Inability of Petite Mutants of Industrial Yeasts to Utilize Various Sugars, and a Comparison with the Ability of the Parent Strains to Ferment the Same Sugars Microaerophilically

J. F. T. Spencer*,**, D. M. Spencer**, and R. Miller*

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A number of industrial strains of *Saccharomyces cerevisiae* were converted to the petite form and tested for the ability to utilize galactose, maltose, sucrose, α-methyl glucoside and raffinose. The parent strains all metabolized these sugars aerobically. Twelve of the petite forms did not utilize galactose, six failed to utilize maltose, 17 did not utilize α-methyl glucoside, and 18 did not utilize raffinose. The petites of two distiller's yeast strains did not utilize sucrose. The respiratory-competent parent strains nearly all fermented galactose, maltose, sucrose and raffinose, though 19 strains did not ferment α-methyl glucoside microaerophilically. Three strains did not ferment galactose, two fermented it only after several days adaptation, one did not ferment raffinose, and two did not ferment sucrose under microaerophilic conditions. Six respiratory-competent strains which did not utilize galactose when in the petite form fermented higher (10%) concentrations of glucose and maltose under microaerophilic conditions, but only three of these fermented galactose. The implications of these findings for the use of such strains in industry are discussed briefly.

Introduction

It has been known for some years that the petite mutation in *Saccharomyces cerevisiae* affects sugar utilization in laboratory strains [1–3]. Evans and Wilkie mention also that in some instances at least, impairment of mitochondrial function by anaerobiosis brings about a similar inhibition of metabolism of the same sugars. Mahler and Wilkie [3] demonstrated that the effect was at the level of transport of the sugars through the cell membrane, rather than by the inhibition of enzyme synthesis. Because of the obvious importance of these findings to the fermentation industries, where the ability of a given yeast strain to metabolize a particular sugar under microaerophilic conditions is a matter of considerable significance, we have tested the ability of the petite forms of a number of brewer's and distiller's yeasts to metabolize a number of common sugars, and compared these results with the ability of the parent strains to ferment these sugars under microaerophilic conditions.

* Department of Biology, Thames Polytechnic, Wellington Street, London SE18, England.
** Department of Biology, Goldsmith's College, University of London, London SE14, England.

Reprint requests to Dr. J. F. T. Spencer.

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Materials and Methods

The yeast strains were obtained from Labatt Brewing Company of Canada, O'Keefe's Breweries of Canada, from Dr. R. E. Simard, Departement des Vivres, Université Laval, Quebec, Canada, from the National Collection of Yeast Cultures, Food Research Institute, Norwich, England, and from miscellaneous sources. They were maintained on malt agar or MYGP agar.

The yeasts were converted to the petite mutants by treatment with acriflavine (approximately 20 μg/ml) in still culture in YEP medium, according to a method suggested by Dr. D. C. Hawthorne. Suspensions were plated on yeast extract-peptone-glucose agar, and petite colonies were isolated and purified. These were plated on yeast extract agar (0.5%) containing the different sugars as sole carbon source (2%), using a multipoint inoculator. They were scored for growth after 24–48 h incubation at 30 °C.

Fermentation tests were done in large Durham tubes (approximately 25 mm) containing medium in depths of about 10 cm (0.5% yeast extract + 2% sugar). The medium was covered with a layer of sterile mineral oil after inoculation.

Results and Discussion

The results are presented in Tables I–III. Of the 24 petite strains, only three metabolized α-methyl

glucoside, and only four metabolized raffinose. All but two metabolized sucrose. Twelve metabolized galactose and maltose, six did not metabolize maltose, and 12 failed to utilize galactose. None of the petite strains metabolized galactose but not maltose.

It is interesting to note that of the petites of the distiller's yeasts, only three metabolized galactose, and one of these failed to metabolize maltose. Two did not metabolize sucrose. Of the petites of the lager yeasts, provided by Labatt's and O'Keefe's breweries, three failed to utilize galactose and all metabolized maltose. Only of the petites from lager and distiller's yeast strains tested utilize α-methyl glucoside, and one used raffinose.

In contrast, of seven British brewing yeasts tested, only two yielded petites which metabolized galactose, and three failed to metabolize maltose. One of these petites metabolized α-methyl glucoside, and two, raffinose. The behavior of a sake yeast (IFO 2193) tested was of interest, as the petite from this strain failed to metabolize galactose or maltose, utilizing only glucose and sucrose.

Table I. Utilization of sugars by petite mutants of industrial yeasts.

Strains	Glu	Gal	Mal	Suc	α-MG	Raff
Distiller's						
YS 2968	+	+	+	−	ND	ND
2969	+	+	+	+	−	−
2970	+	−	+	+	−	−
2972	+	−	+	+	−	−
2973	+	+	+	+	−	−
2974	+	+	+	+	−	−
2975	+	w	+	−	ND	ND
Lager Yeasts						
OK11	+	+	+	+	+w	vw
OK13	+	+	+	+	−	−
OK15	+	+	+	+	−	−
OK17	+	−	+	+	−	−
OK19	+	+	+	+	+w	+
OK20	+	+	+	+	−	−
L-3	+	+	+	+	−	+
L-125	+	−	+	+	−	−
L-164	+	−	+	+	−	−
Sake yeast						
IFO 2193	+	−	−	+	ND	+
British ale yeast						
NCYC 1026	+	+	+	+	−	−
1085	+	−	+	+	−	−
1139	+	+	+	+	+	+
1331	+	−	−	+	−	−
DIB	+	−	−(w)	+	−	−
DICH	+	−	−	+	+	−

Glu = glucose; Gal = galactose; Mal = maltose; Suc = sucrose; α-MG = α-methyl glucoside; Raff = raffinose.

Table II. Fermentation of sugars under microaerophilic conditions by respiratory-competent industrial yeast strains.

Strains	Gal	Mal	Suc	α-MG	Raff
Distiller's					
YS 2968	+	+	−	−	+
2969	+	+	+	−	+
2970	+	+	+	−	+
2971	+	+	+	−	+
2972	+	+	+	−	+
2973	+	+	+	−	+
2974	+	+	+	−	+
2975	+	+	−	−	+
Lager yeasts					
OK11	+	+	+	−	+
OK13	+	+	+	−	+
OK15	+	+	+	+	+(slow)
OK17	+	+	+	−	+
OK19	+	+	+	−	+
OK20	+	+	+	−	+
L-1	+	+	+	+	+
L-3	+	+	+	−	+
L-27	+	+	+	−	+
L-30	+	+	+	+(slow)	+
L-125	+	+	+	+	+
L-164	+	+	+	−	+
Sake yeast					
IFO 2193	−	+	+	−	+
British ale yeasts					
NCYC 1085	+	+	+	−	+
1139	−	+	+	−	+
1331	+(very slow)	+	+	+	+
1335	−	+	+	+	+
DIB	+(slow)	+	+	+	−

Gal = galactose; Mal = maltose; Suc = sucrose; α-MG = α-methyl glucoside; Raff = raffinose.

Table III. Microaerophilic fermentation of higher concentrations of sugars by respiratory-compentent strains of industrial yeasts whose petite forms do not metabolize galactose[a].

Strains	Glu	Gal	Mal
YS 2969	+	+	+
2972	+	w(7 days)	+
2193	+	−	+
DIB	+	−	+
1331	+	−	+
OK17	+	+	+

[a] Sugar concentrations 10%. All tubes covered with sterile mineral oil after inoculation. Petite of OK17 utilized maltose but not galactose. Petites of all other strains failed to utilize either maltose of galactose.

The patterns of fermentation of sugars by the respiratory-competent parent strains, under microaerophilic conditions, was quite unlike that of the petites in most cases. Whereas the parent strains tested by Evans and Wilkie [2] failed to metabolize

the sugars which were not utilized by the corresponding petites forms, when placed under microaerophilic conditions, most of the industrial yeast strains tested metabolized and fermented all of the sugars tested in the present experiments. The exceptions were the sake yeast, which did not ferment galactose, two British brewing yeasts, which also did not ferment galactose, one strain which did not ferment raffinose, 19 which did not ferment α-methyl glucoside, and in particular, two distiller's yeasts which did not ferment sucrose. The petites of these two strains did not utilize sucrose aerobically. In addition, fermentation of raffinose, α-methyl glucoside, and occasionally of galactose, was slow in many strains.

Thus, the results differ considerably from those predicted by the data presented by Evans and Wilkie [1, 2]. Although inhibition of the mitochondrial functions by conversion of these strains to the petite form inhibits the ability of some of the strains to metabolize these sugars, the presumed inhibition of mitochondrial function in the same strains by growth under microaerophilic conditions had no effect on the ability of strains which in the petite form, did not metabolize a particular sugar, to ferment the same sugar, except in the case of α-methyl glucoside, where somewhat better agreement was observed.

When six respiratory-competent strains which did not metabolize galactose in the petite form were tested for the ability to ferment higher (10%) concentrations of glucose, galactose or maltose under microaerophilic conditions, three of them failed to ferment galactose and one other did so only after a delay of 7 days. One (OK17), a lager yeast, which metabolized maltose but not galactose when converted to the petite form, fermented galactose vigorously under these conditions. Glucose and maltose were fermented vigorously by all strains. At this concentration of glucose, mitochondrial function is normally completely inhibited.

Concerning the strains which did not metabolize sucrose when in the petite form (YS 2968 and YS 2975), or ferment it under microaerophilic conditions, it should be noted that this sugar, unlike maltose and galactose, is not transported into the cytoplasm before utilization, but the enzyme responsible (invertase) is normally transported across the cytoplasmic membrane to a location in the periplasmic space or is bound to the outside of the membrane. We have observed (Spencer *et al.* [5]) that invertase is present in the cytoplasm of petite mutants which do not metabolize sucrose, so that apparently the enzyme in not transported across the cytoplasmic membrane to its normal location. We have not as yet determined whether the invertase which we have observed in the petites of these two strains is in the glycosylated or non-glycosylated form. Neither is it yet certain whether this is a particular case of a general phenomenon which may be useful in investigating the problem of protein export from the yeast cell, but we have observed that killer strains which do not metabolize sucrose when in the petite form, remain killers as petites and hence continue to produce and export the proteinaceous killer toxin.

The phenomenon we have described may be of some practical importance in the fermentation industry. While normal brewing and distilling processes use respiratory-competent yeast strains which ferment all of the sugars normally found in cereal grain mashes, if the use of petite strains becomes more widespread, as suggested by Moulin *et al.* [4], then brewers and distillers must consider the nature of the mutant strain and whether it will ferment all of the sugars present in their mashes. It may be significant that the two yeasts whose petite forms do not metabolize sucrose are distillery strains. Presumably they are normally used for the fermentation of cereal mashes, but it would be desirable to investigate yeasts which are used, for instance, for fermentation of molasses mashes where the principal sugars present are sucrose and/or invert sugar. The two strains under consideration do not ferment sucrose under anaerobic conditions, even when in the respiratory-competent form, so that presumably they would utilize sucrose less efficiently during fermentation of a molasses mash.

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