Influence of the Concentration of Glucose and Galactose on the Physiology of *Saccharomyces cerevisiae* in Continuous Culture

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SUMMARY

The effect of increasing concentrations of glucose and galactose on the physiology and composition of *Saccharomyces cerevisiae* was studied in continuous culture. Glucose, at concentrations ranging from 1 to 50 g./l. was degraded predominantly by aerobic fermentation. Galactose catabolism was mainly aerobic at concentrations up to 5 g./l. but became increasingly fermentative at higher sugar concentrations. The fatty-acid content varied with the type of metabolism. Thus at low concentrations of sugar, fatty acids accounted for 8 % dry wt but about 5 % dry wt at high sugar concentrations. High sugar concentrations may be associated with the repression of mitochondrial structures.

The size of the amino acid pool was a function of the availability of ammonia in the medium. Such 'pools' contained appreciable amounts of arginine when NH_4^+ was in excess, whereas in the absence of NH_4^+ from the culture filtrate only trace amounts of arginine were detected. The content of glutamic dehydrogenase in the yeast varied directly with the availability of ammonia and the size of the amino acid pool.

INTRODUCTION

Saccharomyces cerevisiae, grown in the presence of glucose or fructose as the source of carbon and energy, degraded the sugar by aerobic fermentation. When grown on galactose or mannose, degradation proceeded simultaneously by respiration as well as by fermentation (De Deken, 1966). Such a repression of respiratory enzymes by high fermentation rates is known as the 'Crabtree effect'. The concentration of glucose in the medium is of prime importance. Thus Slonimski (1956) showed that up to 6×10^{-3} M-glucose stimulated the rate of respiratory adaptation in anaerobically grown *S. cerevisiae*. At higher concentrations the rate of adaptation decreased while that of fermentation increased. Similarly, Polakis, Bartley & Meek (1965) showed that when grown in the presence of low concentrations of glucose (0.09 %) or galactose (0.9 %) *S. carlsbergensis* synthesized respiratory enzymes and possessed functional mitochondria. In contrast, cells grown in the presence of 0.9 % glucose did not contain recognizable mitochondria and only low levels of respiratory enzymes were detected. The effects of high concentrations of galactose were not determined.

Increasing the glucose concentration from 0.6 % to 20 % in cultures of *Candida parapsilosis* increased the concentrations of several enzymes; in particular the contents of glyceraldehyde phosphate dehydrogenase, phosphoglycerate kinase and pyruvic decarboxylase increased four- to tenfold (Hommes, 1966). Babij, Moss & Ralph (1969) showed that increasing the glucose concentration in cultures of *C. utilis* from 0.1 % to

4.5 % also increased the total fatty-acid content of this yeast from 2 % to 10 % dry weight. The size of the amino acid pool in *Saccharomyces cerevisiae* is dependent on the nature of the limiting substrate in chemostat cultures. In yeasts obtained from glucose-limited cultures this pool is almost three times that from NH_4^+ limited cultures (D. W. Tempest, J. L. Meers & C. M. Brown, unpublished). This paper presents the results of a study of the effects of increasing concentrations of glucose and galactose on the composition and physiology of *S. cerevisiae* in chemostat cultures.

METHODS

Saccharomyces cerevisiae (NCYC712, described by Brown & Hough, 1965) was maintained by monthly subculture on yeast extract (1 %, w/v), peptone (2 %, w/v), glucose, (2 %, w/v) agar.

Growth conditions. Organisms were grown in 0.5 l. Porton-type chemostats at a dilution rate of 0.1 h^{-1} at 25° and at a pH of 4.5, as described by Brown & Rose (1969a). The medium employed was: KH_2PO_4 , 15 mM; MgSO_4 , 4 mM; CaCl_2 , 1 mM; inositol, $1.1 \times 10^{-4} \text{ M}$; pyridoxine HCl, $1.0 \times 10^{-5} \text{ M}$; calcium pantothenate, $1.0 \times 10^{-5} \text{ M}$; thiamine HCl, $1.2 \times 10^{-6} \text{ M}$; biotin $4 \times 10^{-9} \text{ M}$; with trace amounts of Zn^{2+} , MoO_4^{2-} , Cu^{2+} , Fe^{3+} and Mn^{2+} . The nitrogen source was $(\text{NH}_4)_2\text{SO}_4$ 2 mM, and glucose and galactose were added as required. The galactose used (British Drug Houses, Poole, Dorset) contained up to 2 mg./g. glucose. Phosphate-limited media contained KH_2PO_4 , 0.25 mM; $(\text{NH}_4)_2\text{SO}_4$, 10 mM; KCl, 14 mM and glucose at 10 g./l.

Analytical procedures. Samples were taken directly from chemostat cultures and harvested immediately. Yeast concentration and the contents of keto acid, ethanol, glucose and galactose in the culture filtrates were determined by the methods of Brown & Rose (1969a). Ammonia in culture filtrates and amino acid pool samples were determined by the method of Fawcett & Scott (1960). The rates of oxygen uptake of yeast washed in 0.01 M-phosphate buffer (pH 6.5) were determined by conventional manometric methods. Amino acid pools from yeast washed twice in water at room temperature were extracted with boiling water by the method of Dawson (1965) and assayed by the ninhydrin procedure of Harris & Parsons (1958). These 'pools', which contained low concentrations of ammonia, were qualitatively analysed on paper and thin-layer chromatograms as described by Brown & Rose (1969b).

Duplicate samples of culture (containing about 100 mg. dry wt cells) were harvested by centrifuging at $5000g$ for 5 min. and then analysed for fatty acids in the following way. The yeasts were washed twice in ice-cold 10 mM-phosphate buffer (pH 7.0) and suspended in 10 ml. of a mixture of chloroform and methanol (2:1, v/v). To one suspension was added a known amount of myristic acid ($\text{C}_{14:0}$) as an internal standard. These suspensions were transferred to 50 ml. stoppered flasks containing 10 g. of glass beads (1 mm. diam.; Jencons, Hemel Hempstead, Hertfordshire), 5 ml. chloroform/methanol was used for washing. The flasks were then flushed with nitrogen, stoppered, shaken for 1 h. at room temperature on a wrist-action shaker (Griffin & George, London) and left overnight. The turbid supernatant fluid was then decanted from the beads, which were washed twice with 5 ml. chloroform/methanol. The washings were added to the decanted extract and the mixture centrifuged at $3000g$ at room temperature for 5 min. The clear supernatant fluid was then removed and

evaporated to dryness at 40° on a Buckler evapomix (Buckler Instruments Inc., Fort Worth, New Jersey, U.S.A.). The residue was taken up in 5 ml. petroleum spirit (40 to 60°). The solution was removed and again dried. This step was repeated. The material remaining was taken up in 5 ml. of petroleum spirit and this solution transferred to a 50 ml. flask of the design suggested by Kates (1964), and the solvent removed in a stream of nitrogen. The production of methyl esters, their chromatography and quantitative assessment were as described by Brown & Rose (1969*b*).

To prepare enzyme extracts, freshly harvested yeast (about 200 mg. dry wt) was washed twice with 20 ml. of phosphate buffer (10 mM, pH 7.0) containing 1 mM-mercaptoethanol and 0.25 M-sucrose, and then suspended in 7 ml. buffer. This suspension was transferred to chilled vessels containing 5 g. of 1.0 mm. diam. glass beads, shaken at maximum amplitude in a Mickel disintegrator for 30 min. and then centrifuged at 1500g for 10 min., when most of the cell walls and debris sedimented amongst the beads. The supernatant fluid was removed with a pipette, centrifuged at 75,000g for 30 min. and the clear supernatant retained for enzyme analysis. The activities of glutamic dehydrogenase (NADP), pyruvic decarboxylase and alcohol dehydrogenase were measured by recording the rates of oxidation or reduction of coenzyme at 340 nm. in a Unicam SP 500 series II spectrophotometer equipped with an SP 22 log/linear recorder (Pye/Unicam, Cambridge). The test solutions were: for glutamic dehydrogenase, 6.7 mM 2 oxo-glutarate, 2 mM cysteine, 67 mM NH₃, 5 mM EDTA, 0.23 mM NADPH, and 10 mM-phosphate buffer (pH 6.5) to a final volume of 3 ml.; for pyruvic decarboxylase, 3.3 mM Mg²⁺, 3.3 mM cysteine, 1.67 mM thiamine pyrophosphate, 20 µg alcohol dehydrogenase, 0.23 mM NADH, and 10 mM-phosphate buffer (pH 6.5) to 3 ml.; for alcohol dehydrogenase, 25 mM ethanol, 0.23 mM NAD, and 10 mM phosphate buffer (pH 6.5) containing 20 mM semicarboxide hydrochloride to a final volume of 3 ml. The protein content of enzyme extracts was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as standard.

RESULTS

Effect of sugar concentration on growth

The concentration of glucose and galactose employed and their effects on yeast concentration and components of the culture filtrate are shown in Table 1. When the initial concentration of either sugar in the medium was low, only trace amounts could be detected in the culture filtrates, but as the initial concentrations were increased there was a concomitant increase in the residual amounts. Conversely the NH₄⁺ concentrations present in the culture filtrates were high at low concentrations of sugar and virtually absent at high concentrations. Thus the cultures were probably carbon-limited at low concentrations of sugar and NH₄⁺- limited at high sugar concentrations. This was reflected in the changes in amounts of ethanol and keto acid (only pyruvate was detected) in the culture filtrates. The production of ethanol/mg. yeast increased steadily as the concentration of glucose in the medium increased to 10 g./l., above which the rate of increase was slower. Production of ethanol/mg. yeast was less in galactose-grown cultures, little being produced up to 2 g./l. galactose, but appreciable amounts were produced at higher sugar concentrations. With glucose at 1 g./l. keto acid production was high (over 1 mg./mg. organisms) and ethanol production low (0.5 mg./mg.) compared with that of other glucose-grown cultures.

The yields of yeast relative to the sugar utilized are listed in Table 1. With the exception of cultures grown with 1 g./l. glucose, cultures with glucose as carbon source had a uniformly low yield which was consistent with a fermentative degradation of that sugar. In contrast, the yield of yeast grown in galactose medium varied inversely to the concentration of galactose in the medium, reflecting a more fermentative utilization of galactose with increasing concentration of that sugar in the medium. These results suggested that high concentrations of galactose might in fact be repressing the respiratory degradation of the sugar. This notion was tested by comparing in the Warburg apparatus the respiratory activity of yeasts, grown at low and high concen-

Table 1. *Effect of sugar concentration on cell yield and culture filtrate components*

Sugar concentra- tion (g./l.)	Cell concentra- tion (mg./ml.)	Yield (mg. dry wt/mg. sugar utilized)	Culture filtrate				
			Ethanol		Keto acid		NH ₄ ⁺ (μg./ml.)
			μg./ml.	μg./mg. dry wt	μg./ml.	μg./mg. dry wt	
Glucose as carbon source							
1.0	0.05	0.05	25	500	54	1080	45
2.0	0.18	0.12	136	960	34	189	27
5.0	0.44	0.13	590	1345	47	107	1
10.0	0.78	0.14	1440	1850	73	94	1
50.0	1.11	0.12	1800	2120	216	105	0.5
Galactose as carbon source							
1.0	0.34	0.38	9	26	41	121	27
2.0	0.49	0.33	14	29	43	88	6
5.0	0.62	0.22	169	273	61	97	4
10.0	0.83	0.17	584	704	85	103	3
50.0	0.87	0.10	1086	1250	110	126	1

Table 2. *Effect of sugar concentration on the oxidative capacity of Saccharomyces cerevisiae*

Q _{O₂} (μl./mg./h.)	Sugar concentration in culture medium			
	Glucose (g./l.)		Galactose (g./l.)	
	2.0	50.0	1.0	50.0
Endogenous	7.0	9.3	4.3	12.6
Glucose	65.5	22.6	60.0	22.2
Galactose	8.7	11.7	19.0	25.6

trations of sugar. The results obtained (Table 2) demonstrate that at 50 g./l. both glucose and galactose repressed the oxidation of glucose to the same extent. They also show that yeasts grown on glucose lacked the ability to oxidize galactose and that this ability of galactose-grown cells was not affected appreciably by the concentration of galactose in the growth medium.

Over the range of sugar concentration employed the cellular concentrations of protein, carbohydrate and RNA did not vary to any marked extent.

Effect of sugar concentration on fatty-acid composition

Gas chromatography of the methyl esters of fatty acids revealed the major components to be palmitic (C16:0), palmitoleic (C16:1) and oleic acids (C18:1), with smaller amounts of myristic (C14:0) and stearic (C18:0) acids and traces of components corresponding to C14:1, C10:0 and C8:0. The inclusion of a myristic acid standard in the samples enabled the amounts of individual and total components to be expressed as $\mu\text{g. fatty acid/mg. dry weight of yeast}$ (Table 3). Unsaturated fatty acids predominated, with palmitoleic acid consistently accounting for about 50 % total. Because of the low yeast concentration produced in cultures containing glucose at 1 g./l. it was not possible to obtain a reproducible estimate of fatty acids for those

Table 3. *Effect of sugar concentration on fatty-acid content of Saccharomyces cerevisiae*

Sugar concentration (g./l.)	Fatty acid ($\mu\text{g./mg. dry wt yeast}$)							$\text{C}_{14}, \text{C}_{16}, \text{C}_{18}$	
	14:0	14:1	16:0	16:1	18:0	18:1	Total	Total unsaturated	Total un- saturated
Glucose as carbon source									
2.0	1.7	1.1	15.8	45.3	1.3	16.9	82.0	17.5	62.3
5.0	1.2	0.3	10.7	26.3	1.8	9.4	51.3	13.7	36.3
10.0	0.6	0.3	8.9	23.4	2.3	11.5	47.5	11.8	35.2
50.0	1.1	0.0	10.2	28.8	2.7	13.3	56.7	14.0	42.1
Galactose as carbon source									
1.0	1.5	0.2	10.5	45.4	1.5	17.6	77.3	13.5	62.2
2.0	1.5	0.7	10.2	45.7	1.8	18.0	79.0	13.5	62.4
5.0	0.9	0.5	13.7	38.2	5.0	18.9	77.4	19.6	57.6
10.0	0.4	0.0	10.5	30.0	3.3	17.1	62.0	14.2	47.1
50.0	0.6	0.0	6.5	22.2	1.8	12.3	43.7	8.9	34.5

cells. Cultures with glucose at 2 g./l. or galactose at 1, 2 and 5 g./l. contained very similar concentrations of individual and total fatty acids. In these cells fatty acids accounted for about 8 % dry wt. At concentrations of glucose from 5 g./l. and above and galactose from 10 g./l. and above, the total fatty-acid contents decreased. Palmitoleic acid decreased most with a corresponding decrease in the total amounts of unsaturated fatty acids. The percentage composition of these fatty acids remained constant, however, even though the total fatty-acid concentrations were reduced by 40 % to 50 %. Preliminary results obtained by chromatography of lipid fractions on silicic acid impregnated paper (Marinetti, 1962) indicated that changes in fatty-acid composition were closely followed by changes in some phospholipid components. In particular, cardiolipin was present in significant amounts in glucose-limited yeast, but in only trace amounts in yeasts grown at high concentrations of glucose: phosphatidyl ethanolamine and phosphatidyl serine showed similar though smaller changes. The total amounts of neutral lipids (glycerides, sterols and sterol esters) appeared to remain constant throughout.

Effect of sugar concentration on the amino acid pool and the content of glutamic dehydrogenase

The sizes of the amino acid pools of these cultures, corrected for NH_4^+ content, are shown in Table 4. The 'pool' size from both glucose and galactose-grown cells decreased as the sugar concentration was increased and the nitrogen supply of the medium became limiting. The amino acid pools of this organism contain only a limited range of components (Brown, 1970; Tempest, Meers & Brown, unpublished), glutamic acid, alanine and aspartic acid being prominent with smaller amounts of leucine, valine, lysine and arginine. By chromatography on paper and thin layers of cellulose variations in 'pool' size were shown to involve all the components listed above. The amounts of lysine and arginine showed the largest changes, these compounds being present only in trace amounts in 'pools' obtained from yeast grown at high sugar

Table 4. *Effect of sugar concentration on the size of the amino acid pool and the content of glutamic dehydrogenase*

Sugar concentration (g./l.)	Glucose		Galactose	
	Amino acid pool size ($\mu\text{g./mg.}$)	Glutamic dehydrogenase (n-mole/mg. protein/min.)	Amino acid pool size ($\mu\text{g./mg.}$)	Glutamic dehydrogenase (n-mole/mg. protein/min.)
1.0	14.8	620	6.5	1100
2.0	11.3	1140	5.3	1005
5.0	7.6	1850	4.1	1670
10.0	3.6	1730	1.9	1895
50.0	2.5	2390	1.9	2385

concentrations. The glutamic dehydrogenase (EC 1.4.1.3), designated GDH, specific for NADP, has a purely biosynthetic role in metabolism and is therefore of prime importance to organisms growing on NH_4^+ as sole source of nitrogen. The GDH content of both glucose and galactose-grown cells increased as the concentration of sugar was increased (Table 4). Thus the synthesis of GDH was controlled either by the concentration of sugar, the concentration of NH_4^+ remaining in the culture filtrate, or the size of the amino acid pool. To distinguish between these possibilities a phosphate-limited culture was employed which enabled both glucose and NH_4^+ to be present in excess. In this culture the pool size was $9.7 \mu\text{g./mg.}$ while the GDH content was the lowest recorded at $465 \text{ n-mole/mg. protein/min.}$ Thus media with an excess of both NH_4^+ and glucose produce cells with a large amino acid pool and a substantially lowered content of GDH.

Effect of sugar concentration on the content of pyruvic decarboxylase and alcohol dehydrogenase

Pyruvic decarboxylase (EC 4.1.1.1) and alcohol dehydrogenase (EC 1.1.1.1), hereafter abbreviated as PD and ADH respectively, are important to yeast degrading a sugar fermentatively in that they are responsible for the conversion of pyruvic acid to ethanol. These reactions also result in the oxidation of NADH, and the main function of this pathway may be to act as an 'electron sink' when oxidation of pyruvate is restricted. The intracellular contents of PD and ADH were of interest in these experi-

ments because of the increased fermentation induced by high concentrations of sugar. In cultures grown on glucose the contents of both PD and ADH were low at 1 g. glucose/l. but increased with increasing glucose concentration until a plateau value was reached with 10 g./l. (Table 5). In contrast, the contents of these enzymes were independent of sugar concentration in galactose-grown yeasts.

Table 5. *Effect of sugar concentration on the content of pyruvic decarboxylase and alcohol dehydrogenase in Saccharomyces cerevisiae*

Sugar concentration (g./l.)	Glucose		Galactose	
	PD*	ADH*	PD*	ADH*
1.0	45	54	208	253
2.0	125	56	262	222
5.0	147	129	180	206
10.0	260	194	197	194
50.0	271	198	181	202

* n-mole/mg. protein/min.

DISCUSSION

The results reported are in agreement with those of Slonimski (1956) and Polakis *et al.* (1965) in that even at a glucose concentration as low as 1 g./l. oxidative metabolism was not apparent. The very low yield of yeast at this concentration of glucose may have been due to the fact that this glucose concentration, while sufficiently high to prevent the oxidative utilization of the sugar, was apparently too low to completely derepress the synthesis of the 'electron sink' enzymes (PD, ADH) which convert pyruvate to ethanol. This is evident in the low levels of PD and ADH found together with the very high production of pyruvate by such yeasts. Thus such yeasts were at least in part devoid of a mechanism for oxidizing NADH (produced by glycolysis), which in turn would decrease their metabolic efficiency. In medium initially containing 2 g./l. glucose the cultures had a capacity for glucose oxidation (Table 3), a capacity which was lost at higher sugar concentrations. Relating this observation to the fatty-acid composition, it is possible that the 8 % (dry wt) fatty acids found at 2 g. glucose/l. and 1, 2 and 5 g. galactose/l. may have been a reflexion of the presence of mitochondrial lipids. At higher concentrations of glucose and galactose where the oxidative utilization of both sugars was measurably lower, the fatty-acid concentrations of these yeasts were similarly reduced. It is significant that the cell lipids most involved in this alteration were phospholipids and in particular cardiolipin, which is a characteristic component of mitochondrial lipids (Bartley, 1964; Paltauf & Schatz, 1969). Criddle & Schatz (1969) also showed that high concentrations of glucose can bring about such changes. They reported that growth in the presence of 10 % glucose reduced the concentration of mitochondrial protein to 3 % of the total as compared with 11 % for cells grown in the presence of 0.8 % glucose, and they concluded that glucose represses the synthesis of the inner membrane of mitochondria. It is also apparent from the present work that only at concentrations of 5 g./l. and less was galactose a substrate which was utilized predominantly by oxidative mechanisms. Indeed, at higher concentrations the utilization of galactose became almost indistinguishable from that of glucose, with low yield values being obtained

together with a high production of ethanol. The results obtained in the experiments described above differ from those of Babij *et al.* (1969) with *Candida utilis*. *Candida utilis* is an obligatory aerobic organism with little fermentative metabolism and, as was shown by De Deken (1966), does not show a positive Crabtree effect. Thus in the presence of high concentrations of sugar the metabolism of this organism remains oxidative and the 'depot fat' which accumulates as fat droplets under these conditions is possibly a reflexion of an abundance of energy and perhaps acyl-coenzyme A derivatives for biosynthesis.

The amino acid pool of this strain of *Saccharomyces cerevisiae* was dependent upon the availability of both NH_4^+ and carbon skeletons when growing in a defined medium. Thus when both NH_4^+ and glucose were in excess the pool was largest. It fell to a level of about 2 μg . nitrogen/mg. yeast when NH_4^+ limited growth. It is consistent with these observations that the amino acids which varied most were those with more than one nitrogen atom (arginine, lysine). Similar results were recently obtained by Middelhoven (1970), who grew *S. cerevisiae* in a defined medium containing $(\text{NH}_4)_2\text{SO}_4$ as sole source of nitrogen and then starved these cells by shaking in nitrogen-free medium. Under these conditions the pool size fell from 0.744 to 0.583 m-moles/g. dry wt while the arginine content fell from 0.12 to 0.03 m-moles/g. dry wt. This disproportionate decrease in arginine concentration could be correlated with a derepression of arginase in the absence of nitrogen-containing compounds in the medium. These results show that arginine in particular appears to function as a reserve of nitrogen in this organism and is mobilized in the absence of metabolizable nitrogen in the medium. Polakis *et al.* (1965) showed that the NADP-linked GDH of *S. carlsbergensis* was present in highest concentrations in cells grown in synthetic media, the enzyme content being greater in early log phase cells, when glucose remained in the medium, than at later stages of growth when this glucose had been utilized. These authors attributed this change to a decreased rate of growth and protein synthesis in the later stages of batch-cultivation. In the present experiments the rates of growth and protein synthesis were constant throughout and it seems most likely that GDH synthesis was controlled by the NH_4^+ concentration of the medium and the concentration of intracellular amino acids.

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