THE RESPONSE OF A THERMOPHILIC FOOD SPOILAGE ORGANISM TO THIAMIN

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The study of bacterial nutrition has advanced greatly in the past few years. It has been known for some time that, when organisms were grown in a simplified medium containing only inorganic salts and glucose, the addition of vegetable extracts, yeast extract, or liver extract greatly stimulated growth. With the isolation and identification of the vitamins, it was found that these definitely defined substances could be added and would replace the extracts formerly used. Thus it was discovered that some microorganisms require vitamins in addition to the inorganic salts, nitrogen containing compounds, and carbohydrates required by all bacteria.

The organism used for this study was a food spoilage organism known as *Clostridium thermosaccharolyticum*. It is important in commercial canning because of its ability to produce resistant spores and cause acid and gas production in non-acid foods. A study of the organism in various media indicated a correlation between growth and acid production. Throughout this work, acid production as determined by titration was used as a measure of the response of this organism to the substance tested.

It has been shown in previous work (1) that this organism needed thiamin, biotin, and para amino benzoic acid when grown in a medium containing hydrolyzed casein as the nitrogen base. Nicotinic acid and pantothenic acid were needed to maintain maximum growth. The omission of thiamin from a medium prepared as described later and designated as the basal medium resulted in a failure of the organism to grow and produce acid. When one microgram of thiamin was added per ten ml. of medium, maximum growth was obtained. The work reported in this paper represents an attempt to determine the least amount of thiamin that would support maximum growth, and to determine the curve of response to varying amounts of thiamin. A basal medium consisting of vitamin-free hydrolyzed casein, dextrose, sodium acetate, adenine, guanine, uracil, inorganic salts and the vitamins: biotin, p amino benzoic acid, nicotinic acid and pantothenic acid was prepared. Thiamin was omitted from this basal medium, since it was the test substance.

In order to determine the response of the organism, tubes of basal medium were prepared, thiamin added in the following amounts: 0, 0.02, 0.04, 0.08, 0.12, 0.24, 0.40, 0.80 and 1.0 micrograms per ten ml. of medium. After inoculation, the tubes were incubated at 55°C. for 72 hours and the acid produced was titrated with N/10 alkali. The results obtained are reported in table 1.

From the figures (Table 1) a curve can be drawn which will represent the response of the test organism to varying amounts of thiamin. Maximum response as measured by acid production was obtained between the values of 0.4 and 0.8 micrograms

TABLE 1.—RESPONSE OF A THERMOPHILIC FOOD SPOILAGE ORGANISM TO VARYING AMOUNTS OF THIAMIN

Medium	Micrograms of thiamin added	Ml of N/10 NaOH required to neutralize		Average
Basal only	0	1.0	1.0	1.0
Basal inoculated	0	1.4	1.4	1.4
Basal inoculated	0.02	2.4	2.6	2.5
Basal inoculated	0.04	3.5	3.4	3.45
Basal inoculated	0.08	4.2	4.2	4.2
Basal inoculated	0.12	5.7	6.0	5.85
Basal inoculated	0.24	7.5	7.5	7.5
Basal inoculated	0.40	8.9	8.8	8.85
Basal inoculated	0.80	8.9	9.2	9.05
Basal inoculated	1.00	9.0	9.2	.1

of thiamin per 10 ml. of medium. Further additions up to 1.0 micrograms gave no greater response than 0.8 micrograms.

There is a regular increase in response in acid production to the amount of thiamin added between 0.02 and 0.4 micrograms per 10 ml. of medium. Between these values we have almost a straight line curve. Organisms responding in this way to vitamins are often used as a means of assaying extracts of food to determine the content of that vitamin to which they respond.

As this organism gave a good curve of response to thiamin, the possibility of using it as a means of determining the thiamin content in an extract of food or other material was tested. Two feed samples were selected for this work. A standard curve was run to obtain the response to amounts of thiamin between 0.02 and 0.4 micrograms. At the same time extracts of the feed were run in the basal medium, all requirements being supplied except the thiamin. When the results were calculated on the gram basis, Sample 1 contained 5.0 to 5.2 micrograms of thiamin and Sample 2 gave 1.5 micrograms per gram. Chemical determinations using the thiochrome method gave the following results: Sample 1—3.7 micrograms, Sample 2—0.7 micrograms per gram. Thus, the microbiological method gave much higher results than the chemical method, in Sample 2 more than twice as much.

Further tests were conducted in an attempt to determine the reason for the difference in results between the two methods. Previous work (1) had shown this organism responded to the thiazole portion of thiamin when it was added to a medium containing no thiamin. The organism did not respond to the pyrimidine fraction tested. This pyrimidine had an ether linkage in the fifth position of the pyrimidine ring. Schultz, Atkin and Frey (2) have shown, that for yeasts, thiamin treated with sulfite loses 98 percent of its activity for these microorganisms. By this treatment thiamin is broken down into a thiazole fraction and a sulfonic acid pyrimidine. It was assumed that if the feed samples could be treated with sulfite and the activity destroyed, other stimulating substances in the feed could be determined and thus make it possible to include them in the basal medium and thus obtain closer checks between the chemical and microbiological method of determining thiamin. On the basis of

this assumption, extracts of the feed were treated with sulfite according to the method of Schultz, Atkin and Frey. Varying quantities of the treated extract were added to the basal medium. The results obtained after this treatment were identical with those previously reported for the untreated extracts. This seemed to indicate that the organism was able to resynthesize thiamin from the two fractions produced by sulfite cleavage and use it. To test the correctness of this conclusion thiamin itself was treated with sulfite and added to the basal medium in varying amounts. The organism responded to this sulfite treated thiamin in the same way as to the untreated thiamin. Thus it appears that sulfite treatment does not destroy thiamin activity for this thermophilic spoilage organism as it does for yeast. It is possible that pyrimidine structures similar to the above and other types may be encountered in extracts of feeds. If certain thiazole fractions are present the organism will then react as though thiamin were present, since it has the ability to synthesize thiamin from these fractions. The thiochrome method is more or less specific for thiamin and thus would not measure these fractions. This may be one of the reasons why higher results were obtained with this microbiological method than with thiochrome method. There are probably other factors which affect this determination but they were not investigated.

CONCLUSIONS

The food spoilage organism *Clostridium thermosaccharolyticum* responds quantitatively to thiamin in the range of 0.02 to 0.4 micrograms. From this response a standard assay curve can be drawn.

The results of assays of two feeds gave results higher than those obtained by the thiochrome method.

Sulfite treatment of thiamin and of extracts of feeds does not destroy the activity of thiamin for this organism.

REFERENCES

1. Clark, F. M. and Mitchell, Winifred R. 1944. Studies on the Nutritional requirements of *Clostridium thermosaccharolyticum*. Arch. of Biochem. Vol. 3, p. 459-466.

2. Schultz, A. S., Atkin, Lawrence and Frey, C. N. 1942. Determination of Vitamin B_1 by yeast fermentation method. Industrial and Eng. Chem. Vol. 14, p. 35-39.