PRODUCTION OF B-VITAMINS BY HETEROTROPHIC PLANKTONIC BACTERIA ISOLATED FROM PELAGIC ZONE OF THE LAKE JEZIORAK

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SUMMARY: It was found that up to 85% of heterotrophic planktonic bacteria of the pelagic zone of the Lake Jeziorak were capable to produce at least one vitamin of B-group. The most numerous were organisms synthesizing folic acid. The least numerous were producers of biotin. Most bacteria produced one vitamin, only a few strains produced four vitamins synchronically.

Key Words : B-vitamins, planktonic bacteria, pelagic zone.

INTRODUCTION

In the first paper of this series reported about production of B-group vitamins by heterotrophic planktonic bacteria of the littoral zone of an eutrophic Lake Jeziorak. Quantitative and qualitative studies revealed different abilities to produce B-vitamins by strains belonging to different genera and groups of bacteria.

It was demonstrated that this capability depended on the time and place of samplings where the producing organism were isolated.

In the above paper data concerning the origin. Production and occurrence of vitamins in water basins, published by various authors, as well as function and ecological role of those compounds in the life of water basins were discussed.

In this paper the results of studies on B-vitamins production by heterotrophic bacteria isolated from pelagic zone of the Lake Jeziorak are presented.

MATERIAL AND METHODS

Bacteria used in the experiments were isolated from the water of the pelagic zone of an eutrophic Lake Jeziorak. The lake is located in the northern part of Poland in the Ilawa Lakeland. Detailed description of this lake is given by Bittel *et al.* (1) and Bohr (2).

Sampling

Water samples for microbiological analyses were taken in the period of homothemy, in spring (May 13) and autumn (October 29) 1988. The water was collected by means of Isatchenko's sampler into sterile glass ampoules, from the surface layer (from a depth of 30 cm below surface), from mid-depth of the standpoint (2.5 m below surface) and from the layer overlying the sediment (30 cm above bottom sediments). The samples were poured together into sterile bottles, which kept in an ice container and were transported to the laboratory. The time between sampling and plating did not exceed 7 hours.

Counting and isolation of bacteria

The numbers of heterotrophic bacteria were determined the spread plates method using an iron-peptone agar medium according to Ferrer, Stapert and Sokolski (7). The water samples were diluted with sterile buffer water (4). After 10 days incubation at 20°C the colonies of bacteria were counted, and about 130 colonies were picked at random and transferred to semisolid iron peptone agar medium (5.0 g agar per liter). The strains, kept at 4°C and transferred every two months to fresh media as above, were used for further studies.

Identification of bacteria

The bacteria were identified according to the scheme proposed by Shewan, Hobbs and Hodgkins (9), Henrie *et al.* (8) and Buchanan and Gibbons (3).

Vitamins assay

The methods used in the experiments were the same as described previously Strzelczyk and Donderski (10).

RESULTS AND DISCUSSION

Many heterotrophic bacteria are capable of producing various vitamins of B-group. As follows from Table 1, in spring, the most numerous bacteria in the pelagic zone of the Lake Jeziorak were those producing folic acid and riboflavin, while in autumn those producing folic acid and

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Table 1: Number of planktonic bacteria synthesizing B-group vitamins in the pelagic zone of the Lake Jeziorak.

Date of sampling	Vitamin produced	Number of bacteria (10 3/ml)
1988.05.13	Biotin	0.25 (13.5)
	Nicotinic acid	0.36 (19.2)
	Pantothenic acid	0.66 (35.1)
	Riboflavin	0.97 (51.3)
	Folic acid	1.44 (75.7)
	Total number of bacteria	1.90
1988.10.29	Biotin	1.04 (25.0)
	Nicotinic acid	1.07 (25.9)
	Pantothenic acid	2.08 (50.0)
	Riboflavin	1.30 (31.8)
	Folic acid	3.53 (85.0)
	Total number of bacteria	4.15

pantothenic acid. According to Donderski and Sokol (5), in the littoral zone of this lake in the same season, the most numerous were bacteria synthesizing biotin and folic acid, while the least numerous were those producing riboflavin and nicotinic acid. According to present research, the producers of biotin constituted the least numerous group in the pelagic zone. As appears from the studies presented by Strzelczyk and Donderski (10) as well as Donderski and Strzelczyk (6), the producers of folic acid constituted the most numerous group also among benthic bacteria in spring and autumn in lake of different trophy, while the least numerous among those organisms in spring were bacteria synthesizing biotin, and in autumn those producing thiamin. The quantitative-qualitative production of B-vitamins by the strains studies was different. The strains of bacteria isolated from the water of the pelagic zone of the Lake Jeziorak in spring and autumn, through not most numerous, produced in largest amounts nicotinic acid and riboflavin. In least amounts in spring were produced biotin and pantothenic acid, and in autumn also biotin and folic acid (Table 2). The amounts of riboflavin and pantothenic acid produced by strains isolated in autumn were about 3 to 5 times higher than those produced in spring. The amounts of remaining vitamins studied in this work, produced by bacteria isolated both in spring and autumn were very similar. These results are in line with the data obtained by Donderski and Sokol (5), who, while studying the capability of vitamins producing by planktonic bacteria isolated from the littoral zone, found the largest amounts of nicotinic acid and riboflavin and the smallest amounts of biotin.

In parentheses number of bacteria synthesizing the vitamins in percent.

According to Donderski and Strzelczyk (6), the bacteria isolated in isolated in spring and in autumn from the bottom sediments of the mesotrophic Lake Jasne produced similar amounts of B-group vitamins, whereas strains originated from the bttom sediments of an eutrophic Lake Jeziorak (10) in spring synthesized the largest amounts of nicotinic and folic acids, and in autumn of riboflavin and nicotinic acids. Also benthic bacteria of those lakes, isolated in spring and autumn, synthesized the smallest amounts of biotin (6, 10).

Table 2: Amount of vitamins synthesized by the planktonic bacteria isolated from the pelagic zone of the Lake Jeziorak.

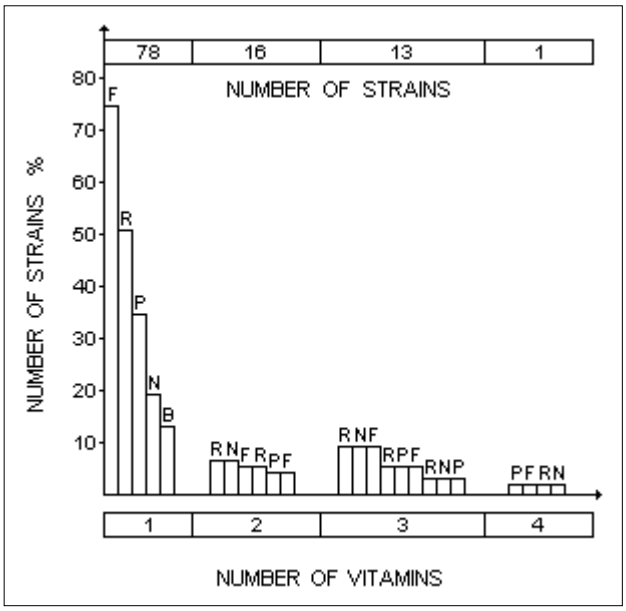
μg/ml						
Vitamins	13.05.1988			29.10.1988		
	Number of strains studied	Range	Mean	Number of strains studied	Range	Mean
Riboflavin	40	0.003-0.325	0.087	27	0.015-0.400	0.186
Nicotinic acid	15	0.090-0.463	0.273	22	0.020-0.475	0.148
Folic acid	28	0.003-0.150	0.048	17	0.008-0.350	0.074
Pantothenic acid	13	0.015-0.105	0.057	10	0.038-0.400	0.215
Biotin*	5	0.018-0.045	0.036	5	0.020-0.060	0.040
μg/g dry wt						
Riboflavin	40	7.04-1378.38	304.51	27	17.04-2767.14	829.52
Nicotinic acid	15	32.23-2577.78	803.61	22	47.62-3400.0	925.68
Folic acid	28	0.92-375.0	61.23	17	1.98-265.63	83.90
Pantothenic acid	13	9.59-131.56	49.67	10	5.58-967.74	256.62
Biotin*	5	11.0-81.0	50.0	5	15.0-145.0	64.00

*:Biotin in ng

Table 3: Quantity of vitamins produced by the planktonic bacteria isolated from the pelogic zone of the Lake Jeziorak (ug/g dry wt. mean).

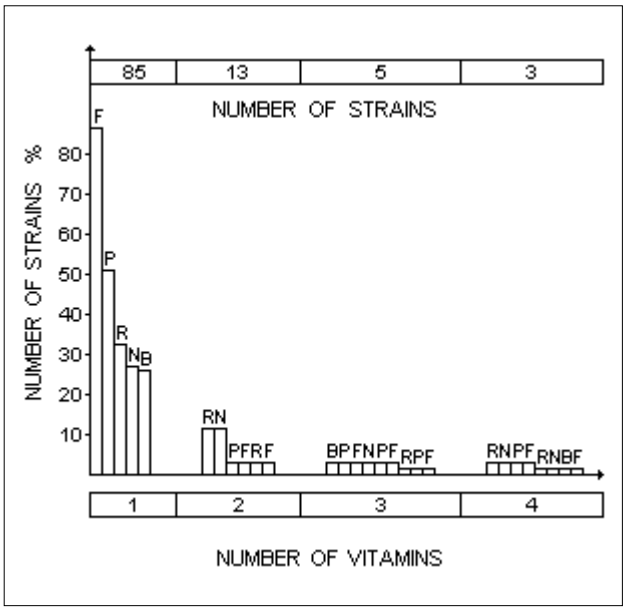
Bacteria	VITAMINS											
	Riboflavin		Nicotenic acid		Biotin*		Pantothenic acid		Falic acid		Number of strains studied	
	S	A	S	A	S	A	S	A	S	A	S	A
Achromobacter sp.	35.77	-	443.60	-	-	-	-	61.59	20.65	10.87	3	1
	(0.018)	-	(0.206)	-	-	-	-	(0.043)	(0.046)	(0.008)		
Aeromonas-Vibrio	440.89	967.51	800.11	1072.45	-	-	63.22	-	8.79	27.11	6	4
	(0.181)	(0.247)	(0.297)	(0.162)	-	-	(0.068)	-	(0.023)	(0.068)		
Alcaligenes sp.	-	462.75	-	-	-	-	-	-	-	-	-	1
	-	(0.118)	-	-	-	-	-	-	-	-		
Arthrobacter-Corynebacterium	-	586.16	-	-	-	0.061	-	-	-	256.63	-	1
	-	(0.233)	-	-	-	(0.00005)	-	-	-	(0.128)		
Bacillus sp.	231.36	-	-	-	-	-	48.32	-	25.21	-	1	-
	(0.113)	-	-	-	-	-	(0.058)	-	(0.030)	-		
Enterobacteriaceae	246.28	1092.14	1299.54	1264.77	55.00	0.145	45.31	485.17	56.67	83.00	30	14
	(0.044)	(0.175)	(0.277)	(0.202)	(0.031)	(0.00004	(0.022)	(0.230)	(0.040)	(0.093)		
Flavobacterium-Cytophaga	438.51	499.00	1247.31	669.05	0.028	-	-	138.82	41.66	81.63	6	17
	(0.105)	(0.184)	(0.290)	(0.131)	(0.00005)	-	-	(0.326)	(0.041)	(0.062)		
Micrococcus sp.	-	2709.68	-	791.37	-	-	-	6.42	-	1.96	-	2
	-	(0.210)	-	(0.117)	-	-	-	(0.130)	-	(0.040)		
Pseudomonas sp.	583.33	-	776.93	-	-	0.015	-	-	-	25.0	4	1
	(0.184)	-	(0.248)	-	-	(0.00002)	-	-	-	(0.078)		
Unidentified	173.30	1006.0	494.59	722.63	-	0.039	-	111.11	139.28	98.27	5	7
	(0.102)	(0.183)	(0.304)	(0.092)	-	(0.00004)	-	(0.080)	(0.085)	(0.055)		

Figure 1: Vitamins synthesized by the planktonic bacteria isolated in spring from the pelagic zone of the Lake Jeziorak.



F: folic acid, R: riboflavin, P: pantothenic acid, N: nicotinic acid, B: biotin.

Figure 2: Vitamins synthesized by the planktonic bacteria isolated in autumn from the pelagic zone of the Lake Jeziorak.



F: folic acid, R: riboflavin, P: pantothenic acid, N: nicotinic acid, B: biotin.

The data presented in Table 3 show clearly that the capability to produce B-vitamins was different in different bacterial strains. Similarly as in the earlier studies not all vitamin investigated in the present work were secreted by bacteria belonging to determined genera or groups. Bacteria of the genera Alcaligenes, Bacillus and of the group Arthrobacter-Corynebacterium produced the fewest number of different vitamins. The remaining strains, referred to definite genera or groups were capable of synthesizing the most vitamins studied in this work. The largest number of different B-vitamins were produced by bacteria of the family Enterobacteriaceae, of the group Flavobacterium-Cytophaga and those belonging to unidentified group.

The most active producers of riboflavin were bacteria of the group Aeromonas-Vibrio, of the family Enterobacteriaceae and strains which proved impossible to be fully identified. Nicotinic acid was secreted most intensively by bacteria of the family Enterobacteriaceae and of the groups Flavobacterium-Cytophaga and Aeromonas-Vibrio. Strains of the family Enterobacteriaceae also the most active producers of biotin. Most of the remaining bacteria did not synthesize biotin at all or only very small amounts. Pantothenic acid was produced most actively by bacteria of the family Enterobacteriaceae are, and folic acid by the same bacteria as well as by the groups Flavobacterium-Cytophaga and unidentified bacteria. These results are similar to those obtained by Strzelczyk and Donderski (10), Donderski and Strzelczyk (6), and Donderski and Sokol (5). Both in the littoral zone and the pelagic zone bacteria of the groups Flavobacterium-Cytophaga and Aeromonas-Vibrio were particularly numerous and active in the production of B-vitamins.

The dominant group among planktonic bacteria of the pelagic zone as well as in the littoral zone (5) and in the bottom sediments (10) organisms capable of synthesizing only one vitamin. From Figures 1 and 2 one can see that in spring it was folic acid and riboflavin, and in autumn folic acid and pantothenic acid.

Among strains producing two vitamins simultaneously, the most numerous group were organisms synthesizing riboflavin and nicotinic acid, and among the producers of three vitamins, strains secreting riboflavin, nicotinic acid and folic acid. No planktonic bacteria of the pelagic zone of the lake Jeziorak were capable to synthesize all five vitamins synchronically. The microbiological synthesis of vitamins seems to be an important link in the vitamin economy of water basins. It is essential to get more information on vitamin producing organisms, the amount of vitamins produced and the factors affecting their synthesis. Therefore studies should be continued in order to get full understanding of the role of vitamins and their producers in the ecology of water basins.

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