
CERTAIN VITAMIN REQUIREMENTS OF BACTERIA

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The existence of a large number of vitamins is now known. Some of these substances were recognized as important in the nutrition of microorganisms before their value in human nutrition was established. It has been known for some time that the addition of liver extract, tomato juice, asparagus juice and similar materials caused an increased growth of microorganisms in culture media. Since these solutions were of indefinite composition the substances causing this increased growth were not definitely known. During the past few years the chemistry of the vitamins has advanced to the point where the chemical structure of many of them is known and they can be obtained for study in bacterial nutrition in pure form. So important are some of these vitamin substances in the nutrition of various strains of bacteria that they fail to grow in a medium that is satisfactory in all other respects.

The work reported in this paper concerns a study of the metabolite (vitamin) requirements of a thermophilic anaerobe,

Clostridium thermosaccharolyticum. This organism is important in the spoilage of non-acid canned foods. The problem was suggested when it was observed that this organism failed to grow consistently in a new improved anaerobic medium introduced by Brewer (1940).

A medium suggested by Snell and Wright (1941) was chosen. It consists of vitamin-free hydrolyzed casein, cystine, tryptophane, sodium acetate, glucose, adenine, guanine and uracil and a mixture of inorganic salts. In this basal medium the test organism failed to give any appreciable growth or acid production. To this basal medium was added the various vitamins to be tested. Anaerobic conditions were maintained by the addition of reduced iron. The substances tested were water soluble vitamins of the B Complex. In all, eight substances were tested, namely:—thiamin (B₁), Riboflavin (B₂), pyridoxin (B₆) biotin, para amino benzoic acid, nicotinic acid, pantothenic acid and folic acid.

The stock culture was stored in liver infusion medium. Transfers were made to the test medium to which had been added all eight of the substances to be tested. Growth appeared as satisfactory in this test medium as in the liver infusion. The inoculum for tests was prepared by growing the organism through three or more transfers in the hydrolyzed casein media, centrifuging, washing the cells with physiological salt solution, again centrifuging and re-suspending these cells in 99 ml. of physiological salt solution. Two drops of this suspension per tube of medium was used as the inoculum. Since the organism produces an appreciable amount of acid, the effect of each vitamin was measured by titrating the acidity produced.

Two methods were used for testing the vitamin requirements of this organism. In the first series tubes were set up so that the first tube contained all eight vitamins, the second contained only seven and so on until the last tube in the series was basal medium only. A second series was run starting with the basal media and adding first one, then two and so forth until all had been added. Using these two techniques it was found that when thiamin, biotin or para amino benzoic acid were omitted from the medium growth and acid production was only slightly better than in the basal medium. In further work it was found that the addition of each of these substances singly to the base medium did not satisfy the requirements of the organism. Combinations of two gave slightly better results, but for maximum acid production all three were required.

After it has been established that these three substances were required, a medium was prepared containing these essential vitamins. Using this as a basal medium the other five substances were added singly and in combination. Riboflavin, pyridoxin and folic acid had no effect on acid production. Nicotinic acid and pantothenic acid alone and when added together gave slight stimulation. From these results it appears that thiamin, biotin and para amino benzoic acid are essential, whereas nicotinic acid and pantothenic acid produce some stimula-

tion but growth and acid production occurs in their absence.

Since the organism responds to thiamin, tests were run to determine its requirements for this vitamin. The basal medium was prepared containing all the necessary substances except thiamin. Thiamin was then added to separate tubes in amounts ranging from .01 micrograms per 10 ml. of medium. Results based on acidity produced indicated maximum acid production when one microgram of thiamin per 10 ml. of medium was used. Acid production decreased as the amount of thiamin was decreased. Addition of amounts greater than 1 microgram per 10 ml. of medium did not increase acid production. No toxicity resulted when as high as 100 micrograms was added.

The thiamin molecule is made up of a pyrimidine and a thiazole structure. Certain organisms can use one or the other of these structures to replace thiamin in their nutrition. Other organisms can combine these two compounds when both are present in the medium and thus produce thiamin. Some organisms require the entire molecule. Tests with *Clostridium thermosaccharolyticum* on these two fractions of thiamin indicated that the pyrimidine structure cannot be used to replace thiamin. The thiazole fraction is about one-tenth as effective in promoting acid production by this organism. When both substances were added together no greater benefit was obtained than when thiazole alone was added.

In conclusion, it may be stated that *Clostridium thermosaccharolyticum* when grown in a hydrolyzed casein glucose medium requires thiamin, biotin, and para amino benzoic acid. Nicotinic acid and pantothenic acid gave some stimulation but are not absolutely essential. The pyrimidine fraction of thiamin is not utilized by this organism whereas the thiazole fraction gives about one-tenth the value of thiamin.

REFERENCES

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