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Studies on the Nutrition of *Candida*

I. Vitamin Requirements of Strain of *Candida*

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SUMMARY

Using 10 vitamins, 17 amino acids and 5 purine pyrimidine bases and Glucose-Simmons' as the basal medium, the nutritional requirements of 65 strains in 11 species of *Candida* were studied. Although no strain tested required any amino acids or purine pyrimidine bases, most of strains were found to be quite exacting for certain kinds of vitamins. Thus, of all strains of 8 species eleven required particular vitamins. While *Candida krusei*, *Candida utilis* and *Candida parapsilosis* (*nov. spec.* 65) did not require vitamins for growth, *Candida albicans*, *C. tropicalis*, *C. guilliermondii*, *C. pulcherrima* and *C. variabilis* were definitely exacting for biotin, *Candida rugosa*, for both biotin and thiamine, *Candida pseudotropicalis*, for biotin, pantothenic acid and niacin and *Candida parakrusei*, for biotin, thiamine, pyridoxine and niacin. In the presence of indispensable vitamins, three vitamins, thiamine, pyridoxine and pantothenic acid, had an additive effect, enhancing the growth of strains of *Candida albicans*, *C. tropicalis* and *C. guilliermondii* respectively. Replacement of biotin by pimelic acid was observed only in a strain of *Candida parakrusei*. The significance of vitamin requirements of *Candida* is discussed in relation to the possibility of identifying strains of *Candida* by this method.

INTRODUCTION

It is known that most strains of *Candida* will not grow in a glucose-ammonia-inorganic salts medium unless it is supplemented by a minute amount of yeast extract, indicating that these strains are exacting for some growth factors (vitamins) present in the extract. However, there are only a few papers on the nutrition of *Candida* and there is little knowledge of vitamin requirements.

In 1953, Fujino *et al* in our laboratory described a method for identifying strains of *Candida* and suggested that further analysis of the vitamin requirements would favour the identification of this organism.

In the present paper are described the results of studies on the vitamin requirements of 65 strains in 11 species of *Candida* and is discussed significance of the results in the identification of the organism.

Materials and Methods

Organisms. Strains used in this study were as follows: *Candida albicans*: 40 strains (RIMD*), *C. tropicalis*: 9 strains (RIMD), *C. krusei*: 2 strains (NI*, IFO*), *C. parakrusei*: 4 strains (RIMD), *C. rugosa*: 1 strain (RIMD), *C. guilliermondii*: 3 strains (IFO, NI, MTU*), *C. pulcherrima*: 2 strains (NI, IFO), *C. parapsilosis*: 1

*Abbreviation

RIMD: Research Institute for Microbial Diseases, Osaka University

NI: Nagao Institute

IFO: Institute for Fermentation, Osaka

MTU: Kyoto University Medical School

strain (RIMD), *C. variabilis*: 1 strain (NI), *C. utilis*: 1 strain (RIMD), and *C. pseudotropicalis*: 1 strain (IFO).

Media. The basal medium employed in the study was mainly Glucose-Simmons' (GS) medium with a formula as follows: NaCl, 5g; MgSO₄·7H₂O, 0.4 g; K₂HPO₄, 1.0 g; (NH₄)₂HPO₄, 1.0 g; glucose, 5.0 g; aq. dist., 1,000 ml and pH 6.8. GS medium supplemented with yeast extract (YG medium) was sometimes used.

Vitamins used. The following vitamins were tested for their effect on the growth of these organisms: biotin 10 mμg/ml, thiamine 100 mμg/ml, pantothenic acid 100 mμg/ml, niacin 500 mμg/ml, pyridoxine 100 mμg/ml, riboflavin 100 mμg/ml, vitamin B₁₂ 10 mμg/ml, *p*-amino benzoic acid 100 mμg/ml, folic acid 100 mμg/ml and inositol 500 mμg/ml., in GS medium. The yeast extract used in this study was the product of the Daigo Eiyokagaku Ltd. Co..

Sugars used. The following sugars were tested: glucose, levulose, mannose, galactose, maltose, sucrose and lactose. The concentration of these sugars was 20 mg/ml in medium.

Cultural method. A small amount 24 hour culture of strains of *Candida* grown on Sabouraud glucose agar was inoculated into GS medium containing vitamins and was incubated at 25°C and 37°C for 48 hours. The 48 hours cultures thus obtained were used as a inoculum for test media. As small amount of the culture which had grown completely after 48 hour incubation was again transferred to a fresh test medium and, finally, the strain with full growth even at the end of the third transfer was regarded as the one which is exacting for the vitamins contained in the medium.

RESULTS AND DISCUSSION

Effect of yeast extract on the growth of strains of Candida in GS medium.

Before determining the specific vitamins required by strains of *Candida*, the effect of an yeast extract on the growth of these strains were observed to eliminate strains not requiring vitamins. The results are shown in Table 1. Three species

Table 1. Effect of yeast extract on the growth of strains of *Candida* in GS medium

Species	GS medium	YG medium	
	37°C—25°C	37°C	25°C
<i>C. albicans</i>	—	+	+
<i>C. tropicalis</i>	—	+	+
<i>C. krusei</i>	+	+	+
<i>C. parakrusei</i>	—	+	+
<i>C. rugosa</i>	—	+	+
<i>C. guilliermondii</i>	—	+	+
<i>C. pulcherrima</i>	—	—	+
<i>C. variabilis</i>	—	—	+
<i>C. utilis</i>	+	+	+
<i>C. pseudotropicalis</i>	—	+	+
<i>C. parapsilosis</i> n. sp. 65	+	+	+

Note: + : growth — : no growth

of *C. krusei*, *C. utilis* and *C. parapsilosis* grew well in GS medium without the addition of yeast extract, indicating that they did not require any specific growth factor. No other strains tested grew in GS medium unless the medium was supplemented by yeast extract. Strains of two species (*C. pulcherrima* and *C. variabilis*) appeared to be temperature sensitive, for they grew well at 25°C but not at 37°C. No temperature sensitivity was observed with other strains whose growth at 25°C was little slower than that at 37°C.

Vitamin requirements of strains of Candida.

The results described in the preceding section clearly show that 8 out of 11 species of *Candida* tested cannot grow in GS medium without a minute amount of yeast extract suggesting that these strains are exacting for specific growth factors contained in the extract. Subsequent experiments with the vitamin mixture proved that this was so. Thus, when GS medium was supplemented by the vitamin mixture, good growth was obtained with these particular strains in the basal medium. Similar results were not obtained when vitamins are replaced by the mixture of amino acids or purine pyrimidine bases.

To identify the particular vitamins required by these strains, a variety of vitamin were added to the test media which was inoculated with 24 hour culture of these strains on Sabouraud glucose agar and then the strains were incubated at 37°C for 48 hours. The cultures were subcultured three times in the same media and at the end of the third transfer, the requirement for the vitamin by each strains was determined by measuring growth. Table 2 shows the results of this

Table 2. Vitamin requirements of strains of *Candida*

Species	No. of strains	Glucose-Simmons' medium				
		Biotin	Pyridoxine	Pantothenic	Niacin	Thiamine
<i>C. albicans</i>	40	+	—	—	—	⊕
<i>C. tropicalis</i>	9	+	⊕	⊕	—	—
<i>C. krusei</i>	2	—	—	—	—	—
<i>C. parakrusei</i>	4	+	+	—	+	+
<i>C. rugosa</i>	1	+	—	—	—	+
<i>C. guilliermondii</i>	3	+	⊕	—	—	—
<i>C. pulcherrima</i>	2	+	—	—	—	—
<i>C. variabilis</i>	1	+	⊕	—	—	—
<i>C. utilis</i>	1	—	—	—	—	—
<i>C. pseudotropicalis</i>	1	+	—	+	+	—
<i>C. parapsilosis</i> n. sp. 65	1	—	—	—	—	—

Note : + : Growth

— : No growth

⊕ : Growth enhanced

experiment. It can be seen that a strain of *C. rugosa* is exacting for both biotin and thiamine, a strain of *C. pseudotropicalis*, for biotin, pantothenic acid and niacin, and a strain of *C. parakrusei*, for biotin, pantothenic acid, niacin and thiamine, while the other strains of the four species only requires biotin for

growth. In addition, it was demonstrated that the growth of some strains of *Candida* was found to be enhanced, even in the presence of indispensable vitamins, by certain vitamins for which those particular strains were not exacting. Thus, the growth of *C. albicans* is enhanced by adding thiamine to a medium containing biotin, *C. tropicalis*, by pyridoxine and pantothenic acid, *C. guilliermondii* and *C. variabilis*, by pyridoxine. Replacement of biotin for pimelic acid, a precursor of biotin, was effective for growth of four strains of *C. parakrusei*, but not for others.

Identification of strains of Candida by a combination of vitamin requirement analysis and sugar utilization.

The results described in the preceding sections demonstrate that, with three exceptions, all strains of 8 species of *Candida* require vitamins for growth and that the requirement appears to be specific for the species but not for the strain. Since the species of *Candida* can be divided into five groups according to their vitamin requirements it may be that analysis of vitamin requirements may have a considerable significance as a subsidiary method for the identification of strains of *Candida*. Sugar utilization has long been used as one of the most important subsidiary methods for identification of strains of *Candida* and, by using this test, one can easily divide the species of the organism into four different groups. Since groups of the species of *Candida* divided by vitamin requirement analysis differ from those divided by the sugar utilization test, a combination of these two analysis must provide a more precise method for identifying these organisms.

The results illustrated in Table 3 were obtained by determining the sugar

Table 3. Sugar utilization tests of *Cadida*, with regard vitamin requirements

Species	No. of strains	Vitamin * added to media	Simmons media						
			Glucose	Levulose	Mannose	Galactose	Maltose	Sucrose	Lactose
<i>C. albicans</i>	40	Biotin	+	+	+	+	+	+	-
<i>C. tropicalis</i>	9	Biotin	+	+	+	+	+	+	-
<i>C. krusei</i>	2	none	+	+	+	-	-	-	-
<i>C. parakrusei</i>	4	Biotin, Niacin, Thiamine, Pyridoxine	+	+	+	-	-	-	-
<i>C. rugosa</i>	1	Biotin, Thiamine	+	+	+	+	+	-	-
<i>C. guilliermondii</i>	3	Biotin	+	+	+	+	+	+	-
<i>C. pulcherrima</i>	2	Biotin	+	+	+	+	+	+	-
<i>C. variabilis</i>	1	Biotin	+	+	+	+	+	+	-
<i>C. utilis</i>	1	none	+	+	+	+	+	+	-
<i>C. pseudo-tropicalis</i>	1	Biotin, Niacin, Pantothenic acid	+	+	+	+	-	+	+
<i>C. parapsilosis</i> n. sp. 65.	1	none	+	+	+	+	+	+	-

Note: + : Growth

- : No growth

* : Vitamins for which the organisms are exacting

utilization of strains of *Candida* in the medium containing particular vitamins for which these strains are exacting. The resulting spectrum of sugar utilization of 11 species of this organisms was in good agreement with that described by Lodder and van Rij (1952). These workers used a basal medium containing yeast extract or 8 mixture of vitamins.

Although the eleven species of *Candida* tested were divided into only four groups by the sugar utilization test alone, they were divided into 6 groups by analysis of their vitamin requirements. Therefore the analysis of the vitamin requirements is, to some extent, useful as a subsidiary method for the identification of these organisms. Analysis of vitamin requirements is important not only for identification. A precise knowledge of the vitamin requirements for each strain is necessary before preparation of a chemically defined medium suitable for the sugar utilization test, as the criterion of the test depends on determination of the growth of the organism resulting from the utilization of test sugars.

In 1952, Lodder and van Rij described a sugar utilization test performed in the chemically defined medium containing a mixture of the following vitamins; biotin, pantothenate, inositol, niacin, *p*-amino benzoic acid, pyridoxine, thiamine and riboflavin. They obviously did not realize that the vitamin requirements of strains of *Candida* differ considerably. Therefore, any strain which requires vitamins other than these 8 will not grow in their medium and so the sugar utilization test will be misleading. For instance, *C. japonica* and *C. mycoderma* var. *tannica* could not grow in their defined media containing various sugar and 8 vitamins and, as a result, would apparently not use any of the sugars tested. Actually, however, these strains can use particular sugars as carbon sources in the presence of the specific growth factors which they require.

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