Journal of Industrial Microbiology & Biotechnology (1999) 22, 627–632

http://www.stockton-press.co.uk/jim

Enhancement of maltose utilisation by *Saccharomyces cerevisiae* in medium containing fermentable hexoses

BW Hazell and PV Attfield

School of Biological Sciences, Macquarie University, Sydney, New South Wales 2109, Australia

Some industrial strains of *Saccharomyces cerevisiae* are unable to maintain high rates of fermentation during transition from catabolism of hexoses to maltose. This phenomenon, termed 'maltose lag', presents problems for the baking, brewing and distilling industries, which rely on yeast catabolism of mixtures of hexoses and maltose. Maltose utilisation requires the presence of maltose permease and α-glucosidase (maltase), encoded by *MAL* genes. Synthesis of these is induced by maltose and repressed by glucose. One strain of baker's yeast used in this work exhibited a marked maltose lag, whereas a second strain exhibited a shorter lag during conversion from hexose to maltose metabolism. The extent of the lag was linked to the levels of maltose permease and maltase in cells at the time of inoculation into mixed sugar medium. This view is supported by results showing that pulsing yeast with maltose to induce expression of *MAL* genes prior to inoculation into mixed sugar medium, enhanced sugar fermentation. Maltose pulsing of yeasts could therefore be useful for enhancing some fermentations relevant to baking and other yeast industries.

Keywords: fermentation; maltose metabolism; yeast; baking; distilling; brewing; *Saccharomyces cerevisiae*

Introduction

Maltose (4-*O*-α-d-glucopyranosyl-d-glucopyranose) is the most abundant fermentable sugar available to yeasts in unsweetened bread doughs, brewing worts and whiskey distilling mashes [21]. Therefore, ability to ferment maltose is crucial to efficient performance of yeast strains in these industries. Strains of yeast used in these traditional industries are mostly *Saccharomyces cerevisiae* or closely related species. They need to carry at least one functional *MAL* locus in order to transport maltose and cleave it intracellularly to produce d-glucose, which is phosphorylated and catabolised. There can be five unlinked *MAL* loci (*MALI-4* and *6*) in strains of *S. cerevisiae* [19]. Each of these consists of *MALX1* that encodes a maltose permease, *MALX2* that encodes α-glucosidase (maltase), and *MALX3* that encodes a positive transcriptional regulator of *MAL*. Some authors describe these as *MALXT*, *MALXS* and *MALXR*, respectively [12]. The *MALX1* and *MALX2* genes are transcribed divergently from a single promoter and the *MALX3* protein interacts with regulatory sequences within the intergenic promoter region. Transcription of *MAL* loci is induced by maltose. Under conditions of zero maltose and low or nil glucose, extremely low levels of maltose permease and maltase are produced without *MALX3* expression being required [22]. It is through this initial low amount of maltose permease that added maltose enters the cell, activating *MALX3* protein, which in turn increases transcription of *MALX1* and *X2* [23]. Glucose repression of maltose utilisation is complex. The promoter regions of the *MAL* loci have MIG1-binding sites and repression is partially *MIG1*-dependent [10], but other factors such as glucose-stimulated mRNA degradation of *MALS* transcript [8] and catabolite inactivation of maltose permease are important too [12].

Generally, a mixture of sugars is available to yeasts in baking, brewing and distiller's fermentation processes. Thus, yeast can be inoculated into relatively complex media that contain hexoses such as glucose and fructose, disaccharides including sucrose and maltose, trisaccharides (eg, maltotriose) and oligosaccharides (eg, maltotetraose). Maltose can be present as 50% or more of the total fermentable sugar in these processes [14,21]. Strains of *S. cerevisiae* normally utilise glucose and fructose in preference to maltose and the more complex sugars. Ideally, for an efficient fermentation process, it is desirable that yeast rapidly convert from metabolism of glucose and fructose to utilise maltose, even while some hexoses persist. If this does not occur, a transient fall in the rate of fermentation results in a drop in production of CO_2 and ethanol. This phenomenon, termed 'maltose lag', is often undesirable in industrial processes as it leads to extended times for fermentations.

In this paper we describe experiments designed to determine the impact of maltose lag in strains of baker's yeast. Ability to ferment maltose in a medium also containing glucose and fructose was assessed along with activities of maltose-utilising enzymes. Yeasts were cultured under conditions that were either inducing, repressing, or non-inducing/non-repressing for *MAL* gene expression. We also demonstrated the potential to reduce the impact of maltose lag by pulsing yeast with maltose prior to inoculation into mixed sugar medium.

Materials and methods

Strains and culture conditions

Two strains of industrial baker's yeast (*S. cerevisiae*) were obtained from Burns Philp R&D Pty Ltd, Sydney, Australia. Strain YPG.01 exhibits a marked maltose lag,

Correspondence: Dr PV Attfield, School of Biological Sciences, Macquarie University, Sydney, New South Wales 2109, Australia
Received 24 December 1988; accepted 18 March 1999

whereas strain YPG.03 has a relatively smaller maltose lag in bread doughs containing no added sugars. Genetic analysis by Ty1 probing revealed they are distinct strains (data not shown). Yeast extract, peptone medium (YP) contained L^{-1}: 10 g bacteriological peptone, 5 g yeast extract and 3 g KH_2PO_4. Media were designated: GYP, glucose, yeast extract, peptone; MYP, maltose, yeast extract, peptone; GlyEtYP, glycerol, ethanol, yeast extract, peptone; 2% GYP contained 20 g L^{-1} glucose, GYP contained 50 g L^{-1} glucose, MYP 50 g L^{-1} maltose, and GlyEtYP contained 30 g L^{-1} glycerol plus 20 g L^{-1} ethanol. Sucrose minimal medium contained L^{-1}: 10 g sucrose, 0.25 g KH_2PO_4 1.2 g $(NH_4)_2SO_4$, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.5 g $MgSO_4 \cdot 7H_2O$, 3.42 g citric acid, 8.9 g trisodium citrate, 6.05 mg ferric citrate, 0.2 mg $CuSO_4 \cdot 5H_2O$ and 0.5 mg $Na_2B_4O_7$. This was autoclaved for 15 min at 115°C and then supplemented with filter-sterilised vitamins to final concentrations L^{-1}, of 1 mg Ca-pantothenate, 1 mg thiamine HCl, 1 mg pyridoxine HCl, 2 mg inositol, 0.5 mg nicotinic acid and 0.2 mg biotin. Yeast colonies were grown on 2% GYP agar (15 g L^{-1} agar) plates at 30°C for 3 days and stored at 4°C to give stock cultures. Inocula were prepared for experiments by transferring three colonies into 50 ml liquid medium in a 250-ml conical flask and incubating the flask at 30°C and 180 rpm for 18 h. Twenty-five millilitres of this culture were used to inoculate 500 ml pre-warmed medium in a 2-L conical flask, which was incubated at 30°C and 180 rpm for 4 h. In the case of GlyEtYP-grown cultures, the full 50 ml of inoculum was added to medium for the second incubation period, which was extended to 8 h. This allowed for the fact that growth of yeast is slower on respirable as opposed to fermentable carbon-sources.

Preparation of cell extracts

Cultures were harvested by centrifugation at 2500 × *g* and 4°C for 5 min. Supernatants were discarded and cells washed with 4°C distilled water. Yeast pellets were resuspended in an approximately equal volume of 4°C 50 mM potassium phosphate buffer, pH 7.25 containing 1 mM phenylmethylsulphonyl fluoride. Glass beads that had been stored at –20°C were added (1.5 g ml^{-1}) to suspended cells which were then disrupted using a CO_2-cooled Braun homogeniser (Crown Scientific, Sydney, Australia) at 4000 oscillations min^{-1} for 30 s. Disrupted samples were transferred to 50 ml-centrifuge tubes and centrifuged at 2500 × *g* for 10 min at 4°C. Supernatants were used for enzyme assays and determination of protein concentrations.

Enzyme assays

The substrate for assay of maltase was *para*-nitrophenyl-α-d-glucopyranoside (pNPG). It was prepared by dissolving 50 mg in 50 ml of 50 mM potassium phosphate buffer, pH 7.25. Reactions were started by addition of 100 μl cell extract to 5 ml pNPG solution incubated at 30°C. Aliquots (1 ml) were removed at intervals and vortex mixed immediately with 1 ml 0.1 M sodium carbonate. Absorbance by free *para*-nitrophenol formed by cleavage of pNPG was measured at 410 nm. Enzyme activities are given as units where one unit equals one μmol pNP released min^{-1} mg $protein^{-1}$. Proteins in cell extracts were assayed using the enhanced bicinchoninic protein assay protocol (Pierce Chemical Co, Sydney, Australia) according to the manufacturer's instruction.

Uptake of maltose was measured in whole cells using a hydrogen symport assay. Cells were harvested, washed and resuspended in distilled water. Final cell density was adjusted to approximately 10^8 cells ml^{-1} in 10 ml distilled water (measured by absorbance at 640 nm and checked by microscopy for total counts). Iodoacetamide was added (100 μl of fresh 1 M stock) to inhibit glycolysis and extrusion of protons. The suspension of cells, contained in a Universal bottle, was placed in a 30°C water bath over a magnetic stirrer. A small magnetic stirrer bar was added and a pH probe inserted into the solution. The suspension was incubated for 10 min and then adjusted to exactly pH 6.0. One millilitre of 20 g L^{-1} maltose was added and pH change recorded over the initial 15-s period. The rise in pH related to stoichiometric proton and maltose symport. Maltose permease activities are given as units, where one unit equals one nmol maltose transported min^{-1} 10^9 $cells^{-1}$.

Isocitrate lyase was assayed as described in [4]. Succinate dehydrogenase and cytochrome *c* oxidase were assayed according to [5]. Cell breakage and assay buffer for these enzymes was 0.6 M sorbitol, 1 mM EDTA and 10 mM KH_2PO_4 adjusted to pH 7.25. Activities of these enzymes are given as nmoles substrate utilised, or product formed min^{-1} mg $protein^{-1}$.

Fermentation testing

Yeast were grown in sucrose minimal medium and harvested in late respiratory phase as ethanol was depleted, according to [17]. In some experiments yeasts were 'maltose pulsed' by culturing in sucrose minimal medium until ethanol was depleted to 1.5 g L^{-1} then adding maltose at 4 g L^{-1}. These cultures were then incubated to allow fermentation and respiration of maltose before harvesting them when ethanol had been depleted. Fermentation testing medium contained L^{-1}: 43 g maltose, 3.75 g glucose, 3.25 g fructose, 2.07 g KH_2PO_4, 2.76 g $MgSO_4 \cdot 7H_2O$, 0.67 g $(NH_4)_2SO_4$, 2.07 g casein hydrolysate, 4.02 g citric acid, 44.25 g trisodium citrate, 9.2 mg thiamine, 9.2 mg riboflavin, 46 mg nicotinic acid, 18.2 mg pantothenic acid and 231 μg biotin. Yeasts were assayed for fermentative activity by measuring CO_2 production as follows: 1.2 ± 0.005 g filtered yeast was added to 5 ml fermentation medium prewarmed at 30°C. This was then mixed into 45 ml of the same medium contained in a stirred 'risograph' vessel that was submerged in a 30°C water bath. Gas evolution was measured for up to 3 h using a Risograph instrument (R Design Model 1260, Pullman, Washington, USA). To permit analysis of supernatant media for ethanol and sugars, parallel cultures were set up but not connected to the Risograph instrument. Samples of culture were removed at indicated times, microcentrifuged and assayed as described below.

Assay of ethanol and sugars

Ethanol was determined in culture supernatant against known standards using a Perkin Elmer gas chromatograph with flame ionisation detector (Perkin Elmer, Sydney, Australia). Separation was on a 25 m × 0.33 mm bonded phase, fused silica capillary column with a film thickness

of 0.25 μm. Flow rate for hydrogen carrier gas was 2.7 ml min^{-1}. Run time was 2 min with column temperature of 150°C. Injector and detector temperatures were 180°C and 200°C, respectively.

Sugars were derivatised prior to gas chromatography. Trimethylsilane oxime derivatives were formed using the procedure: 50 μl of each sample was transferred to 5-ml reaction vials. Samples were dried in a water bath at 70°C using a constant stream of dry nitrogen gas. Hydroxylamine HCl (300 μl of a 25 mg ml^{-1} stock in pyridine) was added. Samples were mixed and placed at 75°C for 20 min, then re-mixed and incubated for a further 10 min. Silylation was performed by addition of 1 ml hexamethyldisilazine followed by 100 μl trifluoroacetic acid, mixing and allowing the mixture to stand at room temperature for 30 min prior to gas chromatography. A standard of each sugar was made and treated similarly. A known concentration of xylose was included as an internal marker in all samples and sugar standards.

Results

Yeast strains YPG.01 and YPG.03 were tested for their abilities to ferment a mixed sugar medium that contained 3.75 g L^{-1} glucose and 3.25 g L^{-1} fructose, plus 43 g L^{-1} maltose as fermentable carbon. These sugar concentrations were chosen because they mimic the levels of sugars in yeasted bread doughs immediately after the doughs are mixed [21]. Figure 1 shows that both strains had similar fermentative activities as measured by CO_2 evolution up to 40 min. From 50 to about 75 min, strain YPG.03 produced a flat rate, whereas strain YPG.01 exhibited a drop in fermentation. Thereafter fermentation rates increased but not to the same level as observed in the initial 40 min of incubation. Parallel studies showed that ethanol production followed the same strain-dependent patterns as CO_2 evolution (data not shown).

Concentrations of sugars were measured in order to determine their relative utilisation by the yeasts. Strain YPG.03 produced more total CO_2 than strain YPG.01 for less consumption of glucose and fructose in the first hour of incubation (Table 1). Up to this time, strain YPG.03 had utilised 10% of the total maltose whereas strain YPG.01 had only consumed 6%. By 90 min, strain YPG.03 had utilised almost 30% of maltose whereas strain YPG.01 had only used 10% (Table 1).

Relatively slow fermentation of maltose by strain YPG.01 could be due to intrinsically lower levels of maltose utilising enzymes in this yeast relative to strain YPG.03. To test this, the strains were assayed for maltose permease and maltase activities after growth on GlyEtYP (non-inducing/non-repressing), GYP (repressing) or MYP (inducing). Both strains showed the lowest levels of maltose-utilising activities when grown in glucose medium and the highest when grown in maltose (Table 2). Comparing the two strains, YPG.01 produced lower activities than YPG.03. Relative activities were closest when strains were fully induced (maltose). Strain YPG.01 produced particularly low maltase activity when grown in glucose. This strain also had notably lower maltose permease and maltase

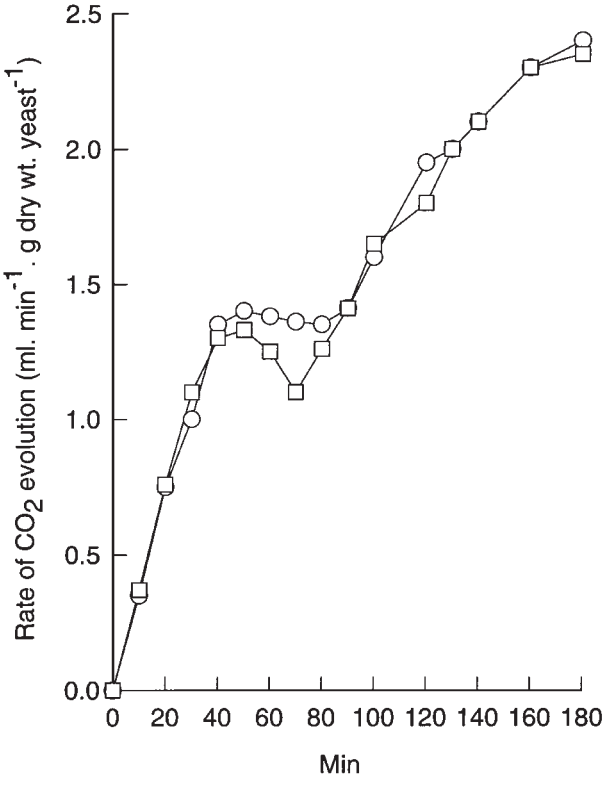

Figure 1 Fermentation rates of yeast strains in mixed sugar medium. Strains YPG.01 and YPG.03 were grown on sucrose minimal medium until all sugar and ethanol was depleted. They were then inoculated separately into fermentation medium containing 3.75 g L^{-1} glucose, 3.25 g L^{-1} fructose, and 43 g L^{-1} maltose as described in Materials and Methods. □ Strain YPG.01; ○, strain YPG.03. Data shown are typical of three separate experiments assayed in duplicate. Errors between duplicates were ≤2% and between experiments <5%.

than strain YPG.03 after growth in glycerol/ethanol (Table 2).

Both yeast strains had low maltose permease and maltase activities when grown on glucose (Table 2), indicating sensitivity to catabolite repression. The possibility arises that differences in maltose fermentation between strains YPG.01 and YPG.03 result from differences in sensitivities to catabolite repression, or inactivation (of maltose permease). Therefore the strains were assayed for activities of other enzymes subject to catabolite repression. Isocitrate lyase, succinate dehydrogenase and cytochrome *c* oxidase were similarly high in both yeasts grown on glycerol/ethanol (Table 3). Activities of these enzymes were lower but similar for both strains when the strains were grown on glucose.

An alternative explanation for reduced maltose utilisation by strain YPG.01 relative to strain YPG.03, is that the maltose permease of the former strain is more sensitive to catabolite inactivation. To test this, cells were assayed for permease after growth in MYP and subsequent incubation for 90 min in 50 mM potassium phosphate buffer, pH 6.0, which tested for intrinsic stability of permease. Separate samples of cells were also incubated in buffer that contained either glucose (inactivation), maltose (non-inactivation) or glucose and maltose. The latter tested the ability of maltose to counteract any inactivating effect of

Table 1 Sugar depletion by yeasts during fermentation of mixed sugar medium

Time (min)	CO_2 evolved (ml g dry yeast^{-1})	Total glucose (g L^{-1})	Total fructose (g L^{-1})	Total maltose (g L^{-1})
Strain YPG.01:				
50	47	0.75	1.9	–
60	59	0.34	1.5	40
70	69	0.17	0.98	–
80	81	0.12	0.65	–
90	96	0.072	0.46	38
100	112	0.038	0.30	–
110	131	ND	0.19	–
120	150	ND	0.13	34
Strain YPG.03:				
50	51	0.94	2.0	–
60	65	0.56	1.7	38
70	79	0.29	0.94	–
80	93	0.18	0.88	–
90	107	0.11	0.62	31
100	122	0.078	0.46	–
110	139	0.060	0.31	–
120	158	ND	0.24	25

Values for sugars are given to two significant figures and refer to concentrations remaining in the medium at the given time. Initial (time 0) sugar concentrations were: 3.75 g L^{-1} glucose, 3.25 g L^{-1} fructose and 43 g L^{-1} maltose. ND = none detected. Data are typical of duplicate assays from two separate experiments. Errors between duplicates were $<5\%$ and between experiments $\leq 8\%$.

Table 2 Maltose permease and maltase activities of yeast strains

Strain	Glucose	Glucose	Glycerol/ethanol	Glycerol/ethanol	Maltose	Maltose
	Permease	Maltase	Permease	Maltase	Permease	Maltase
YPG.01	ND	0.0051 (± 0.00015)	1.2 (± 0.061)	0.099 (± 0.0019)	2.0 (± 0.070)	0.62 (± 0.049)
YPG.03	ND	0.025 (± 0.0011)	3.1 (± 0.15)	0.40 (± 0.012)	3.7 (± 0.034)	0.69 (± 0.041)
YPG.03[a] / YPG.01	–	4.9	2.6	4.0	1.9	1.1

Strains were grown as described in Materials and Methods, with the carbon sources indicated. Enzyme activities (defined in Materials and Methods) are means (± standard deviations) given to two significant figures, derived from three to five repeated experiments.
ND = none detected.
[a]Relative mean activities between the two strains.

Table 3 Specific activities of enzymes sensitive to carbon catabolite repression in yeast

Strain	Carbon source	Isocitrate lyase	Succinate dehydrogenase	Cytochrome *c* oxidase
YPG.01	Glucose	0.51 (± 0.025)	2.6 (± 0.031)	49 (± 0.38)
YPG.01	Glycerol/ethanol	18 (± 0.95)	14 (± 0.81)	87 (± 2.1)
YPG.03	Glucose	0.63 (± 0.024)	2.8 (± 0.026)	45 (± 1.2)
YPG.03	Glycerol/ethanol	35 (± 2.8)	13 (± 0.66)	91 (± 2.7)

Yeasts were grown in either GYP or GlyEtYP before being harvested, disrupted and extracts assayed for activities as described in Materials and Methods. Enzyme activities (defined in Materials and Methods) are means (± standard deviations) given to two significant figures, derived from three to five repeated experiments.

glucose. Sugars were added at 20 g L^{-1}, a concentration at which glucose effects catabolite inactivation [9]. Strain YPG.01 grown in MYP, had an initial maltose permease activity of 2.0 (± 0.07) units and lost 20% of this activity when incubated in potassium phosphate buffer without sugar for 90 min (data not shown). Strain YPG.03 had an initial activity of 3.7 (± 0.034) and lost 8% when incubated in buffer without sugar. This suggests the intrinsic stability of maltose permease may be lower in strain YPG.01 relative to strain YPG.03. Both strains lost 40% of their initial maltose permease activity when incubated in glucose. Addition of maltose with glucose failed to alleviate inactivation effects of the hexose in either strain (data not shown). These results indicate that maltose permease in

strain YPG.01 may be more intrinsically unstable than in strain YPG.03, but that it is not particularly sensitive to catabolite inactivation.

These results raise the possibility that maltose fermentation by strain YPG.01 could be improved if it had higher levels of maltose permease and maltase at the time of inoculation into fermentation medium. To test this, strain YPG.01 was grown in sucrose minimal medium until the sugar was consumed and ethanol was respired to 1.5 g L^{-1}. Maltose pulsing was then applied as described in Materials and Methods and yeast was assayed for fermentative activity and maltose-utilising enzymes. Figure 2 shows that yeast pulsed with maltose maintained a higher rate of fermentation after about 45 min. Improved fermentation by maltose-pulsed strain YPG.01 relative to non-pulsed strain YPG.01 was most marked in the second hour of incubation, when glucose and fructose would have been significantly depleted and maltose was the major carbon source available (Table 1). Yeast that had not been pulsed with maltose had 2.1 (± 0.074) units of maltose permease and 0.24 (± 0.012) units of maltase, whereas the maltose-pulsed yeast produced 5.1 (± 0.22) and 0.64 (± 0.032) units of these activities, respectively. By comparison, strain YPG.03 grown in sucrose minimal medium without maltose pulsing produced 3.2 (± 0.16) units of maltose permease and 0.50 (± 0.021) units of maltase.

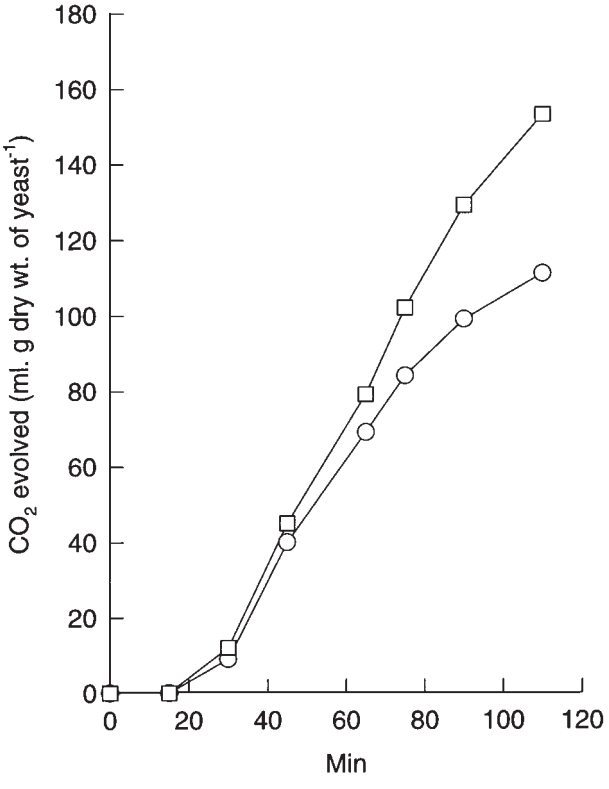

Figure 2 Fermentation by maltose-pulsed and -non-pulsed yeast in mixed sugar medium. Strain YPG.01 was grown in sucrose minimal medium and subjected to a pulse of maltose as described in Materials and Methods. Yeast samples were inoculated into fermentation medium containing 3.75 g L^{-1} glucose, 3.25 g L^{-1} fructose, and 43 g L^{-1} maltose as described in Materials and Methods. □, Strain YPG.01 pulsed with maltose; ○, strain YPG.01 nonpulsed. Data shown are typical of three separate experiments assayed in duplicate. Errors between duplicates were ≤2% and between experiments <5%.

Discussion

Many of the traditional yeast fermentation industries require strains that are able to ferment maltose efficiently in the presence of relatively lower levels of hexoses. Ideally, strains should be able to maintain a steady rate of fermentation whilst undergoing transition from catabolism of glucose or fructose to maltose. As shown in this work, some strains of yeast actually exhibit a fall in fermentation rate when glucose and fructose are nearing exhaustion and maltose is the major fermentable carbon source remaining. This lag in maltose fermentation appears to be unrelated to general catabolic controls such as carbon catabolite repression or inactivation carried over from the period of fermentation on glucose and fructose. This view is supported by the data indicating that catabolite-repressed activities were similar in the two strains (one strongly maltose lagging, the other less so) after growth on repressing medium containing glucose. Also, maltose permease activity of the strongly maltose lagging strain, strain YPG.01, was not more sensitive to catabolite inactivation than that of the lesser lagging strain, YPG.03.

The strain exhibiting marked maltose lag was able to produce similar high levels of maltose permease and maltase in comparison to the lesser lagging strain when grown on maltose medium. Moreover, the two strains eventually achieved similar rates of fermentation on maltose. This indicates an intrinsic ability of both strains to synthesise sufficient levels of the maltose-utilising activities when maltose is present in the absence of glucose or fructose. However when grown under the non-inducing and non-repressing conditions (glycerol/ethanol), strain YPG.03 had four-fold more maltase and over two-fold higher maltose permease activities than strain YPG.01. Prior to fermentation testing, yeasts were grown in aerobic batch culture in sucrose minimal medium to a point where ethanol was completely respired. This procedure was followed because it provided yeast biomass in a physiological state that is similar to that of yeast used in baking and distiller's applications. Yeast biomass for use in these industries, is grown by aerobic fed-batch methods on molasses or glucose syrups that have very little or no maltose [3,21]. As a result yeast is uninduced with respect to maltose metabolism. Maltose-utilising activities of the yeasts were non-induced and non-repressed by growth in sucrose minimal or glycerol/ethanol media. However, strain YPG.03 had higher levels of these enzymes in both types of media, and produced better maltose utilisation than strain YPG.01 after growth in sucrose minimal medium.

Others have found high activities of maltase in the presence of glucose in yeast strains that do not exhibit maltose lag [20]. Our results imply that the extent of maltose lag is linked to the activity of maltose-utilising enzymes at the time of inoculation into mixed sugar medium. Whilst capacity for *de novo* synthesis may be important for final fermentation rates on maltose, it is the initial activities of maltose-utilising enzymes that determine the ability of yeasts to ferment maltose in the presence of hexoses. This

is important for diminishing the maltose lag phenomenon. This view is supported by the fact that strain YPG.03 had higher maltose-utilising activities than strain YPG.01 after growth in sucrose minimal medium and had a lesser maltose lag. Moreover, strain YPG.01 was able to ferment maltose more efficiently after undergoing a maltose pulse to induce maltose permease and maltase activities, prior to inoculation into mixed sugar medium. Other workers have reported the improvement of maltose metabolism in mixed sugar medium (brewer's wort) following growth of brewer's yeast in medium that contains maltose as the sole carbon source [7]. Our results indicate that, at least in the case of baker's yeast, it is only necessary to pulse the biomass with maltose to induce the MAL system in order to reduce the impact of maltose lag.

What is the nature of the difference in maltose metabolism between strains YPG.01 and YPG0.3? It is unlikely that functional *MAL* gene copy number is the cause because both strains produced similar levels of maltose-utilising activities after growth on maltose. It is more likely that mutations in the *MAL* loci are relevant to the maltose lag characteristics of these yeasts. Mutant laboratory strains of *S. cerevisiae* with altered maltose utilisation have been isolated. These include strains with mutations in general carbon metabolism [1,6,15,25]. The implication of results reported here is that carbon catabolite repression or inactivation is not relevant to maltose fermentation by these particular industrial strains of yeast in mixed sugar medium that contains hexoses at concentrations similar to those occurring in unsweetened bread doughs. Other mutant strains of yeast have been isolated with altered expression of maltose-utilising genes, and there has been extensive research characterising the *MAL* loci, maltose utilisation phenotypes and constitutive expression of maltose permease and maltase in laboratory and naturally occurring strains of yeast. In particular, mutants that have increased maltose permease and maltase expression have been shown to map to the *MALX3* (regulatory) gene [2,11,13,16,18,24]. It is feasible that altered positive regulation of *MAL* loci is responsible for differences between strains YPG.01 and YPG.03. However, the exact nature of differences remains to be clarified. The genetic and molecular nature of variations between *MAL* loci of industrial yeast strains is the subject of further work.

In conclusion, the results reported indicate that levels of maltose permease and maltase in yeast cells at the time of inoculation into mixed sugar medium impact on the extent of maltose lag observed. Improved fermentation by yeast pulsed with maltose prior to inoculation into mixed sugar medium supports this view and provides a potential means of conditioning yeast for more efficient fermentations in baking, brewing and distiller's applications.

References

1 Carlson M, BC Osmond, L Neighbourne and D Botstein. 1984. A suppressor of *SNF1* mutations causes high level invertase synthesis in yeast. Genetics 107: 19–32.

2 Charron MJ and CA Michels. 1988. The naturally occurring alleles of *MALI* in *Saccharomyces* species evolved by various mutagenic processes including chromosomal rearrangement. Genetics 120: 83–93.

3 Chen SL and M Chiger. 1985. Production of baker's yeast. In: Comprehensive Biotechnology (Blanch HW, S Drew, DIC Wang and M Moo-Young, eds), pp 429–462, Pergamon Press, New York.

4 Dixon GH and T Kornberg. 1959. Assay methods for key enzymes of the glyoxalate cycle. J Biol Chem 72: 3P.

5 Duncan HM and B Mackler. 1966. Electron transport systems of yeast. III. Preparation and properties of cytochrome oxidase. J Biol Chem 241: 1964–1967.

6 Entian KD. 1980. Genetic and biochemical evidence for hexokinase PII as a key enzyme involved in carbon catabolite repression in yeast. Mol Gen Genet 178: 633–637.

7 Ernandes JR, JW Williams, I Russell and GG Stewart. 1993. Effect of yeast adaptation to maltose utilisation on sugar uptake during the fermentation of brewer's wort. J Inst Brew 99: 67–71.

8 Federoff HJ, TR Ecclesall and J Marmur. 1983 Carbon catabolite repression of maltase synthesis in *Saccharomyces cerevisiae*. J Bacteriol 156: 301–307.

9 Gorts CPM. 1969. Effects of glucose on activity and the kinetics of the maltose uptake and of α-glucosidase in *Saccharomyces cerevisiae*. Biochim Biophys Acta 146: 173–180.

10 Hu Z, JO Nehlin, H Ronne and CA Michels. 1995. *MIG1*-dependent and *MIG1*-independent glucose regulation of *MAL* gene expression in *Saccharomyces cerevisiae*. Curr Genet 28: 258–266.

11 Khan NA and NR Eaton. 1971. Genetic control of maltose formation in yeast. I. Strains producing high and low basal levels of enzyme. Mol Gen Genet 112: 317–322.

12 Klein CJL, L Olsson and J Nielsen. 1998. Glucose control in *Saccharomyces cerevisiae*: the role of *MIG1* in metabolic functions. Microbiology 144: 13–24.

13 Kopetski E, E Zellner, G Schunecher and FK Zimmerman. 1989. Nucleotide sequence of the *Saccharomyces* positive regulatory mutant gene *MAL2-8^{c}p. Nucl Acid Res 17: 5390.*

14 MacWilliam IC. 1968. Wort composition: a review. J Inst Brew 74: 38–54.

15 Michels CA and A Romanowski. 1980. Glucose repression resistant mutation in *Saccharomyces carlsbergensis.* J Bacteriol 143: 674–679.

16 Mowshowitz DB. 1981. The effects of three different loci on the regulation of maltase synthesis in yeast. Genetics 95: 713–728.

17 Myers DK, DTM Lawlor and PV Attfield. 1997. Influence of invertase activity and glycerol synthesis and retention on fermentation of media with a high sugar concentration by *Saccharomyces cerevisiae*. Appl Environ Micro 63: 145–150.

18 Naumov GI, ES Naumova and CA Michels. 1994. Genetic variation of repeated *MAL* loci in natural populations of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus*. Genetics 136: 803–812.

19 Needleman RB and CA Michels. 1983. Repeated family of genes controlling maltose fermentation in *Saccharomyces carlsbergensis*. Mol Cell Biol 3: 796–802.

20 Oda Y and K Ouchi. 1990. Role of yeast maltose fermentation genes in CO_2 production rate from sponge dough. Food Microbiol 7: 43–47.

21 Reed G and TW Nagodawithana. 1991. Yeast Technology, 2nd edn. Van Nostrand Reinhold, NY.

22 Rodicio R and FK Zimmermann. 1985. Cloning of the maltose regulatory genes of *Saccharomyces cerevisiae*. Curr Genet 9: 539–545.

23 Vanoni M, P Sollitti and J Marmur. 1989. Structure and regulation of the multigene family controlling maltose fermentation in budding yeast. Prog Nucl Acid Res 37: 281–322.

24 Zimmerman FK and NR Eaton. 1974. Genetics of induction and catabolite repression of maltose synthesis in *Saccharomyces cerevisiae*. Mol Gen Genet 134: 261–272.

25 Zimmerman FK and I Scheel. 1977. Mutants of *Saccharomyces cerevisiae* resistant to carbon catabolite repression. Mol Gen Genet 154: 75–82.