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

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SUMMARY: Different genera and groups of heterotrophic planktonic bacteria of the littoral zone of the lake Jeziorak produced different B-group vitamins. Most numerous among the planktonic bacteria of this zone of the lake were biotin producers and least numerous were organisms synthesizing riboflavin and nicotinic acid. Most bacteria produced one or two vitamins. Three or more vitamins were produced by only a few strains. In autumn no strain produced all five vitamins studied in this work.

Key Words: B-group vitamins, planktonic bacteria, littoral.

INTRODUCTION

The vitamins occurring in water basins originate from microbiological biodegradation of plant and animal remains, water plant secretions, sewage and rainwater falling into the basins (18, 21). Also many bacterial strains isolated from lakes and sea water are capable of producing vitamins (3, 8,10,13, 23).

Vitamins of the B group are the precursors or components of many enzymes, which indicates their important role in the cellular metabolism of the producers, consumers and destroyers, including bacteria themselves. The ecological role of vitamins as compounds affecting the development and succession of algae in water basins has been reported by a number of researches (6,11,12, 20). According to Hagedorn (9), bacteria, not algae, are the main producers of vitamins in water basins. Ohwada and Taga (16) claim that bottom sediments are the main vitamin source for water.

The present investigations were undertaken to get a better knowledge very scarce so far of quantitative-qualitative production of the B group vitamins by bacteria of water basins including planktonic ones, which would contribute to a better understanding of the role and importance of these vitamins in the life of water basins.

MATERIALS AND METHODS

Bacteria used in the experiments were isolated from the water of the littoral zone of the eutrophic lake Jeziorak situated in the lawa Lakeland. Detailed data about the lake have been given by Bittel *et al.* (1).

Sampling

Water samples were collected in spring (May, 13) and autumn (October, 29) 1988 from a depth of 30 cm below surface by means of Isatchenko's sample into sterile glass ampules, which stored in an ice container were transported to the laboratory. The time between sampling and analyzing did not exceeded seven hours.

Counting and isolation of bacteria

The numbers of heterotrophic bacteria were determined by the spread plates method. Water samples, diluted with sterile buffer water (4), were plated on iron-peptone agar medium according to ferrer, Stapert and Sokolski (7). 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-pepton agar medium (5.0 g agar per litre). The strains, kept at 4°C and transferred to fresh media every 2 months, were used for further studies.

Identification of bacteria

The bacteria were identified according to the scheme proposed by Shewan, Hobbs and Hodgkins (22), and Buchanan and Gibbons (2).

Vitamins assay

The methods used in the experiments were the same as described previously Strzelczyk and Donderski (24).

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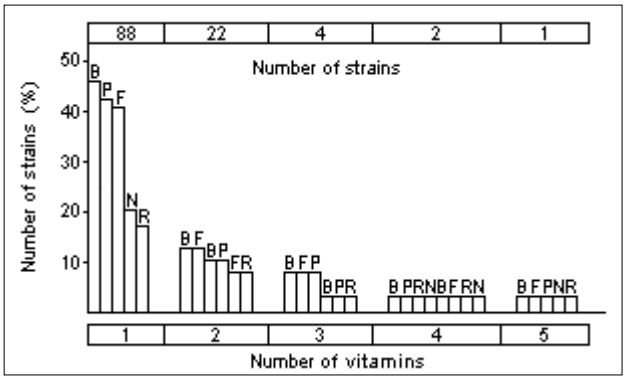
RESULTS

The results of our studies are presented in Tables 1–3 and Figures 1 and 2. As follows from Table 1, the most numerous bacteria in the water of the littoral were those producing biotin and folic acid. The least numerous were those producing riboflavin and nicotinic acid. They were more numerous in autumn than in spring.

The quantitative-qualitative vitamins production by the bacteria under study showed variations. The strains isolated from the water of the littoral zone both in spring and in autumn produced riboflavin and nicotinic acid in the largest amounts, and biotin and folic acid in the smallest amounts (Table 2). The bacteria isolated in autumn produced about 3 times as much nicotinic acid and about twice as much biotin as those isolated in spring. The amounts of other vitamins produced by bacteria isolated is spring and in autumn were similar.

Figure 1: Vitamins synthesized by tho planktonic bacteria isolated in spring from the littoral zone of the lake Jeziorak.



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

It appears from Table 3 that different strains show different capabilities for synthesizing vitamins. Bacteria of the genus Micrococcus produced the fewest of different vitamins. The remaining bacterial strains referred to definite genus or group were capable of synthesizing all the vitamins under study (one or several simultaneously).

The most active biotin producers were bacteria which have not been fully identified and of the family Enterobacteriaceae. The largest amounts of riboflavin were found in cultures of Achromobacter and Alcaligenes. Nicotinic acid was secreted most intensively by bacteria of the unidentified group, by Flavobacterium-Cytophaga and representatives of the genera Alcaligenes and Achromobacter. Folic acid was produced most actively by stains of the group Aeromonas-Vibrio and unidentified, while pantothenic acid by bacteria belonging to the general Alcaligenes, micrococcus and of the family Enterobacteriaceae.

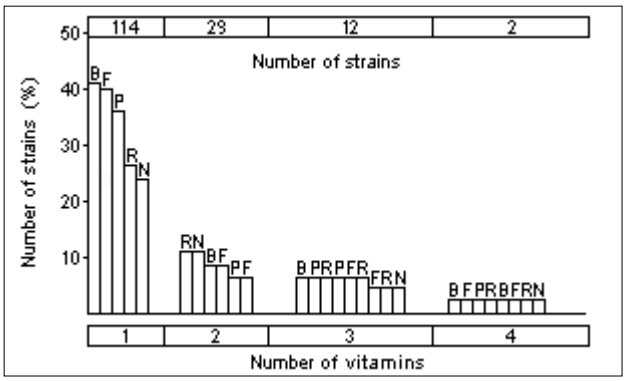
Figures 1 and 2 show the number of vitamins produced by the strains used for study. It can be seen that most bacteria produced one vitamin. In spring this was biotin and pantothenic acid, and in autumn also biotin and folic acid. Two or more vitamins were synthesized by much fewer strains, more being produced by bacteria iso-

Table 1: Numbers of planktonic bacteria synthesizing B group vitamins in the littoral zone of the lake Jeziorak.

Date of sampling	Vitamin produced	Number of Bacteria (10^3/ml)
1988.05.13	Riboflavin	1.14 (17.0)
	Nicotinic acid	1.37 (20.4)
	Folic acid	2.74 (40.9)
	Pantothenic acid	2.82 (42.1)
	Biotin	3.05 (45.5)
	Total number of bacteria	6.70
1988.10.29	Riboflavin	3.42 (26.7)
	Nicotinic acid	3.08 (24.1)
	Folic acid	5.15 (40.2)
	Pantothenic acid	4.69 (36.6)
	Biotin	5.26 (41.1)
	Total number of bacteria	12.80

In parentheses number of bacteria synthesizing the vitamins in percent.

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



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

lated in spring than by those isolated in autumn. None of the bacterial strains isolated in autumn was found to synthesize all five vitamins synchronically.

DISCUSSION

Many authors have been interested in the occurrence of the B group vitamins in water basins, their seasonal, vertical and horizontal distribution (12,14-16, 20). According to Provasoli (19), Hagedon (11), Nishijima and Hata (13), some vitamins, and especially cobalamin, thiamin and biotin separately or in various combinations are necessary to algae affecting significantly their development and succession in the water basins. Hagedorn (11) claims that it is the bacteria and not the algae that are the main vitamins producer in water basins. It appears from our studies that about 50% of the heterotrophic planktonic bacteria of the littoral zone of lake Jeziorak were capable of producing different vitamins of group B. The most numerous group among them were biotin producing strains. According to Strzelczyk and Donderski (24), about 70% of the benthic bacteria of the same lake were capable of synthesizing group B vitamins, and the most numerous group among them being folic acid and pantothenic acid producing organism.

As follows from our studies as well as those of Strzelczyk and Donderski (24), biotin was produced in smallest amounts by bacteria from both habitats in contrast to riboflavin and nicotinic acid, which were produced in largest amounts. On the whole, the planktonic bacteria of the littoral zone of the lake Jeziorak produced larger amounts of vitamins, except pantothenic acid, than the benthic bacteria of that lake (24). As concerns biotin synthesis, Nishijima and Hata (13,15) found a converse phenomenon.

It also follows from the above data that the quantity and quality of vitamins secreted were different genera and groups of bacteria and depended of the time and place of sampling. This corresponds with the data obtained by Strzelczyk and Donderski (24) and Donderski and Strzelczyk (5).

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

μg/ml						
Vitamins	13.05.1986			29.10.1986		
	Number of strains studied	Range	Mean	Number of strains studied	Range	Mean
Riboflavin	15	0.007–0.328	0.128	31	0.005–0.310	0.065
Nicotinic acid	18	0.010–0.370	0.134	28	0.020–0.500	0.204
Folic acid	24	0.058–0.063	0.106	25	0.045–0.263	0.113
Pantothenic acid	24	0.020–0.152	0.072	22	0.040–0.300	0.111
Biotin*	23	0.040–0.400	0.156	23	0.028–0.400	0.193
μg/g dry wt						
Riboflavin	15	14.28–923.94	402.16	31	26.23–1275.00	313.59
Nicotinic acid	18	52.26–903.23	367.91	28	35.09–4542.86	910.35
Folic acid	24	4.84–294.12	29.26	25	4.98–155.56	32.63
Pantothenic acid	24	3.81–130.43	34.17	22	4.47–192.31	47.00
Biotin*	23	2.00–219.00	34.00	23	2.00–214.00	63.00

*: Biotin in ng

Table 3: Quantity of vitamins produced by the planktonic bacteria isolated from the littoral zone of the lake Jeziorak (μg/g dry wt-mean).

Bacteria	Vitamins										Number of strains studies	
	Riboflavin		Nicotinic acid		Folic acid		Pantothenic acid		Biotin*			
	S	A	S	A	S	A	S	A	S	A		
Achromobacter sp.	9.33	378	-	994	-	18	-	11	-	2	1	16
	(0.053)	(0.043)	-	(0.149)	-	(0.135)	-	(0.040)	-	(0.400)		
Aeromanas-Vibrio	501	269	348	909	13	72	8	40	21	57	10	21
	(0.238)	(0.076)	(0.113)	(0.296)	(0.170)	(0.114)	(0.092)	(0.109)	(0.207)	(0.173)		
Alcaligenes sp.	-	443	-	1142	-	46	-	117	-	53	0	21
	-	(0.073)	-	(0.183)	-	(0.092)	-	(0.140)	-	(0.182)		
Enterobacteriaceae	342	250	399	511	33	13	43	47	43	95	58	28
	(0.097)	(0.041)	(0.093)	(0.170)	(0.097)	(0.188)	(0.071)	(0.120)	(0.143)	(0.156)		
Flavobacterium-Cytophaga	181	128	346	1304	10	8	9	9	8	-	17	5
	(0.067)	(0.103)	(0.176)	(0.365)	(0.095)	(0.089)	(0.058)	(0.100)	(0.160)	-		
Micrococcus sp.	-	286	-	-	-	18	-	50	-	-	0	3
	-	(0.140)	-	-	-	(0.053)	-	(0.150)	-	-		
Pseudomonas sp.	-	269	-	746	-	21	-	34	-	25	0	14
	-	(0.188)	-	(0.271)	-	(0.114)	-	(0.072)	-	(0.078)		
Unidentified	-	93	360	2314	-	105	5	48	-	97	2	6
	-	(0.025)	(0.262)	(0.295)	-	(0.110)	(0.040)	(0.130)	-	(0.400)		

*: Biotin in ng, S: spring, A: autumn values in parentheses in μg/ml of media.

Since microbiological synthesis of vitamins may be important from the scientific as well as the practical and economic point of view, further studies are necessary for the better understanding of the role of vitamins and their producers in the ecology of water basins and other environments too.

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REFERENCES

1. Bittel L, Grimn S, Ochocki S : The horizontal distribution of zooplankton in the southern pat of lake Jeziorak. Zesz Nauk UMK Torun, Papers from Limnol Stn in Ikawa, 1:3, 1965.

2. Buchanan RE, Gibbons NE : Bergery's Manual of Determinative Bacteriology. Williams and Wilkins Comp, Baltimore, 1974.

3. Burkholder PR, Burkholder LN : Vitamin B_{12} in suspended solids and marsh muds collected along the coast of Georgia. Limnol Oceannogr 1 (3):202,1958.

4. Daubner I : Mikrobiologia Vodl Slov Akad Vied, Bratislava, 1967.

5. Donderski W, Strezelczyk E : Studies on B vitamins production by heterotrophic benthic bacteria of lakes of different trophy. II. Mesotrophic lake Jasne. Acta Microbiol Polon 4:357, 1987.

6. Droop MR : Organic micronutrients. In : Biochemistry and Physiology of Algae. Ed Lewin RA, Academic Press, New York, p 131, 1962.

7. Ferrer EB, Stopert EM, Sokolski WT : A medium for improved recovery of bacteria from water. Can J Microbiol 9:420, 1963.

8. Gillespite PA, Morita RY : Vitamin B_{12} production and depletion in a naturally occurring eutrophic lake. Appl Microbiol 23:341,1972.

9. Hagedorn H : Untersuchungen uber das Vorkommen und die Entstehung des Thiamins in zwei Ostholsteinischen Seen. Arch Hydrobiol, 3:283, 1969.

10. Hagedorn H : Die vertikale Verteilung von Thiamin, Bakterien und Phytoplankton in drei Ostholsteinischen Seen. Ber Deutsch Bot Ges, 42:223, 1969.

11. Hagedorn H : Die okologische Bedeutung des Thiamins.

Ber Deutsch Bot Ges, 84:479, 1971.

12. Kurata A, Saraceni C, Kadota H : The status of B group vitamins in macrophyte and pelagic zones of Lake Biwa. Mem Ist ital Idrobiol, 37:63, 1979.

13. Nishijima T, Hata Y : Distribution of thiamine, biotin and vitamin B_{12} in lake Kojima. II Distribution in the bottom sediments. Bull Japan Soc Sci Fish, 44:815, 1978.

14. Nishijima T, Hata Y : Seasonal variation in the concentration of dissolved vitamin B_{12}, thiamine and biotin in Susaki Harbor and Nomi Inlet. Rep USA Mar Biol Inst, 1:1, 1979.

15. Nishijima T, Hata Y : Distribution of vitamin B_{12}, thiamine and biotin in the water of Harima-Nada Sea. Bull Japan Soc Sci Fish, 52:1533, 1986.

16. Ohwada K, Otsuhata M, Taga N : Seasonal cycles of vitamin B_{12} thiamin and biotin in the surface of lake Tsukui. Bull Japan Soc Sci Fish, 37:817, 1972.

17. Ohwada K, Taga N : Vitamin B_{12}, thiamin and biotin in lake Sagami. Limnol Oceanogr, 17:315, 1972.

18. Parker BC, Wachtel MA : Seasonal distribution of cobalamin, biotin, and niacin in rainwater. Virg Polytech Inst and State University, p 301, 1970.

19. Provasali I : Organic regulation of phytoplankton fertility. In: The Sea Ed Hill MN, Inters Publishers Inc New York, p 165, 1963.

20. Rheinheimer G : Mikrobiologia wod. PWRIL Warszawa, 1987.

21. Schmidt EL : Soil microorganisms and plant growth substances. Soil Sci, 2:129, 1951.

22. Shewan JM, Hobbs G, Hodgkins W : A determinative scheme for the identification of certain genera of Gram-negative bacteria with special reference to the Pseudomonadaceae. J Appl Bacteriol, 23:379, 1960.

23. Starr TJ, Jones ME, Martinez C : The production of vitamin B_{12} active substances by marine bacteria. Limnol Oceanogr, 2:114, 1957.

24. Strzelczyk E, Donderski W : Studies on B vitamins production by heterotrophic benthic bacteria of lakes of different trophy. I Eutrophic lake Jeziorak. Acta Microbiol Polon, 4:345, 1987.

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