PHYSIOLOGICAL CONSEQUENCES OF POLYPLOIDY IN YEASTS

II. Growth and Yield Characteristics of the Diploid and the Autotetraploid Brewery Yeasts

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Summary

1. The improvement in the fermentative ability on duplication of the chromosome complement of a diploid brewery yeast necessitated a detailed study of the yield and growth characteristics of the diploid and the autotetraploid with a view to possible exploitation in the food yeast industry.

2. Experiments under a variety of physiological conditions show that the criterion employed for assessment influences the interpretation of the data obtained. Under conditions of vigorous aeration, the four chromosome strain is found to give higher yield of yeast than the diploid. It has been demonstrated that the tetraploid is more sensitive to carbon dioxide tension than the diploid.

3. The implications of the results, both theoretical and technical, are discussed.

4. That chromosomal duplication decreases the moisture content of the material has also been recorded.

Introduction

The improvement in the fermentative ability of the strain of brewery yeast on duplication of its chromosome complement (Mitra, 1948, 1952) indicated the possibilities of a genetical improvement of yeast types for exploitation in the alcohol industry. It was thought desirable to find out whether such a change in the chromosome constitution also brings about any alteration in the proliferation characteristics of the organism which would be capable of exploitation in the food yeast industry.

Prema Bai and Subramaniam (1947) have already reported on the characteristics of the growth curves obtained with the diploid strain, BY 1, and the autotetraploid, BY 3. The results indicated that the rate of proliferation of the four chromosome strain is superior to that of the diploid control in the sense that the end of the logarithmic phase of growth is reached earlier in the case of the former. No precise data were given on the comparative yield of the crop of yeast obtained at the end of the period of incubation.

A study was therefore undertaken to secure quantitative data on the crop of yeast that could be obtained from the two strains under identical conditions of culture. In view of the finding that physiological conditions influence the performance of diploid and tetraploid material (Duraiswami, 1952), it was deemed imperative to investigate the two strains under various conditions of aerobiosis.

EXPERIMENTAL

Since the nutritional requirements of the two strains have been shown to be identical (Prema Bai, 1947), a simple natural medium could be used for comparative studies. Barley wort of specific gravity 1·02 and pH 4·6, prepared in a single lot, was used for each set of experiments. One hundred ml. aliquots of this medium were distributed in the different types of reaction vessels as detailed below:

(i) two Erlenmeyer flasks of 250 ml. capacity,

(ii) a litre Roux flask, and

(iii) a litre round-bottomed flask with arrangement for aeration by continuous passage of sterile air by means of a suction pump.

The flasks with the medium were sterilised by autoclaving at 10 lb. steam pressure for 30 minutes. The medium in one of the Erlenmeyer flasks was saturated with CO_2 by passing sterile carbon dioxide from a Kipp for about 15 minutes.

The inocula for the two strains were built up in the following manner:

Slant culture on wort agar	24 hours → one loop	5 ml. of wort	24 hours → contents transferred	20 ml. wort in 250 ml. flask

After growth for 24 hours in the flask the contents were thoroughly agitated to obtain a uniform suspension, 5 ml. of this being used for inoculation of each vessel. Incubation was at room temperature. The Roux flasks were kept in a flat position to facilitate aeration. In the case of the cultures under active aeration care was taken to see that the flasks in which the two strains were being cultured received the same amount of air. This could be adjusted by noting the number of bubbles passing through per unit time.

After growth for 18 hours, the crops obtained in all the cases were washed repeatedly with 0·89% saline and finally suspended in a known volume of the saline. Estimation of the yield was by two independent methods. In the gravimetric procedure, aliquots of the suspensions were dispersed into weighed assay tubes and centrifuged at a constant speed for a known length of time. The supernatant was poured out and the last traces of moisture removed by means of filter-paper bits. The tubes were then weighed for the moist weight and left overnight in an oven at 90° C. After cooling, they were weighed again to get the dry weight. It was thus possible to estimate the total yield of the crop on the basis of both moist and dry weights. In the second method, aliquots of the suspensions were centrifuged at constant speed for a known length of time in tubes having calibrated bottoms. The lengths of the columns obtained were measured and the volume of the yeast so packed calculated.

Table I gives the result of a typical set of observations, the difference in the amount of dry matter obtained in the two cases being presented on a percentage basis.

TABLE I

Yield of the diploid and the autotetraploid strains under different conditions of aeration

Cultural conditions	Yield of yeast per 100 ml. (in g.)					Volume of cells		% Mois
	Moist		Dry			mm.^{3}/ml.		
	BY 1	BY 3	BY 1	BY 3	BY 1/BY 3 %	BY 1	BY 3	BY 1
Aeration in round-bottomed flask ..	1·6796	2·0014	0·3952	0·6054	153·1	18·17	19·59	76·47
Roux flask ..	1·5516	1·3592	0·3076	0·3128	101·6	12·49	12·31	80·19
Conical flask ..	1·0416	0·8504	0·1896	0·1976	104·2	8·14	7·27	81·79
Conical flask with medium saturated with CO_2	0·7856	0·4528	0·1400	0·1048	74·8	6·43	4·48	82·17

A perusal of Table I shows that under conditions of vigorous aeration the yield of the tetraploid strain is much higher than that of the diploid and this can be demonstrated by taking the two independent methods for the determination of the quantity of yeast. The degree of aeration determines the yield in both the cases. The poorer the aeration the less is the yield. However, under all the conditions the yield of the tetraploid as determined on the basis of moist weight or volume on centrifugation is less than that obtained in the case of the diploid. But since the higher moisture content of the diploid yeast more than outbalances the difference in the yield, the ultimate dry weight shows a slight superiority in the tetraploid in all instances except where the medium was saturated with carbon dioxide. According to all the three different criteria, the yield shown by the tetraploid is significantly poorer than the diploid under the last mentioned condition.

The marked superiority in the proliferating capacity of the tetraploid strain under adequate aeration necessitated a study of the behaviour of the two strains under ideal conditions of propagation. A method closely simulating that in the manufacture of food yeast was chosen for the following set of experiments.

Quantity of yeast obtained, under ideal conditions of propagation, with the diploid and the autotetraploid.—To provide optimum conditions for the propagation of yeast, the inoculum for the final aeration should be built up in gradual steps under conditions ensuring competition between the cells for the nutrient (Walter, 1941), so that the final pitch is composed of actively dividing cells. Final culture naturally should be in a dilute sugar medium with vigorous aeration.

The same medium as was used in the foregoing experiments was employed throughout the present investigations also. Forty-eight-hour old cells from a wort-agar slant were inoculated to 10 ml. of the medium contained in a 100 ml. conical flask. At the end of 24 hours the contents of the flask were transferred to a tall jar containing 40 ml. of the medium and having an arrangement for aeration by a suction pump. Active aeration was provided for 24 hours at the end of which a fairly thick suspension of yeast was obtained in both the cases. This was used as the inoculum for the final propagation of the strain under conditions of vigorous aeration.

The arrangement consisted of a 500 ml. round-bottomed flask with connections for the bubbling of air through the medium. The portion of the inlet tubing submerged in the medium was made into a perforated bulb to facilitate the dispersion of air. The outlet tube was connected to a wash bottle arrangement which served as a trap for the collection of the medium overflowing due to foam and entrainment. Air at moderate pressure was supplied by an air compressor.

The flasks contained 100 ml. aliquots of the medium. Aeration was started immediately after inoculation and at the end of 24 hours of growth at room temperature, the yields of the diploid and the tetraploid were estimated on the basis cf moist as well as dry weight. The experiments for the two strains were run simultaneously and under strictly identical conditions. A representative set of results is given in Table II.

TABLE II

Yield of the diploid and the tetraploid under ideal conditions of propagation

Strain	Total yield	
	Moist weight	Dry weight
Diploid, BY 1. ..	3·240 g.	0·714 g.
Autotetraploid, BY 3 ..	4·040 g.	1·252 g.
BY 3/BY 1 (%) ..	124·7	175·3

DISCUSSION

Before passing on to a consideration of the yield of yeast, it will be relevant to discuss briefly the findings on the mcisture relationship of the two strains. The results recorded in Table I indicate that the moisture content of any given strain of yeast is a function of its physiological condition. With vigorous aeration of the medium, both the two-chromosome and the four-chromosome strains give values significantly lower than those of the samples obtained under conditions of less efficient aeration or even under complete anaerobiosis. It is nevertheless significant that whatever the physiological condition, the tetraploid has less of moisture than the diploid. Duplication of the chromosome complement seems therefore to have influenced moisture relationship adversely. It also appears obvious that the physiological conditions under which yeast cells are examined affect the actual value of the moisture content. It is interesting to note that more dry matter can be obtained per given weight of moist yeast under conditions of vigorous aeration.

Two important points should be borne in mind in interpreting the results of the yield experiments: (1) The dry matter per unit weight of moist yeast is much greater in the case of the tetraploid than in that of the diploid. (2) The metabolic activity of the tetraploid is much greater than that of the two-chromosome strain, as would be evident from studies on the fermentative abilities of the two strains (Mitra, 1948, 1952). For active proliferation, therefore, a greater supply of oxygen will be necessary for the four chromosome strain.

Under conditions of inadequate aeration, the tetraploid passes very easily to a fermentative mode of life. Death and disintegration is the only fate of these fermenting cells which are highly endopolyploid in nature (Subramaniam, 1947, 1948). On the other hand, the diploid cells maintain a slow but sustained rate of reproduction even under fairly anaerobic conditions and thus show practically equal or even greater yield than the tetraploid. With vigorous aeration the metabolic need of the tetraploid being fully satisfied, the strain shows a much higher yield due to its higher metabolic activity and higher rate of proliferation. This improvement in the yield of dry matter is about 75% over the diploid. The implication of the results from the point of view of yeast manufacture is evident.

A perusal of the results in Table I indicates that duplication of the chromosome complement has resulted in an increased sensitivity to high concentrations of carbon dioxide. The interesting implication therefore follows that the inhibition of glycolysis (fermentation) on admission of air, *i.e.*, the "Pasteur effect" (Burk, 1939) may be operative to a much greater extent in the case of the tetraploid than in the diploid. However, no definite conclusions can be drawn from the present investigations, since parallel estimations of sugar balance as well as the alcohol formed were not carried out. The data justify the belief that a quantitative study of the "Pasteur effect" in these two strains would be of interest not only from the biochemical point of view but from a genetical angle as well.

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