PHYSIOLOGICAL CONSEQUENCES OF POLYPLOIDY IN YEASTS

III. Influence of Alcohol on the Glucose Metabolism of the Diploid and Autotetraploid Brewery Yeasts

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SUMMARY

1. The interesting discovery in this laboratory that the tetraploid yeast shows an accelerated rate of fermentation though producing only the same percentage of alcohol as the diploid necessitated a series of investigations on the effect of alcohol in the medium on glucose utilisation under comparable conditions.

2. An analysis of the results indicates that the quality of the pitch influences the performance of both the diploid and the autotetraploid. The actual percentage of sugar metabolized at any given level of alcohol is higher in the case of the tetraploid but this does not necessarily imply that it has a higher alcohol tolerance than the diploid. This difference in the actual glucose consumed is demonstrated to be due only to the differing fermenting abilities of the two strains.

3. The necessity for a redefinition of alcohol tolerance is discussed. The only reliable method of ascertaining the alcohol tolerance is to study the performance of the yeast in regular fermentation trials.

4. The relative alcohol tolerance of the two strains depends on the physiological condition of the cells. Storage for different periods produces different effects on the diploid and the autotetraploid.

INTRODUCTION

Investigations on the attenuation characteristics of the two chromosome brewery yeast, BY 1, and its autotetraploid, BY 3, led Mitra (1948) to conclude that duplication of the chromosome complement had resulted *not* in an increase in the alcohol produced but only in an acceleration of the *rate* of fermentation. In the context of this result it was thought of interest to elucidate in detail the fermentative behaviour of the two strains under various physiological conditions. It is well known that in spite of favourable environmental conditions fermentation stops much earlier than would be expected on the basis of the sugar supplied, when a particular concentration

of alcohol is attained in the medium. The level of alcohol necessary to inhibit any further fermentation is characteristic of the strain of yeast used. This phenomenon has been studied in detail by Gray (1941) who concludes that for each strain of yeast there is what he terms a definite "alcohol tolerance" which determines the maximum alcohol that can be produced by it irrespective of the concentration of sugar in the medium. In the present investigation, an attempt has been made to assess the influence of alcohol on the fermentation of glucose by the two- and the four-chromosome strains respectively under different physiological conditions.

Noggle (1947) has pointed out that when comparing the physiological behaviour of a diploid and its autopolyploid, it is necessary to take into consideration the fact that one of the primary effects of induced polyploidy is the alteration in the rate of growth. He suggests that comparisons should be made on the basis not only of the "chronological age" but also of the "physiological age", the latter term connoting that they are at identical stages of development even though chronologically one may be older than the other. This distinction has led to the discovery that depending upon the criterion used the results obtained may differ. The experiments to be reported were planned in such a way as to account for any differences arising from the above distinction.

EXPERIMENTAL

For purposes of the present study, the method described by Gray (1941) was followed in all the essentials. The fermentation medium was compounded of 0·15% potassium di-hydrogen phosphate, 0·15% ammonium sulphate and approximately 1·5% dextrose, adjusted to a pH of 4·6. The alcohol concentrations were calculated from determinations of the specific gravity of the absolute alcohol by the pycnometer method. Pyrex erlenmeyer flasks of 50 ml. capacity were used for the test fermentations. The experiments were conducted simultaneously for both the strains in order to get strictly comparable results.

The adjustment of the inoculum for the trial fermentations was as follows. The crop obtained in each case was repeatedly washed with 0·89% saline and finally suspended in 6 ml. of the saline. One ml. of the well-dispersed suspension was pipetted out into a tared assay tube. This was centrifuged at constant speed for 10 minutes and after pouring out the supernatant and removing the last traces of moisture sticking to the sides of the tube by means of a filter-paper, the weight of the moist yeast was determined. The suspension was then diluted on this basis to give in all experiments an inoculum of 0·125 gm. per ml. of moist yeast.

Immediately after inoculation the initial glucose was determined for each series. The flasks were then incubated at 28° C. for 18 hours at the end of which period, they were taken out and the fermentation stopped by the addition of 1 ml. of 5N sodium hydroxide. The residual sugar in each flask was then estimated. The method of Stiles, Peterson and Fred (1926) was adopted for all sugar estimations.

In the first series of experiments attention was focussed on the effect of alcohol on the sugar metabolism of the two strains grown under conditions of vigorous aeration for chronologically identical periods. A loop of material from the stock agar slant of each strain was inoculated into 5 ml. of barley wort in a sterile bacteriological tube. At the end of 24 hours the contents were transferred to a one litre Lister flask containing 100 ml. of wort. The flask was provided with arrangements for aeration. At the end of aeration under sterile conditions for 24 hours, the crop obtained was recovered and used as inoculum for the test fermentations. The results of a series of such trials are presented in Fig. 1.

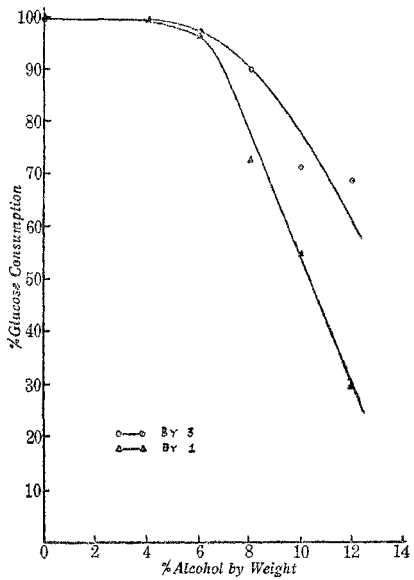

FIG. 1. Alcohol Concentration *Vs.* Glucose Consumption. Inocula of Identical Chronological Age

Prema Bai and Subramaniam (1947) demonstrated that the 4-chromosome strain, BY 3, has a quicker rate of growth than the diploid, BY 1. They found that under the conditions employed by them the logarithmic growth phase ended at 16 hours for BY 3, whereas for BY 1 it lasted till 21 hours. In order to study the influence of alcohol on material of comparable physiological age, the tetraploid was allowed to proliferate for 16 hours, whereas the diploid was cultured for 21 hours. The starting material, as before, was a loop from the stock agar slant inoculated into 5 ml. of wort. At the end of 24 hours the contents were transferred to 100 ml. of wort in a one-litre Lister flask and aerated as usual under sterile conditions. The experiments were timed to give crops of the two strains of the same physiological age. The results are presented in Fig. 2.

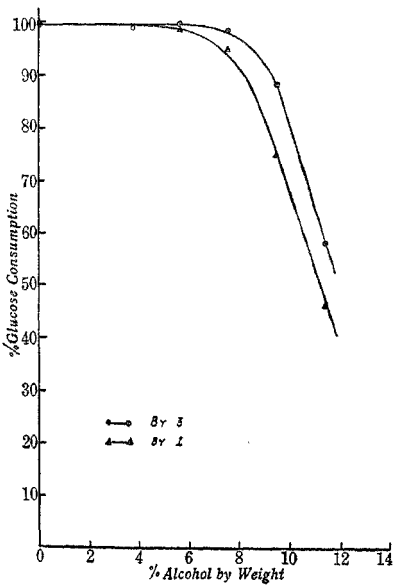

FIG. 2. Alcohol Concentration *Vs.* Glucose Consumption. Inocula of Identical Physiological Age

In the series of experiments detailed above, control of the temperature during the preparation of the yeast cultures for the trial fermentation was

not possible. In the next batch of experiments, therefore, yeast crops cultivated at a constant temperature were utilised. Five ml. of wort in a bacteriological tube was inoculated from the stock agar slant, kept for 24 hours at 28° C. and transferred to a one litre Roux flask containing 100 ml. of wort. This was incubated for 16 hours at the same temperature and the material obtained was used for the test fermentations. By limiting the growth period to 16 hours, the risk of anaerobic conditions setting in during the period of incubation was avoided. This was necessary because the medium was not being aerated. The results are to be found represented graphically in Fig. 3.

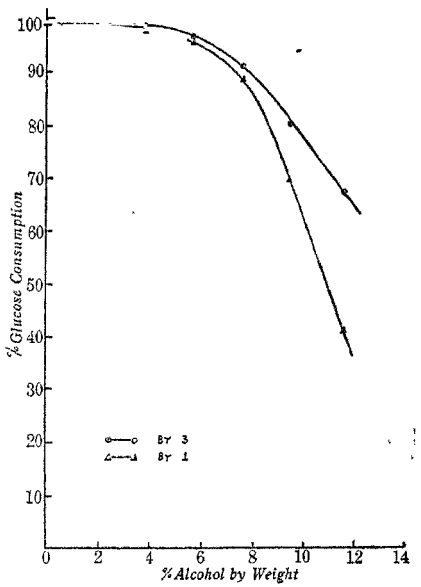

FIG. 3. Alcohol Concentration *Vs.* Glucose Consumption. Inocula from Roux Flask Cultures

For a study of the physiological effects of storing the inoculum the following procedure was adopted. The two strains were cultured in Lister flasks as described earlier and crops of identical physiological age were stored successively for 3, 6, 12 and 24 hours respectively at room temperature in

the spent wort. Since most of the nutriment would have been used up during growth, it is to be expected that the restricted availability and the later complete lack of nutrients would affect the cell storage products and the fermentative ability. At the end of the specific storage period, trial fermentations were run as before using the above as inocula. The results are presented in Figs. 4 and 5.

DISCUSSION

Fig. 1 represents the results with yeast crops of identical chronological age, *viz.*, 24 hours. It is found that within a range of 6%, both the diploid and the autotetraploid work efficiently even though the 4-chromosome strain, BY 3, shows a slight superiority in its performance. If one compares the slope of the two curves, it is found that BY 1 shows a sharper drop than BY 3. The obvious conclusion is that the tetraploid has a greater ability to withstand high alcohol concentrations than the diploid under these condi-

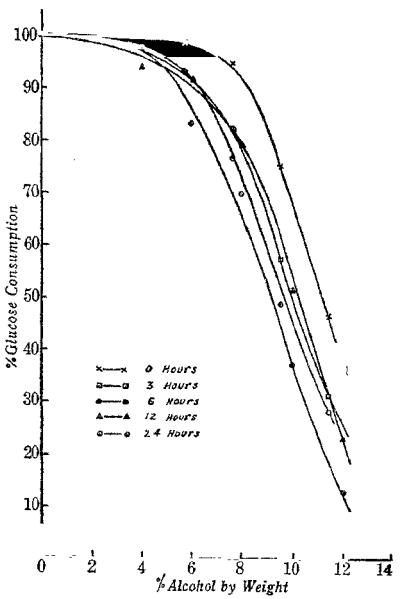

FIG. 4. Alcohol Concentration *Vs.* Glucose Consumption. Effect of Storage. BY 1

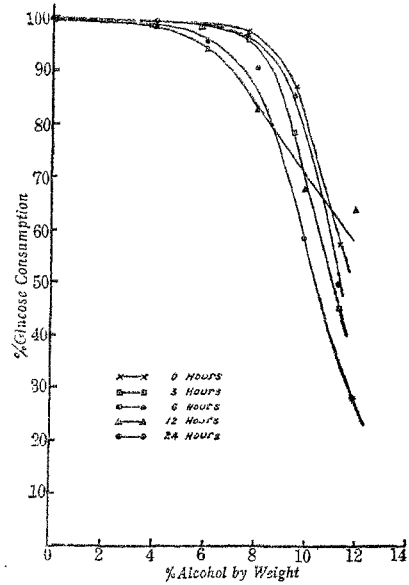

FIG. 5. Alcohol Concentration *Vs.* Glucose Consumption. Effect of Storage. BY 3

tions. Similarity in behaviour is exhibited by the two strains up to a level of 5·7% of alcohol when crops of the same physiological age are used (Fig. 2). The subsequent variation follows the pattern observed in the previous case, except that the difference in the percentage of glucose utilised at the maximum alcohol concentration, *viz.*, 12%, is not so large. The nature of the curves in Fig. 3 which represent the results of the Roux flask experiments is different. Apart from the fact that there is no clear-cut region parallel to the X-axis as in the other two cases, the slope of the curves for both BY 1 and BY 3 is not steep till about 8% of alcohol after which it increases sharply in the case of BY 1, whereas BY 3 still shows a high percentage of sugar utilisation at a concentration of nearly 12% of alcohol. It follows that the metabolism of sugar by the diploid is more susceptible to increasing alcohol concentrations than that by the tetraploid. Under these conditions, therefore, the tetraploid is able to stand higher concentrations of alcohol than the diploid.

A comparison of the effect of storage of the inoculum for varying periods on the utilisation of glucose in the presence of alcohol by the two strains is interesting. Taking the diploid, one finds from the curves in Fig. 4 that with increasing periods of storage there is a steady decline in the performance of the strain. It is clear that storage of the cells has throughout a detrimental effect on the utilisation of sugar by the diploid. In the case of the autotetraploid, however, no regularity is observed. Storage for 6 and 12 hours seems to have increased its capacity to tolerate high concentrations of alcohol, though on storage for 3 and 24 hours the effect was just the opposite.

The considerations detailed above indicate that depending on its physiological condition, the organism changes its response to varying levels of alcohol. The necessity for a study of genetically related strains under widely varying physiological conditions for a proper evaluation of the actual effect of the genetical alteration is hence obvious. For instance, from Fig. 1 it would appear that the autotetraploid is able to withstand increasing alcohol concentrations better than the diploid. A perusal of Fig. 2, however, would indicate that the two strains have a similar response.

That the diploid and the autotetraploid differ in their fermentative ability has been elucidated by Mitra (1948, 1952). It is therefore reasonable to expect that this difference should influence their reaction to increasing concentrations of alcohol. Such, in fact, is the case. This is very clearly brought out by the data incorporated in Fig. 2. At alcohol levels of 7·6%, 9·5% and 11·4%, BY 1 shows a glucose utilisation of 94·4%, 75·0% and 46·2% respectively. For the same alcohol concentrations, BY 3 has utilised 98·2%, 86·0% and 57·9% respectively of the sugar. An examination of the slope of the two curves after the phase parallel to the X-axis shows that the *rate of fall* in the percentage utilisation of glucose is the same in both the diploid and the autotetraploid. But the actual starting points of the slopes of the curves do not coincide, indicating that though the rate of fall in the consumption of glucose is the same for both, the 4-chromosome strain always metabolizes a higher percentage of the sugar. This is merely because of the greater fermentative ability it possesses. However, if the fermentation had proceeded to the finish in both the cases, the diploid could have made up for its slower rate of fermentation by a longer duration of the activity.

It may be relevant to consider in detail the conclusions reached by Gray (1941) on "alcohol tolerance" in yeasts in the light of the results presented here since in both, the same experimental procedure has been followed. The above author defines alcohol tolerance as "the maximum

percentage of alcohol (by weight) at which percentage of glucose utilisation is no more than 1% below the percentage utilisation in the control flask of the same series". If one should draw a curve with the alcohol concentration on the X-axis and the percentage glucose utilised on the Y-axis, then according to Gray, the alcohol tolerance will be represented by the point of departure of the curve from the initial 'plateau', *i.e.*, the phase parallel to the X-axis. From this point of view, this definition will hold only when there is a clear cut plateau in the curve. If instead of this the variation should be continuous, it will not be possible to assess the alcohol tolerance of the material. Such an example will be found in the curve for BY 1 stored for 12 hours (Fig. 4). One cannot be justified in concluding that in this case the strain has a very poor alcohol tolerance since even at a concentration of 8% of alcohol, more than 80% of the sugar has been metabolised. Yet another drawback in the above definition arises from the fact that there is no way of finding out whether the superiority of a given strain over another in respect of glucose utilisation is due to its inherently high alcohol tolerance or merely due to the fact that it has a better rate of fermentation—a character totally unrelated to its alcohol tolerance.

Attention may in this connection be invited to the results presented by Gray (1941) in Table V. These represent the data for the strains 26, 1 and 18, all of which according to him have the same alcohol tolerance. Represented graphically, as has been done in Fig. 6, one comes across certain remarkable facts. The characteristics of the three strains vary very widely in relation to each other. The slope of the curve for strain 26 is less steep than that of either strain 1 or 18. This signifies that even though the point of departure from the plateau of the curve may correspond with those of the other two, the rate of fall in the glucose metabolism with increasing concentrations of alcohol is less marked. It is therefore obvious that strain 26 is superior to both 1 and 18. The curve for strain 18 is also peculiar. After a steady fall in the rate of glucose metabolism it suddenly shows a small but significant change in the slope at an alcohol level of 11%. The slope resumes its original character from a concentration of 13%. In the absence of graphical representation and a detailed comparison of the *rate of fall* in glucose consumption, these significant differences have apparently been missed by the above author.

It has to be concluded, therefore, that the point of departure of the curve from the plateau does not represent the "alcohol tolerance", *i.e.*, the concentration of alcohol at which the activity of the yeast ceases. Even at much higher concentrations there is considerable metabolism of sugar though

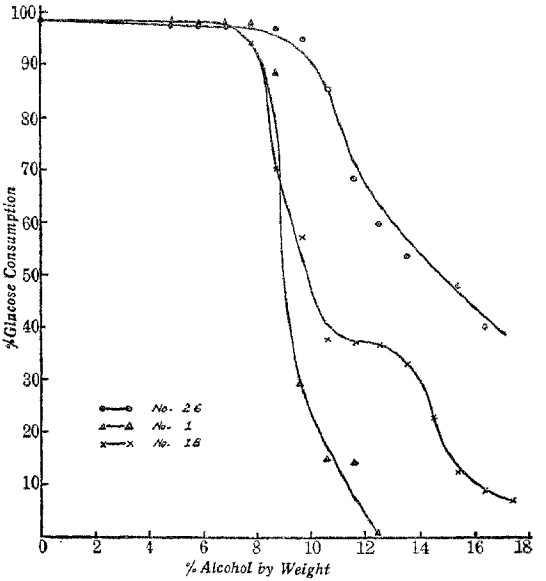

FIG. 6. Alcohol Concentration *Vs.* Glucose Consumption (Gray, 1941, Table 5)

perhaps at a slower rate. The actual susceptibility of the strain to increasing levels of alcohol could be evaluated only on the basis of the slope of the curves. This would obviate difficulties due to the strains having differing fermentative abilities. A quantitative idea of the alcohol tolerance leading to the assignment of a numerical value can be obtained only by actual test fermentations. The present author (Duraiswami, 1950) had suggested a modification of the definition of Gray to the effect that the alcohol concentration above which the glucose utilisation falls below 90% be taken to represent alcohol tolerance. At that time the investigations on the effect of storage of the inoculum on the sugar utilisation had not been completed. Judged from the present discussion, this definition will also be found inadequate.

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