institute of Biology, Nicolaus Copernicus University, Torun, Poland.

MATERIALS AND METHODS

The subject of the study were heterotrophic bacteria isolated from the water of the littoral and pelagic zone of the mesotrophic, tending towards distrophy, lake Jasne. The lake is a small, forest surrounded water basin, 10.7 ha in area, situated in the north-eastern part of Poland, in the Ilawa Lakeland. Its maximum length is 500 m, its width about 300 m, and its maximum depth 19.8 m. The water pH ranges from 4.3 to 4.8. More data about the lake are given by Zytkowicz (26).

Sampling

Water samples for study were taken at the time of homothermy of the water mass, in spring (April 14) and in autumn (October 21) 1990. The samples were taken in the littoral and pelagic zone, from the surface layer (about 30 cm below water surface), from the medial layer (half way down, at 3.5 m in the littoral and 9.0 m in the pelagic zone) and from the near bottom layer (about 30 cm above sediment - at 7.0 m and 18.0 m deep respectively). The water was collected by means of an Isatchenko sampler into sterile glass ampoules, which were

placed in an ice container (where the temperature was about ±7°C), taken to the laboratory and analyzed immediately. The time between sampling and analyzing did not exceed eight hours.

Isolation and counting of bacteria

For the isolation and counting of bacteria the spread plate method was applied. Water samples diluted with sterile buffer water (4), were plated on the surface iron-peptone agar medium, after Ferrer, Stapert, Sokolski (8).

After 10 days of incubation at 20°C the bacterial colonies were counted, then from each sample 50 colonies were randomly picked and transferred to a semisolid iron-peptone agar medium (5.0 g agar/litre). The strains were stored at ±4°C and transferred to a fresh semisolid medium every second month to be used for further studies.

Identification of bacteria

The bacteria were identified according to the scheme proposed by Shewan, Hobbs and Hodgkins (23), Hendrie, Mitchell and Shewan (12) and Buchanan and Gibbons (2).

Vitamins assay

The methods used to study the synthesis of B-vitamins by the bacteria under study were the same as described earlier by Strzelczyk and Donderski (25).

RESULTS

In the littoral zone of the lake, in spring the most numerous group were bacteria producing nicotinic acid and pantothenic acid (Table 1). In autumn the most numerous were also bacteria synthesizing nicotinic acid, then riboflavin and folic acid. The least numerous group both in spring and in autumn were bacteria synthesizing thiamin and biotin but their number in autumn, particularly of those producing thiamin, was 30-60 times higher than in spring in the littoral zone, and 5-480 times in the pelagic zone. In the vertical plane of the water mass, in the littoral zone of the lake, in spring the percentage of producers of particular vitamins was much alike, while in autumn the numbers of the vitamins producers was generally decreasing with the depth.

In the pelagic zone the number of B-vitamins producers among the heterotrophic bacteria was much smaller. Among bacteria of this zone the predominant group both in spring and autumn were strains producing folic acid. In spring the second largest group were bacteria producing biotin and nicotinic acid. In this zone of the lake the vitamins producers were generally more numerous in spring than in autumn. The vertical distribution of B-vitamins producers occurring in the surface layer and those above the sediments were but small.

Table 1: Number of heterotrophic bacteria synthesizing B-vitamins in water of the lake Jasne*.

Vitamin produced	Source of sampling	Littoral zone		Pelagic zone	
		14.04.90	21.10.90	14.04.90	21.10.90
Nicotinic acid	a	1.65	19.62	0.17	0.04
	b	2.33	14.25	0.43	1.40
	c	2.29	8.99	0.024	0.65
Pantothenic acid	a	1.24	15.35	0.07	0.12
	b	1.27	7.98	0.27	1.83
	c	1.31	2.62	0.012	1.29
Riboflavin	a	0.70	18.76	0.07	0.16
	b	1.27	16.52	0.17	1.31
	c	1.31	11.23	0.015	2.21
Folic acid	a	0.75	18.76	0.39	1.00
	b	1.08	13.66	1.37	9.91
	c	1.21	7.12	0.10	12.35
Biotin	a	0.12	13.63	0.22	0.31
	b	0.24	6.26	0.67	4.46
	c	0.05	3.74	0.06	2.48
Thiamine	a	0.16	5.13	0.05	0.26
	b	0.13	3.99	0.13	1.42
	c	0.05	3.00	0.012	5.77
Total number of bacteria	a	1.98	30.70	0.45	1.24
	b	3.00	23.36	1.50	11.90
	c	2.62	17.97	0.12	17.78

*: number of bacteria $x10^3/cm^3$ of water a. water from surface layer (about 30 cm depth), b: water middle layer of littoral and pelagic zone (respectively: 3,5 and 9 m depth), c: water from the near bottom sediments (about 30 cm above the bottom).

The quality of vitamins production by the bacteria under study was much the same in both lake zones, but the quantity varied depending on the place and time of isolation of the strains. The bacteria isolated from the water of the littoral zone, both in spring and in autumn, produced the largest amounts of pantothenic acid and nicotinic acid, and the smallest amounts of thiamin and biotin (Table 2).

The strains isolated from the water of the pelagic zone (Table 3) in spring also produced pantothenic acid and nicotinic acid in largest amounts, and biotin and thiamin in smallest amounts, but the amounts of vitamins produced of the bacteria of this zone were 2-14 times smaller than those produced by the strains coming from the littoral zone. The bacteria isolated from the water of the pelagic zone in autumn produced pantothenic acid, thiamin and folic acid in the largest amounts. Biotin was also produced

Table 2: Amount of vitamins produced by the planktonic bacteria isolated from the littoral zone of the lake Jasne (in ug/g d.w.).

Vitamins	14.041990				21.10.1990			
	X	Y	Range	Mean	X	Y	Range	Mean
Pantothenic acid	a	11	30.91-7850.00	2720.67	a	7	74.84-4695.49	2225.29
	b	11	64.26-7277.42	2130.15	b	6	743.36-5323.31	3245.47
	c	8	333.33-11532.35	5131.40	c	6	22.35-13452.63	4892.27
Nicotinic acid	a	7	142.34-604.84	235.98	a	6	126.37-220.17	183.62
	b	7	102.27-6062.50	1246.80	b	9	89.04-564.22	242.01
	c	7	98.36-5083.33	1172.79	c	11	38.27-1125.56	381.62
Riboflavin	a	7	26.25-332.93	133.83	a	5	66.96-229.65	124.04
	b	5	13.95-206.02	74.58	b	6	26.91-265.31	128.09
	c	7	17.82-307.63	97.81	c	9	41.71-205.37	107.31
Folic acid	a	19	12.90-192.10	65.82	a	22	12.00-113.60	59.93
	b	18	18.20-145.80	75.17	b	24	11.80-117.10	57.43
	c	23	12.00-150.30	72.05	c	19	10.20-177.80	103.80
Thiamine	a	2	6.58-80.63	43.61	a	5	4.18-100.49	29.25
	b	1	0.41-0.42	0.41	b	7	4.17-72.53	35.24
	c	1	88.82-88.85	88.83	c	6	3.93-46.04	22.49
Biotin	a	3	0.25-1.27	0.85	a	16	0.06-0.84	0.50
	b	4	0.26-2.12	0.82	b	11	0.18-0.64	0.35
	c	1	0.24-0.26	0.25	c	10	0.01-0.42	0.12

X: source of sampling (as footnote in Table 1), Y: number of strains studied.

but in the smallest amounts. The amounts of thiamin produced in autumn by the bacteria of the pelagic zone were about 2-14 times as large, and of folic acid about 3 times as large as those produced by the strains isolated from the littoral zone in the same season. The production of biotin by bacteria isolated from the pelagic zone was 3-14 times lower than by those from the littoral zone. In the littoral zone more biotin was produced by bacteria in spring, and less in autumn. In the pelagic zone the reverse phenomenon was found.

It follows from Table 4 that different strains show different abilities to synthesize vitamins of group B. The majority of bacteria referred to particular genera or groups were able to synthesize 2-4 vitamins simultaneously. Strains of the genus Achromobacter, Pseudomonas and of the group Flavobacterium-Cytophaga in the littoral zone, as well as those of the family Enterobacteriaceae in the pelagic zone were able to produce and secrete simultaneously all six mentioned vitamins. On the other hand, bacteria of the genus Bacillus isolated from the water of the littoral zone and those of the genus Alcaligenes isolated from the water of the pelagic zone were able to produce only one B-vitamin.

The most active biotin producers in the littoral zone were bacteria of the groups Arthrobacter-Corynebacterium and Flavobacterium-Cytophaga. The largest amounts of folic acid were found in the cultures of Achromobacter and Pseudomonas, and nicotinic acid was secreted most intensely by bacteria of the genus Pseudomonas and the group Flavobacterium-Cytophaga. Pantothenic acid was produced most actively by bacteria of the genera Pseudomonas and Alcaligenes, of the group Flavobacterium, Cytophaga and of the family Enterobacteriaceae. The most active producers of riboflavin in littoral zone were bacteria of the genus Pseudomonas and of the group Aeromonas-Vibrio, and of thiamin those of the genera Achromobacter and Pseudomonas.

Among bacteria isolated from the water of the pelagic zone most strains produced biotin in similar amounts, but the strains isolated in autumn produced somewhat more of it than those isolated in spring. Folic acid was produced most intensely by bacteria of the genus Acinetobacter, of the group Flavobacterium-Cytophaga and of the family Enterobacteriaceae. The most active producers of nicotinic acid proved to be bacteria of the group Flavobacterium-Cytophaga, the family Enterobacteriaceae and of the genus Pseudomonas. The largest

Table 3: Amount of vitamins produced by the planktonic bacteria isolated from the pelagic zone of the lake Jasne (in ug/g d.w.).

Vitamins	14.04.1990				21.10.1990			
	X	Y	Range	Mean	X	Y	Range	Mean
Pantothenic acid	a	6	69.24-3592.08	1114.97	a	5	495.65-2427.27	1098.15
	b	6	400.00-3975.00	1266.64	b	6	865.38-1243.63	1017.55
	c	7	485.52-1640.00	845.36	c	6	51.21-1833.33	1114.44
Nicotinic acid	a	7	39.21-1309.09	422.90	a	5	25.30-376.47	122.73
	b	7	1.54-1650.00	328.72	b	8	26.66-1273.33	353.23
	c	8	52.01-800.00	307.72	c	5	148.81-61.53	32.27
Riboflavin	a	7	38.37-1871.55	402.80	a	8	0.74-124.52	38.85
	b	5	31.32-150.00	66.07	b	8	24.32-215.87	72.55
	c	8	51.02-800.00	307.72	c	5	14.81-61.53	32.27
Folic acid	a	27	18.20-882.40	224.40	a	26	16.90-937.50	189.56
	b	25	6.10-708.30	132.50	b	24	19.60-659.40	149.12
	c	25	11.40-550.00	186.08	c	25	17.10-860.00	289.48
Thiamine	a	4	5.86-214.29	82.67	a	10	179.14-617.90	407.25
	b	3	24.67-211.59	87.74	b	10	38.79-343.75	138.34
	c	4	51.56-227.06	169.29	c	8	12.50-124.04	53.86
Biotin	a	7	0.01-0.32	0.09	a	12	0.02-027	0.13
	b	8	0.01-0.44	0.06	b	18	0.05-0.51	0.14
	c	6	0.01-0.20	0.07	c	6	0.06-0.44	0.23

X: source of sampling (as footnote in Table 1), Y: number of strains studied.

amounts of pantothenic acid were secreted by bacteria of the family Enterobacteriaceae, of the genus Achromobacter and also of the group Flavobacterium-Cytophaga. The most active producers of riboflavin were bacteria of the genus Pseudomonas. Thiamin was produced most intensely by strains of the groups Aeromonas-Vibrio and Flavobacterium-Cytopha-ga and of the genera Acinetobacter and Achromobacter.

DISCUSSION

Many bacterial strains isolated from lakes and seas are able to produce various vitamins of group B (3, 5, 6, 9,15,16, 24, 25).

Our studies have demonstrated that in the surface water of the littoral zone of lake Jasne bacteria producing B-vitamins averaged about 38-49%, and in the water of the pelagic zone about 25-35% of the total microflora. In the eutrophic lake Jeziorak, according to Donderski and Sokol (6), in the littoral zone there were about 50% of such bacteria, and in the pelagic zone of the same lake there were about 40% of them, as reported by Donderski (7). The larger numbers of B-vitamins producers in the littoral zone than in the pelagic zone are in agreement with the data reported by Kurata, Saraceni and Kadota (15), who, while studying over the year the content of thiamin, biotin and vitamin B_{12} in the horizontal plane of the eutrophic lake Biwa, found that besides the seasonal variations in their content there were always larger amounts of those vitamins in the littoral zone than in the pelagic one. This must explicitly suggest a higher number of producers of those vitamins in the littoral zone. Among the planktonic bacteria of the littoral zone (Figure 1) of lake Jasne the predominant strains were those producing nicotinic acid and riboflavin. This ability was exhibited by 87.5% and 70.7% of bacteria respectively. In the pelagic zone (Figure 2) the most numerous group were folic acid producers (up to 91.1%), next producers of biotin (up to 51.4%), nicotinic acid (37.2%) and thiamin (32.6%). In the eutrophic lake Jeziorak, according to Donderski and Sokol (6), the producers of nicotinic acid and riboflavin constituted the least numerous group (on average about 20% of the total number of bacteria) in the littoral zone, while the predominant group consisted of strains synthesizing biotin and folic acid (40% each on the average). In the pelagic zones of lake Jeziorak (7) similarly as in the lake Jasne, the most numerous group was that of folic acid producers. This ability was

Table 4: Quantity of vitamins produced by different bacteria isolated from the littoral and the pelagic zone of the lake Jasne (in ug/g d.w.-mean).

Bacteria	Vitamins produced in the littoral zone												Number of strains	
	Biotin		Folic acid		Nicotinic acid		Pantothen acid		Riboflavin		Thiamine			
	S	A	S	A	S	A	S	A	S	A	S	A	S	A
Achromobacter sp.	0.368	0.323	74	86	216	337	2716	1851	21	115	88	31	56	65
Acinetobacter sp.	-	-	-	29	-	418	-	-	-	-	-	-	-	3
Aeromonas-Vibrio	-	-	-	67	102	-	1831	-	332	95	-	-	3	2
Alcaligenes sp.	-	-	-	-	355	-	3592	-	-	41	-	-	3	1
Arthrobacter-Corynebacterium	1.27	-	58	-	-	-	-	-	-	-	-	-	2	-
Bacillus sp.	-	-	-	-	-	-	988	-	-	-	-	-	1	-
Enterobacteriaceae	-	0.412	83	54	369	390	3077	1787	62	109	-	-	22	42
Flavobacterium-Cytophaga	1.19	0.014	56	-	1055	187	3306	-	53	100	-	6	37	4
Pseudomonas sp.	-	0.156	90	53	1667	154	4342	5567	145	152	40	31	16	74
Vitamins produced in the littoral zone														
Achromobacter sp.	0.081	0.206	160	213	234	91	799	1367	66	43	5	273	99	45
Acinetobacter sp.	-	-	-	399	-	43	-	873	-	58	-	398	-	25
Aeromonas-Vibrio	0.045	0.091	8.3	122	-	-	-	495	41	45	-	490	4	7
Alcaligenes sp.	-	-	-	-	-	-	-	-	-	-	115	179	2	1
Arthrobacter-Corynebacterium	0.101	-	67	-	-	-	-	-	-	-	-	-	2	-
Enterobacteriaceae	0.080	0.151	270	116	484	107	1151	1603	132	43	115	200	70	86
Flavobacterium-Cytophaga	0.013	0.121	213	213	-	661	1282	664	65	131	211	298	13	21
Pseudomonas sp.	0.132	-	166	69	562	-	764	-	615	175	150	124	22	4

S: spring, A: autumn.

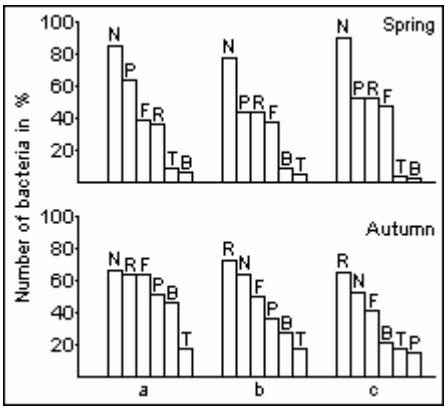
exhibited by about 90% strains in spring and 80% in autumn. Folic acid producers were also the most numerous group in the bottom sediments of both, lake Jeziorak (25) and lake Jasne (5).

The present studies on the quantities of vitamins of B-group have shown that those varied depending on the time and place of sampling and on the genus or group of the bacteria. This corresponds with the results obtained by Donderski and Sokol (6) and Donderski (7) in their studies on the planktonic bacteria of lake Jeziorak and those obtained by Strzelczyk and Donderski (25) and Donderski and Strzelczyk (5) on benthic bacteria of lakes of different trophies.

Our studies have demonstrated that in both zones of the lake Jasne pantothenic acid and nicotinic acid were secreted in largest amounts, and biotin and thiamin in smallest ones. According to Donderski and Sokol (6), and Donderski (7), the planktonic bacteria of the littoral and pelagic zone of lake Jeziorak synthesized nicotinic acid and riboflavin in largest amounts, and biotin, folic acid or pantothenic acid in smallest amounts. The data obtained by Strzelczyk and Donderski (25) for benthic bacteria of the lake Jeziorak indicate that they produced nicotinic acid and folic acid in the largest amounts. On the other hand, the benthic bacteria of lake Jasne, according to Donderski and Strzelczyk (5), secreted the largest amounts of folic acid and riboflavin. The benthic bacteria of both lakes, according to mentioned authors, produced biotin and thiamin in smallest amounts, like in the case of planktonic bacteria (Figure 1 and Figure 2).

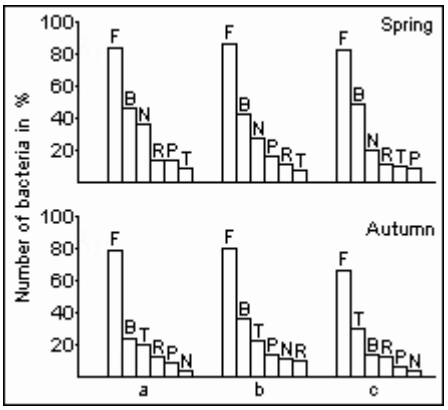
It follows from the analysis of the data that amounts of vitamins secreted by the planktonic bacteria of the mesotrophic lake Jasne were in most cases 2-13 times higher than those produced by the benthic bacteria of that lake (5), whereas, the amounts of vitamins produced by bacteria isolated from those two environments in the eutrophic lake Jeziorak were similar (5, 7, 25).

Figure 1: Vitamins synthesized by the planktonic bacteria isolated from the littoral zone of the lake Jasne.



a: surface water (about 30 cm depth), b: water from the middle layer (about 3.5 m depth), c: water from the near bottom (about 30 cm above the bottom), N: nicotinic acid, P: pantothenic acid, F: folic acid, R: riboflavin, T: thiamine, B: biotin.

Figure 2: Vitamins synthesized by the planktonic bacteria isolated from the pelagic zone of the lake Jasne.



a: surface water (about 30 cm depth), b: water from the middle layer (about 9.0 m depth), c: water from the near bottom (about 30 cm above the bottom), N: nicotinic acid, P: pantothenic acid, F: folic acid, R: riboflavin, T: thiamine, B: biotin.

Considering the fact that the microbiological synthesis of vitamins from the scientific, practical as well as economic point of view seems to be very important for natural environments and human economy, further research is necessary in order to understand better the role of vitamins and their producers.

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Correspondence:
Wojciech DONDERSKI
Department of Water
Microbiology and Biotechnology,
Institute of Biology,
N. Copernicus University,
Gagarina 9, PL 87-100 Torun,
POLAND.