

**NUTRITIONAL REQUIREMENTS OF A  
SACCHAROMYCES Sp.? ISOLATED  
FROM COCONUT TODDY**

A SAMPLE of coconut toddy obtained from Ceylon was found to contain an unusually large-sized yeast. Attempts to isolate this organism on wort-agar proved unsuccessful. It, however, grew on a synthetic medium fortified with thiamin, riboflavin, pyridoxin, pantothen, niacin, biotin, inositol and *p*-amino-benzoic acid.

It was, therefore, of interest to determine the essential vitamin and other nutritive requirements. The big size of the cell ( $15\mu \times 5\mu$ ) facilitated cytochemical investigation of the syndromes of avitaminosis.

Basal media with no vitamins were compounded with solutions of salts, citrate buffer (pH 4.6), ammonium sulphate (0.4 gm./100 ml) and glucose (5 gm./100 ml.). The all-vitamin media, those lacking a given vitamin or an essential amino-acid were compounded, 5 ml.

TABLE I  
Yeast-Growth on Media With and Without Essential Nutrients

Media composition	Percentage of absorption	Average size of cell in microns	Remarks
Basal medium (no vitamins)	16	9.5 × 3	Vacuolated cells
B. M. with all Vitamins & amino acids	55	15.0 × 5	Granulated cells
Lacking thiamin	50	12.5 × 3	Thin cells
Lacking riboflavin	53	15.0 × 5	
Lacking pyridoxine	53	12.5 × 5	Highly vacuolated
Lacking calcium pantothenate	41	12.5 × 5	Cytoplasm thin
Lacking nicotinic acid	16	12.5 × 5	Few granules
Lacking <i>p</i> -amino-benzoic acid	53	10.0 × 3	Granulated cells
Lacking biotin	37	12.5 × 5	Refractive granules
Lacking inositol	24	12.5 × 5	Less of cytoplasm
Lacking choline	50	12.5 × 5	
Lacking <i>L</i> -aspartic acid	53	10.0 × 5	Large vacuoles
Lacking <i>L</i> -tryptophane	52	12.0 × 5	Thin cells
Lacking <i>L</i> -cystine	50	12.0 × 5	
Lacking <i>DL</i> -methionine	53	12.0 × 5	
With Lily liver extract	62	12.5 × 5	Granulated cells
With inositol, niacin, biotin and calcium pantothenate	53	12.0 × 5	Granulated cells

of each sterilised and distributed into sterile test-tubes (22 mm. × 150 mm.), and inoculated with 0.5 ml. of a uniform yeast suspension of a 24-hour culture containing 0.4 mgm. of wet yeast per ml. The tubes were placed at a slant and incubated at 28° C. for 24 hours.

Turbidities representing growths were photoelectrically measured and expressed as the percentages of absorption (see Table I).

The results show that:—

(1) Nicotinic acid and inositol are the essential vitamins for the growth of the organism; biotin and pantothen are stimulatory. Lack of these vitamins result in vacuolation or in a poor differentiation of cells.

(2) Other vitamins exert little effect on growth. This is supported by the fact that the growth-rate on a basal medium fortified with only niacin, inositol, biotin and pantothen is as good as that on the all-vitamin medium.

(3) The microscopic appearance of the two cultures are similar showing that the four vitamins satisfy all the normal requirements of the organism.

(4) Liver extract which has given a significantly higher growth and induced a distinctive microscopic picture, appears to contain a growth factor or factors essential to the organism—vitamin or amino acid—other than those investigated.

(5) The adaptability of this yeast as a test organism for the microbiological assay of niacin and inositol is indicated.

Our sincere thanks are due to Sir J. C. Ghosh for his kind interest.

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#### MICROBIOLOGICAL ASSAY OF NIACIN WITH A SACCHAROMYCES Sp.? ISOLATED FROM COCONUT TODDY

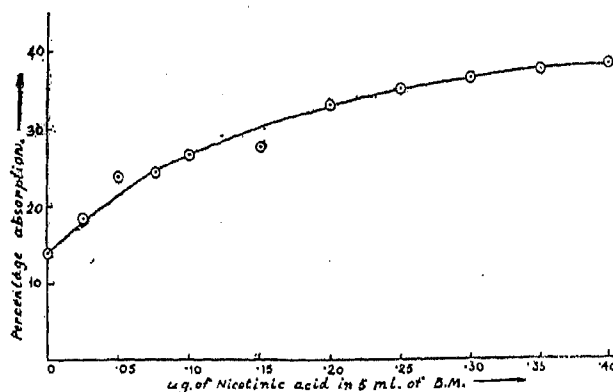
THE vitamin requirements of this organism have been determined and the indispensability of niacin and inositol for its growth established.<sup>1</sup> It was of interest to examine the adaptability of the organism for the assay of niacin, and determine the range of concentration which could be estimated.

Basal media containing all vitamins but niacin were compounded. 100 ml. of the medium contained:—Glucose 5 gms., ammonium sulphate 0.4 gm., *l*-aspartic acid 10 mg., *l*-tryptophane 1.2 mg., *l*-cystine 4 mg., *dl*-methionine 4 mg., thiamin 80 μg, riboflavin 80 μg, pyridoxine 80 μg, pantothen 80 μg, *p*-amino-benzoic acid 80 μg, biotin 100 m μg inositol 200 μg, solution of salts 12.5 ml. and citrate buffer (pH 4.6) 10.0 ml.

Aliquots of the medium (2 ml.) were distributed into sterile tubes (22 mm. × 150 mm.), graded amounts of niacin added and the volume made up to 4.5 ml. with sterile water. The tubes were inoculated with a washed and uniform suspension of the organism (previously grown on an all-vitamin medium for 24 hours)

and incubated for 24 hours at 28° C. Growths of the organism were photoelectrically measured and the results expressed as percentages of absorption (see Table I and Fig. 1).

Medium with μg Niacin	0.0	0.025	0.05	0.075	0.1	0.15
Per cent. absorption	14.5	18.5	24.0	24.5	26.0	27.0
Medium with μg Niacin	0.20	0.25	0.3	0.35	0.4	0.5
Per cent. absorption	32.5	34.5	36.0	37.0	37.5	38.5



It is concluded that (1) the organism is adaptable for the assay of niacin and (2) the assay-range lies between 0 and 0.04 μg per ml., and this method appears to represent a more sensitive method of assay than others so far known.

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<sup>1</sup> Ramachandra Rao, Mistry and Sreenivasaya, *Curr. Sci.*, 1947, 16, 145.

#### PHYTOPHTHORA PALMIVORA BUTLER ON CYPHOMANDRA BETACEA SENDT. AND CARICA PAPAYA LINN.

Two isolates of *Phytophthora* were obtained from two independent sources, one from the stem of tree tomato (*Cyphomandra betacea* Sendt.) with a patch canker from the Fruit Station at Burliar at the foot of the Nilgiris, the other from a rotten hollow stem of a papaw tree in Coimbatore.

Both these isolates did not produce any oospores in pure culture even after three months although sporangia and chlamydo-spores were produced in abundance in old cultures. Therefore, they were grown in paired cultures with known plus and minus strains of *P. palmivora* available in the Government My-