CARBOHYDRATE METABOLISM OF *DEBARYOMYCES GUILLIERMONDII*

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ABSTRACT. — Metabolism in strain Y-1448 of *Debaryomyces guilliermondii* was studied by incorporating glucose-1-C^{14} in the medium. C_1 of glucose underwent oxidation to CO_2 at a more rapid rate at the initial period of metabolism than in the later phase. The results suggest that an initial rapid utilization by the pentose phosphate pathway which decreases in rate with the time while another one, presumably the Embden-Meyerhof pathway, continues to operate; that lactose was utilized by the organism; and that the organism can oxidize galactose.

Debaryomyces guilliermondii is a membrane-forming yeast, the taxonomy of which has been studied only in recent years (Lodder and Kreger van Rij, 1952). A few reports are available on certain aspects of the physiology and on the functional requirements of the organism (Stelling-Dekker, 1931; Wickerham and Burton, 1948; Wickerham and Burton, 1954; Etchells and Bell, 1950; and Merdinger and Shair, 1962). According to Giovannozzi, 1941, and Mrak and Bonar, 1939, *D. guilliermondii* is not able to utilize lactose, starch, or dextrin, but the same authors are in disagreement as to its ability to utilize galactose.

Because of the limited knowledge in this area, the present study of the intermediary metabolism of *D. guilliermondii* was undertaken. As previous studies from this laboratory had shown that the strain N R R L Y-1448 of *D. guilliermondii* utilized lactose with the evolution of CO_2, the intent of the present investigation was to determine the amount of CO_2 released when lactose was used as a source of carbon and the amount of $C^{14}O_2$ given off when glucose-1-C^{14} was used as a substrate over intervals of 3 and 5 days.

MATERIALS AND METHODS

Malt extract, yeast extract, lactose, glucose, galactose, soluble starch and dextrin were Difco preparations. Glucose-1-C^{14} was obtained from the Nuclear Instrument Corporation and Volk Radiochemical Co.

The basal culture medium contained 0.3% malt extract, 0.3% yeast extract, 2% NaCl, nutrient salt mixture recommended by Wickerham, 1946, and either 5% glucose, 5% lactose, 5% galactose, 1.5% soluble starch, 2% dextrin or lactose broth with additional 4.5% lactose without Wickerham's nutrient salt mixture. A loopful of a 48 hr culture of *D. guilliermondii* strain NRRL Y-1448 was introduced into a 50 ml Erlenmeyer flask containing 20 ml of sterile glucose medium. The phosphate salts were sterilized separately and added to the sterilized medium. After 48 hr of incubation at 30°C the medium was transferred into a 500 ml flask containing 230 ml of sterile glucose medium. A total of 2 ml of

solution containing approximately 4.75 μ of glucose-1-C^{14} was then added and the contents shaken on a reciprocating shaker or by a magnetic stirrer. The flask was then attached to a series of 3 interconnected flasks on one side and 4 on the other. The first two flasks each contained 150 ml of 10% NaOH, the third, 300 ml of saturated $Ba(OH)_2$, the fourth, substrate and the organism; the fifth, 1000 ml of saturated $Ba(OH)_2$, and the remaining three, 500 ml each of the last solution. The temperature of the medium was 31°C. Air was drawn through the train of the 8 flasks at a slow constant rate.

Several 1 ml samples of the substrate containing the glucose-1-C^{14} were pipetted and emptied into either stainless steel planchets or suitable glass vials before and at the end of 3 and 5 day experimental periods for measurement of radioactivity. $BaCO_3$ was recovered from the last four flasks of the train and washed with water and ethanol and then dried at 200°C for 2 hr. Radioactivity was ascertained in the planchets with a Gas Proportional Counter. A Packard Tri-Carb liquid scintillation spectrometer was employed with the samples in the glass vials.

Similar procedures were applied to the substrate containing the basal medium and 5% lactose and for the broth to which 4.5% lactose was added but without Wickerham's salt mixture. Galactose utilization was tested with cells which had been transferred three times in a medium containing only galactose. Each experiment was accompanied by controls which were identical except for the absence of the organism. In the experiments with labeled glucose, the corresponding controls were devoid of any tagged glucose.

Results and Discussion

The $BaCO_3$ obtained from the experiment with glucose-1-C^{14} amounted to 1.91 gm after 3 days and 3.1 gm after 5 days as shown in Table 1, whereas the controls yielded 1.87 gm and 3.06 gm of $BaCO_3$ for the respective intervals. The rate at which $C^{14}O_2$ was produced in 3 days was more rapid than the one in the 5 day run. The decrease in the rate of $C^{14}O_2$ production is also reflected by the values given as total percent of $C^{14}O_2$ collected.

An increasing amount of evidence is being presented relative to the fact that certain organisms catabolize glucose at first much faster through the hexosemonophosphate shunt by converting glucose-C_1 into CO_2 rath-

Table 1.—Utilization of Glucose-1-C^{14} by *Debaryomyces quilliermondii*. In each experiment 4.75 μ of Glucose-1-C^{14} was added to the incubation flask.

Days of incubation	Grams of $BaCO_3$ collected	Specific activity of $BaCO_3$ counts/g	Total activity recovered in microcuries	Average glucose-1-C^{14} per day in microcuries	Percent of glucose-1-C^{14} recovered
3	1.91	0.231	0.438	0.146	9.2
5	3.10	0.165	0.512	0.102	10.8

er than through other pathways. In the later phases, however, the Embden-Meyerhof scheme prevails (Koshland and Westheimer, 1950; Blumenthal et al., 1954; Horecker et al., 1954; Claridge and Werkman, 1954; and Clark and Wallace, 1958). The present findings obtained with glucose-1-C^{14} indicate that the hexosemonophosphate scheme is a pathway of glucose breakdown in *D. guilliermondii*. That the Embden-Meyerhof scheme also occurs is supported by the findings of Merdinger and Shair, 1962, who isolated pyruvate as well as ethanol and acetate.

The amount of $BaCO_3$ obtained from 5% lactose with the nutrient salt mixture and lactose broth without the nutrient salts over a period of 5 days was 3.2 gm and 2.1 gm, respectively. The higher rate of metabolic activity in the former medium was probably due to the presence of the nutrient salts. Since the organism produced nearly the same amount of CO_2 with lactose as with glucose during the same interval of time, it can be inferred that *D. guilliermondii* can utilize both carbohydrates equally well and that it possesses the enzyme system for the hydrolysis of lactose.

In corroboration of the finding of Giovannozzi, 1941, qualitative tests, carried out with 5% galactose in the basal medium, point to the ability of the organism to metabolize this carbohydrate. In those experiments employing starch or dextrin as the source of carbohydrate, the organism failed to grow, which confirms previous reports.

Summary

Experiments in which glucose-1-C^{14} was administered to a strain of *Debaryomyces guilliermondii* showed that it oxidizes C_1 as C_1O_2 at first much faster than other carbon atoms of glucose. The organism appears to utilize lactose at the same rate as it does glucose since the amounts of $BaCO_3$ obtained with either sugar, collected after 5 days, are practically the same. The organism grows well with galactose as a single source of carbon.

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