

METHANOL TOLERANCE OF THREE STRAINS OF *SACCHAROMYCES CEREVISIAE* HANSEN¹

JAMES R. TROYER²

Department of Botany and Plant Pathology, The Ohio State University, Columbus 10

That the ethanol produced in the fermentation of glucose by yeast eventually reaches a concentration at which it prevents further conversion of substrate is a well-known fact. In a systematic study of a number of yeasts Gray (1941) has shown that this inhibitory concentration, while varying widely among different yeasts, is characteristic enough for a given species or strain that definite "alcohol (ethanol) tolerance" values may be assigned. An empirical relationship between these alcohol tolerance values and the amounts of reserve materials formed, especially fats, has been described (Gray, 1948).

Results of a preliminary attempt to acquire information concerning the mechanism of this ethanol inhibition indicated that the alcohol may exert its effect on glucose utilization in a culture by limiting the size of the active cell population (Troyer, 1953). Since limitation of multiplication is a rather general effect, it is important to know whether the phenomenon of alcohol tolerance hinges around specific properties of ethanol alone or whether related substances may not produce similar effects. In the present work this question has been investigated by ascertaining the effects of added methanol upon the utilization of glucose by three yeasts.

EXPERIMENTAL

The three strains of *Saccharomyces cerevisiae* Hansen employed in this study were Nos. 1 (D. C. L. strain), 28 (Rasse M strain), and 31 (Brown-Forman strain) of the culture collection of the Ohio State University Department of Botany and Plant Pathology. The ethanol tolerances of these yeasts have been studied (Gray, 1941). A liquid glucose-phosphate-yeast extract medium (Gray, 1946) was used in 125-ml. Erlenmeyer flasks, each of which contained 50 ml. of liquid. Procedural details in the preparation, sterilization, and inoculation of the cultures were similar to those previously described (Troyer, 1953), with the sole exception that methanol instead of ethanol was added in varying amounts to the vessels. Cultures were maintained without shaking at 30° C for 24 hours. Initial and final glucose concentrations were estimated by the method of Stiles, Peterson, and Fred (1926). The initial cell count in each case was 1×10^6 per ml., while the initial glucose concentration was 10.10–11.12 mg. per ml.

RESULTS AND DISCUSSION

The results obtained in this study are summarized in figure 1. Increasing the concentration of methanol in the culture medium up to a certain point had little effect on the utilization of glucose by the three yeasts. Above this critical level, which appeared to be characteristic for each yeast, a further increase in methanol concentration was accompanied by decreased glucose utilization. In general the sigmoid curves of figure 1 resemble the response curves usually obtained in studies of this kind. In this respect these results are similar to those reported for the inhibition of glucose utilization in these yeasts by ethanol (Gray, 1941). It may be supposed that for each yeast there is a critical methanol concentration

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²Present address: Department of Biology, University of Alabama, University, Alabama.

above which the utilization of glucose is inhibited—in other words, that each yeast possesses a certain methanol tolerance. By analogy with the concept of Gray (1941) this tolerance value might be defined as the maximum concentration of methanol at which the percentage of glucose utilized is no more than one percent below the percentage of glucose utilized in the absence of methanol. Other definitions would, of course, be possible.

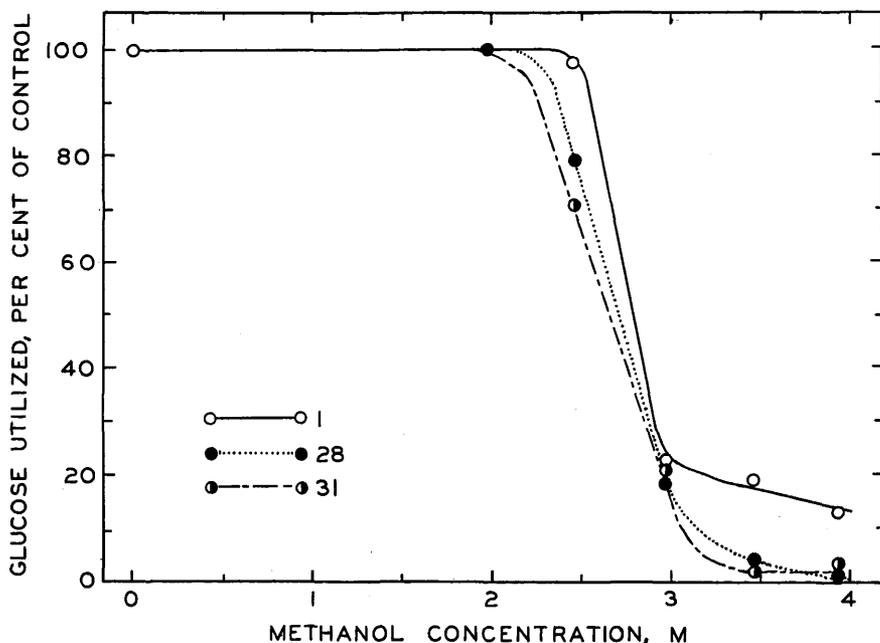


FIGURE 1. The effect of added methanol on glucose utilization by three strains of *Saccharomyces cerevisiae* Hansen. Methanol concentration is expressed in terms of molarity of the final solution in the culture flasks. The amount of glucose utilized in each instance is expressed as a percentage of that utilized in the control cultures to which no methanol was added. Each point represents the mean value for duplicate cultures.

As might be expected, yeasts appear to vary in methanol tolerance. If the strains studied here are ranked on this basis, the order is $1 < 28 < 31$. Curiously, this order is exactly the reverse of that reported for the ethanol tolerances of these same yeasts; when ethanol tolerance is measured as reductions in either glucose utilization (Gray, 1941) or population size (Troyer, 1953), the order is $31 < 28 < 1$. Since only three strains were considered in this investigation, little importance should now be attached to this observation; it seems likely that the inversion of order might not hold if additional yeasts were to be studied. If the present results are compared with those of Gray (1941), methanol appears to be less inhibitory to the processes of glucose utilization in these yeasts than is ethanol. The methanol tolerance values (as molarities) for yeasts 1, 28, and 31, respectively, were 2.42 M, 2.20 M, and 2.00 M. If the data of Gray are expressed as molarities to facilitate comparison, the ethanol tolerance values are, in the same order, 1.29 M, 1.77 M, and 1.93 M.

The overall similarity of the results reported here to those previously secured in studies of the effects of ethanol on the same yeasts suggests that the phenomenon of alcohol tolerance may be rather non-specific in nature: the action of ethanol in

inhibiting glucose utilization may be associated with its properties as a member of a general class of substances, rather than with its role as a yeast metabolite. Of course, the economic significance of the inhibition is not lessened by the conclusion that limitation of fermentation by ethanol is perhaps only an incidental consequence of its formation.

SUMMARY

The effect of added initial methanol on the utilization of glucose by three strains of *Saccharomyces cerevisiae* Hansen has been studied. The observed inhibition resembles that previously obtained with ethanol. It is suggested that this result indicates that the ethanol tolerance phenomenon may not devolve directly upon the fact that ethanol is a yeast metabolite.

ACKNOWLEDGMENT

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LITERATURE CITED

- Gray, W. D. 1941. Studies on the alcohol tolerance of yeasts. *Jour. Bact.* 42: 561-574.
———. 1946. The acclimatization of yeast to high concentrations of glucose: its subsequent effect upon alcohol tolerance. *Jour. Bact.* 52: 703-709.
———. 1948. Further studies on the alcohol tolerance of yeast: its relationship to cell storage products. *Jour. Bact.* 55: 53-59.
Stiles, H. R., W. H. Peterson, and E. B. Fred. 1926. A rapid method for the determination of sugars in bacterial cultures. *Jour. Bact.* 12: 428-435.
Troyer, J. R. 1953. A relation between cell multiplication and alcohol tolerance in yeasts. *Mycologia* 45: 20-39.
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