The production of Vitamin B_{12} like substances by Bacteria from alimentary canal of Mediterranean fishes

by

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Much attention is presently given to the part played by bacteria in the productivity of water basins as initial producers of biologically active compounds, such as vitamin B_{12}-like substances. This attention has been brought about by the investigations which disclosed the significance of vitamin B_{12}-like substances as a limiting and controlling factor for the development of phytoplankton, the main link of the trophic chain. The evidence on the role played by bacteria in the production and supplement of different trophic levels of the system water - plankton - fishes with vitamin B_{12}-like substances is extremely poor. To cite a single examp'e, from the paper by STARR a. oth. [1957, 1959] it is known that microflora of alimentary canal of grey mullet (6 of 8 strains of bacteria) produces vitamin B_{12} in amounts of 0.4 up to 5.0 ng/ml.

Unfortunately, in literature available we have found no evidence for vitamin-synthesizing activity of the microflora of alimentary canal in other fishes.

We have studied the productivity of heterotrophic bacteria isolated from the alimentary canal of six Black-Sea fishes with different nutrition and way of life : *Scorpaena porcus*, *Trachurus mediterraneus ponticus*, *Mugil auratus*, *Platichthys flesus luscus*, *Mullus barbatus ponticus* and *Spicara smaris*.

Identification of the bacterial cultures was carried out by N.A. KRASILNIKOV'S determiner [1949]. The biosynthesis of vitamin B_{12} was studied by culturing the bacteria on solid nutrient medium which besides nitrogen and carbon sources also comprised main vitamins of vital importance belonging to group B [BENZHITSKY, GUTVEIB, LEBEDEVA, 1970]. Two variants of nutrient medium were used :

1. medium based on seawater deficient in vitamin B_{12} (exposed to UV irradiation, source type ПРК-4, 6 hours exposition;

2. non-irradiated seawater with natural, previously measured concentration of vitamin B_{12} (0.008ng/ml).

The content of vitamin B_{12}-like substances was determined by highly sensitive microbiological method — test-tube procedure (test organism *Escherichia coli*, strain 113 - 3) in reference to 1 g of bacteria, net weight [KUTSEVA, 1961].

As a result, 68 bacterial cultures were isolated; 28 of them (40 p. 100) exhibited vitamin-synthesizing activity. They were placed into 7 genera *(Bacterium, Pseudobacterium, Pseudomonas, Vibrio, Bacillus, Micrococcus, Sarcina)* and 16 species. Most strains (67.9 p. 100) produced insignificant amounts (up to 10 ng/g) of vitamin B_{12}; 21.4 p. 100 strains synthesized the vitamin at the rate of up to 30 ng/g, and 10.7 p. 100 strains exhibited maximum activity (124.6 to 317.3 ng/g). Of the two most active species *(Bacterium halophilum* and *Bacillus oligonitrophillus)* the former belongs to bacteria abundant in seas of Mediterranean type.

As a whole, vitamin-synthesizing bacteria are most common among non-spore-bearing bacteria (50 p. 100 the number of active cultures), the second place occupy cocci (32.2 p. 100) and then follow spore-bearing rods (17.8 p. 100).

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The majority of strains (17 cultures, 9 species) showed lower activity on the irradiated medium, deficient in vitamin B_{12}, than on the non-irradiated one.

It should be noted, that the amount of vitamin B_{12}, produced by bacteria, considerably exceeds its content in a medium. Obviously, extraction of this vitamin out of nutrient medium and its accumulation by the cells of bacteria for the present instance had no decisive importance, and the amount of the vitamin found in the cells could be considered as the result of biosynthesis.

At the same time, nine strains (7 species) were found to have higher vitamin-synthesizing ability on the irradiated medium. Some of these (*Bac. cereus*, *Bacterium halophilum*) exhibited 10 times as high activity as compared to the medium based on non-irradiated seawater. It is likely that when a medium is exposed to irradiation for most efficient freeing from vitamin B_{12}, inactivation of inhibitors affecting productive activity of some cultures occur.

Bacteria able to produce vitamin B_{12}-like substances were found in the alimentary canal of Black-Sea fishes investigated. Some of them were found only in a single species (e.g. Bac. *cereus* was found only in *Platichthys flesus luscus*, *Bac. virgatus* only in *Scorpaena porcus*, etc.), the other were isolated from the alimentary canal of two or three species (e.g. *Bac. oligonitrophilus* was found in *Platichthys flesus luscus*, *Scorpaena porcus* and grey mullet; *Sar. flava* was found in *Mullus barbatus ponticus*, *Spicara smaris* and grey mullet).

It was found that strains *Sar. flava* isolated from alimentary canal of the three fishes sometimes differed sharply (up to 35 times) in vitamin-synthesizing ability. On the other hand, there were cases when strains (*Bact. halophilum* and *Vibr. nigricans*) isolated from the same species (*Scorpaena porcus* and *Trachurus mediterraneus ponticus* - respectively) also differed (by up to 23 times) in vitamin B_{12} activity.

It is worth noting that the most quantity of bacteria was isolated from the alimentary canal of bottom fishes *Scorpaena porcus* and *Platichthys flesus luscus* (31 strains), 13 of which (46.4 p. 100 of active ones) were vitamin-producing. The most active producers of the vitamin also were found among these cultures. So, *Bac. halophilum* from *S. porcus* produced the most amount of vitamin B_{12}-like substances on both media.

In chromatographic analysis of vitamin B_{12}-like substances produced by the bacteria cultured on the two variants of media on bioautogram in addition to true vitamin B_{12} (cyanocobalamin) there were also found two or three its analogues. Comparison of the location of the spots produced by vitamin B_{12}-like substances of bacterial origin with those produced by test compounds enables one to suppose these analogues to be pseudovitamin B_{12} (adenine-cobalamin) and also factor III (oxybenzimidazol-cobalamin) and factor A (2-methyladenine-cobalamin).

So, as a result of the present investigations it was found that Black-Sea fishes of different nutrition patterns possess useful internal microflora able to synthesize vitamin B_{12}-like substances.

This microflora may apparently provide one of the sources of such compounds of vital necessity as vitamin B_{12} and its natural analogues.

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