VITAMIN REQUIREMENTS OF TORULOPSIS GLABRATA, A MARINE YEAST

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ABSTRACT

The feasibility of employing a marine yeast, Torulopsis glabrata for assaying micro-quantities $(5-40 \text{ m}\mu g.)$ of thiamine has been suggested.

It has been reported¹⁻⁴ that certain select strains of yeasts such as Saccharomyces cerevisiæ, Torula cremoris and Klæckera brevis could be successfully employed for the microbiological assay of the vitamins of the B-complex. In a previous communication,⁵ the growth response of a marine yeast, Cryptococcus laurentii, to graded doses of thiamine was reported although this strain, it was pointed out, could not be used for the assay of this vitamin.

In the present report, another marine yeast, isolated and identified by Bhat and Kachwalla as Torulopsis glabrata⁶ was studied for its vitamin requirements and was found to demand thiamine as an essential growth factor. The basal medium employed was a modified Burkholder's⁷ medium, the modification being with respect to 3% extra pure sodium chloride incorporated into the medium to simulate the marine environs. Several vitamins, namely, thiamine, pyridoxine, calcium pantothenate, inositol, nicotinic acid and biotin were omitted singly from the medium otherwise complete and the growth requirements of this organism was tested. After incubation of the culture flasks for 24 hours at 24-26° C., it was noticed that excepting those combinations which lacked thiamine and pyridoxine, all the other culture flasks showed appreciable growth response. The culture flasks lacking pyridoxine showed only very poor response indicating thereby that it acted as growth-promoting factor. The culture lacking in thiamine, on the other hand, showed not even the slightest growth, although all the other B-complex vitamins were present in the medium. Experiments carried out repeatedly for a period of over five months on the above lines showed consistently that the organism's requirement for thiamine was absolute.

In the next series of experiments, a series of culture flasks were prepared by addition of graded dosages of thiamine ranging from 10-200 mµg./ml. The inoculum in this and all subsequent sets of experiments was prepared from a 24hour enrichment agar slant. A loopful was inoculated into 10 ml. of basal medium containing 1 mµg. of thiamine. After 18 hours of growth, the yeast was centrifuged, washed three times in saline and the wet yeast was weighed and resuspended in measured quantity of saline so as to contain 0.1 mg./ml. One ml. of this suspension was used as inoculum. Growth measurements were read in triplicate 259 at the end of 18-20 hours of incubation on a Klett Summerson colorimeter using 42 filter.

Figure 1 shows the general growth response of this organism to graded dosages of thiamine. Further studies were conducted at even a lower range, namely,



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FIG. 1

5-40 mµg./ml. and at all times the response was found to be consistent at this level and hence assays were undertaken by incorporating into the basal medium from 5-40 mµg. of thiamine at graded levels. Fig. 2 shows the standard curve which is fairly linear thus indicating the possibility of using this organism for the estimation of microquantities of thiamine.

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