Direct Evidence That Maltose Transport Activity Is Affected by the Lipid Composition of Brewer's Yeast

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ABSTRACT

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A brewer's yeast strain was grown with maltose as sole carbon source under strictly anaerobic conditions with and without ergosterol and/or unsaturated fatty acid (Tween 80) supplements. Under all these conditions the *MALx1* genes for maltose transporters were strongly expressed during growth. The fatty acid unsaturation indices of growing and stationary phase yeast were increased from about 20% to 56–69% by supplementation with Tween 80. Ergosterol contents were increased up to at least 4-fold by supplementation with ergosterol and Tween 80. Maltose transport activity measured at 20°C was not increased by supplementation with Tween 80 alone, but was increased 2-fold and 3-fold, respectively, in growing and stationary phase yeast by supplementation with ergosterol together with Tween 80. The stimulation of maltose transport by ergosterol was greater when the transport was measured at temperatures (10°C and 0°C) lower than 20°C. The results show that proper function of maltose transporters requires adequate amounts of ergosterol in the yeast. This effect may partly explain the low maltose (and maltotriose) uptake rates both in the second half of brewery fermentations, when the sterol content of yeast has fallen, and when fresh wort is pitched with sterol-deficient cropped yeast.

Key words: Cropped yeast, ergosterol, lipid composition, maltose transport, temperature effects, unsaturated fatty acids.

INTRODUCTION

Yeast cropped from industrial scale wort fermentations has low contents of unsaturated fatty acids (UFA) and sterols because it has grown with very limited access to oxygen[2,8,17]. For this reason, wort must be aerated at the start of fermentations when, as is the usual case, cropped yeast is re-pitched to start a new fermentation. It is often stated that the low UFA and sterol contents of the plasma membrane prevent the proper function of sugar transporters, so that for the first few hours after re-pitching yeast is dependent upon its internal supplies of carbohydrate (glycogen and trehalose) because it is unable to transport wort sugars into the cells. Glycogen and trehalose are rapidly mobilised after pitching[21,23]. Glycogen mobilisation is believed to provide the energy needed to drive the oxygen-dependent synthesis of sterols and UFA. Efficient transport of sugars is said to be restored and wort fermentation commences only when adequate levels of sterols and UFA are reached.

Although this scenario is frequently described, we have not been able to find a direct demonstration that the sterol or fatty acid composition of the yeast plasma membrane affects the function of sugar transporters. Quain et al.[21] found no glucose uptake during the first 2 h after pitching, during which time the wort oxygen was consumed. They suggested that the lack of glucose uptake might reflect regulation of glucose transporters by the membrane lipid composition. However, although lack of glucose uptake is consistent with the proposed inability of glucose transporters to function properly in UFA- and sterol-deficient membranes, it does not prove such an inability. Because glucose transport is passive, failure to further metabolise intracellular glucose for any reason will rapidly prevent further glucose uptake. Keenan and Rose[15] could not find a significant difference between the kinetics of glucose transport by yeast enriched with, respectively, oleyl or linoleyl residues. In contrast to glucose, the transport kinetics of several amino acids are affected by the fatty acyl composition of yeast[6,15,20]. Calderbank et al.[6] found that the amount of general amino acid permease, but not its affinity towards alanine, was increased in yeast enriched with linoleyl residues, and suggested that in this case the altered fatty acid composition promoted intracellular trafficking of newly synthesised permease into the plasma membrane.

In mammalian cells the activity of the GLUT1-encoded glucose transporter is acutely sensitive to the phospholipid and sterol (cholesterol) composition of the membrane, as shown both by experiments with transporter reconstituted into artificial membranes and by using cells with manipulated lipid compositions[4,7,30]. In the present work we manipulated the sterol and fatty acid composition of yeast by growing it, with maltose as sole carbon source, under strictly anaerobic conditions in the presence or absence of lipid supplements. The rates of maltose and maltotriose transport and subsequent fermentation are known to be strongly regulated at transcription and post-translation levels by glucose repression of α-glucoside transporters and maltases and by glucose-triggered

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catabolite inactivation of the transporters[19,27]. Our objective was to investigate whether the rate of maltose transport is further limited by other physiological factors of practical importance during brewery fermentations.

MATERIALS AND METHODS

Yeast and cultivation

The yeast used was an industrial brewer's strain, A24, from VTT's collection. Possibly functional maltose transporter genes in this strain include *MAL11, MAL21, MAL31* and *MAL41,* whereas its *AGT1* gene is non-functional[28]. It also contains the recently discovered[9,24] maltose/maltotriose transporter gene, *MTT1* (Vidgren, V., Rautio J.J. and Londesborough, J., unpublished observations). Yeast for stocks was grown into stationary phase on YP (1% yeast extract, 2% peptone) containing 40 g maltose $\cdot L^{-1}$, collected and stored at –80°C as suspensions in 30% glycerol containing 200 mg fresh yeast mass $\cdot mL^{-1}$. Yeast suspension (150 µL) was inoculated into 300 mL of synthetic complete (SC) medium[26] containing 40 g maltose $\cdot L^{-1}$ and the indicated supplements in a 500 mL bottle. SC medium consists of (mg $\cdot L^{-1}$) yeast nitrogen base without amino acids, 6700; adenine, 13.5; arginine, 348; aspartate, 266; histidine, 58; myo-inositol, 36; isoleucine, 525; leucine 262; lysine, 91; methionine, 149; phenylalanine, 83; serine, 105; threonine, 119; tryptophan, 82; tyrosine, 30; uracil, 22 and valine, 117. The bottle was fitted with a magnetic stirrer, a glycerol-filled bubble trap and an inlet tube that reached below the surface of the medium. After inoculation air was purged from the medium and head space by passing CO_2 through the inlet tube and out of the bubble trap. Growths were conducted at 24°C. Biomass was estimated by turbidity (OD600) measurements on small samples withdrawn from one of each pair of replicate bottles anaerobically by syringe through the inlet tube. Where indicated the growth media (300 mL) were supplemented with either 600 µL of a 50:50 mixture of Tween 80 (Fluka Chemika) and ethanol (giving a final concentration of 0.1% (v/v) Tween 80) or with 600 µL of the same Tween 80/ethanol mixture containing also 7.5 mg ergosterol $\cdot mL^{-1}$ (giving final concentrations of 0.1% (v/v) Tween 80 and 15 mg ergosterol $\cdot L^{-1}$). Ergosterol was from Sigma (minimum purity, 90%). Aerobic growths were carried out, as stated in the text, in either SC or YP medium containing 40 g maltose $\cdot L^{-1}$ in conical flasks fitted with porous plugs and shaken (180 rpm) at 26°C.

Maltose transport

Standard maltose transport assays were made essentially as described[19]. Yeast were harvested by centrifugation (10 min at 5000 × g), washed with ice-cold distilled water and suspended to 200 mg fresh yeast $\cdot mL^{-1}$ in ice-cold 0.1 M tartrate/Tris buffer pH 4.2. Portions of about 1 mL were equilibrated to 20°C for 5 min immediately before assay. Reactions were started by adding 40 µL of yeast suspension to 20 µL of 15 mM ^{14}C-maltose (900 dpm $\cdot$ nmol^{-1}) in 15 mL tubes with conical bottoms. Reactions were stopped after 20 s by addition of 10 mL ice-cold water. The yeast was collected on a 0.45 µm HVLP filter (Millipore) and rinsed with 10 mL ice-cold water. The filter was transferred to scintillation cocktail and counted. Assays at 10 and 0°C were done with appropriately longer reaction times. Observed rates were at least 90% of the rates observed with 50% shorter reaction times. One U catalyses the uptake of 1 µmol of maltose $\cdot$ min^{-1} under the stated conditions. Rates were normalised to total yeast protein, determined by extracting the yeast overnight with 1 M NaOH and assaying the extract by biuret[11], or to yeast dry mass, determined by washing the yeast with water and drying it at 105°C for 16 h. Protein contents were close to 40% of dry masses.

Lipid analyses

Yeast was harvested by centrifugation (5 min at 5000 × g) and the pellets were washed twice with ice-cold distilled water and then suspended in ice-cold distilled water (5.0 mL $\cdot$ g yeast^{-1}). Four 500 µL portions were transferred to 12 mL flat-bottomed screw-cap tubes (Kimax) and centrifuged 10 min at 5000 × g. The supernatants were removed by Pasteur pipette and the pellets (83 mg yeast) stored under N_2 at –20°C. Duplicate samples for total fatty acids were saponified with 3 M NaOH in 50% (v/v) methanol and then esterified with 4 M HCl in methanol and the methyl esters extracted into hexane/methyl-t-butyl ether (1:1) and washed with 0.3 M NaOH. The organic phase was saturated with anhydrous Na_2SO_4 and the methyl fatty acid esters analysed by gas chromatography (GC) using an HP5890 gas chromatograph fitted with FID. The column was 30 m HP-Innowax, i.d. 0.258 mm and 0.5 µm film. Injector and detector were at 270°C. After 3 min at 50°C column temperature was raised at 25°C $\cdot$ min^{-1} to 180°C and then at 10°C $\cdot$ min^{-1} to 250°C, held for 7 min and further raised at 10°C $\cdot$ min^{-1} to 270°C and held for 20 min. The carrier gas was He and detector gases H_2 and synthetic air. Duplicate samples for total sterols were hydrolysed under N_2 with 20% KOH in ethanol and sterols extracted into pentane. The pentane was evaporated at room temperature under a stream of N_2 and the sample was silylated at room temperature by adding 180 µL of a mixture of N,O-bis(trimethylsilyl)-trifluoroacetamide and trimethylchlorosilane (2:1) and analysed in an HP 6890 gas chromatograph equipped with FID and on-column injector. The column was 15 m DB-1, i.d. 0.53 mm and 0.15 µm film. After 1.5 min at 100°C the temperature was raised at 12°C $\cdot$ min^{-1} to 330°C and held for 1.0 min.

Northern analyses

Total RNA was isolated from yeast using TRIzol (Life Technologies, Carlsbad, CA, U.S.A.) according to the manufacturer's instructions and quantified by measuring its A260 assuming 1 µg $\cdot mL^{-1}$ has an A260 of 25. RNA was denatured by treatment with glyoxal/dimethyl sulfoxide[5], subjected to electrophoresis in 1% agarose, and blotted onto a nylon membrane (Hybond, Amersham). Probes were made by PCR (the 779 nucleotide *MAL61* probe started from nucleotide 361 of the *MAL61* gene and the 986 nucleotide *AGT1* probe started from nucleotide 842 of the *AGT1* gene) and labelled by the Random primed DNA Labeling kit (Roche). After hybridisation the membrane was washed and band intensities were read with a phosphorimager.

RESULTS

Typical growth curves are shown in Fig. 1. Similar results were obtained in two other replicate experiments. In media without supplements the yeast grew slowly to a final OD 600 of 4.1. Addition of Tween 80 alone increased the growth rate, and raised the final OD 600 to 5.0. Addition of Tween 80 containing ergosterol further increased the growth rate and raised the final OD 600 to 7.6. Yeast in bottles that were not sampled during the first 23 h (open symbols in Fig. 1) did not grow more slowly than yeast in the sampled bottles, suggesting that the sampling procedure did not introduce air. Growing yeast was collected at 23.3 h by harvesting the unsampled bottles and stationary phase yeast at 48 h from the sampled bottles.

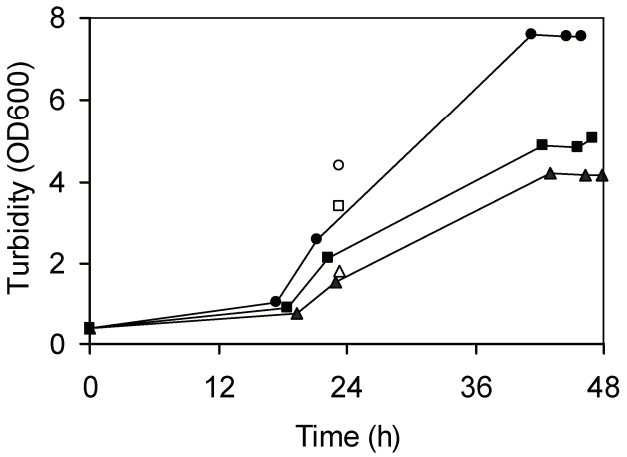

Fig. 1. Anaerobic growths. Media were supplemented with no addition (▲, △), Tween 80 alone (■, □) or Tween 80 containing ergosterol (●, ○). Solid symbols are from growths that were sampled at intervals and open symbols from growths that were not sampled until 23.3 h.

Lipid analyses and maltose transport activities of yeast samples from the Fig. 1 growths are shown in Tables I and II. As expected from earlier work (see, e.g.,[15,16]), yeast grown anaerobically without supplements contained low amounts of both palmitoleic (C16:1) and oleic (C18:1) acids (which *S. cerevisiae* can synthesise) and of linolic (C18:2) and linoleic (C18:3) acids (which *S. cerevisiae* cannot synthesise[10,25]) and had low unsaturation indices both during growth (21%) and in the stationary phase (20%) (Table I). When the yeast was grown aerobically (in a shake flask) in SC containing 40 g maltose · L^{-1} to stationary phase, it contained larger amounts of total fatty acids and much higher proportions of palmitoleic and oleic acids and had an unsaturation index of 80% (the fatty acid composition and ergosterol content of yeast grown aerobically was the same in minimal (SC) and rich (YP) media). Lauric (C12:0) and myristic (C14:0) acids accounted for much higher proportions of total fatty acids in the unsupplemented anaerobically grown yeast than in the aerobically grown control. The changes reflected absolute increases in the amounts of C12:0 and C14:0 per

Table I. Fatty acid compositions of anaerobically grown yeasts and aerobic control*.

	Anaerobic growth phase			Anaerobic stationary phase			Aerobic
Lipid supplement	**None**	**T**	**T + E**	**None**	**T**	**T + E**	**None**
Lauric (C12:0)	19.8	1.9	1.4	14.4	2.6	2.4	1.9
Myristic (C14:0)	12.5	10.9	6.7	4.5	11.8	5.8	1.2
Palmitic (C16:0)	42.3	23.0	21.2	52.3	26.8	24.2	13.9
Palmitoleic (C16:1)	17.5	5.6	6.4	15.7	5.3	6.0	57.8
Stearic (C18:0)	4.0	2.4	2.3	8.6	3.3	3.0	3.0
Oleic (C18:1)	3.7	56.0	61.7	4.6	50.0	58.1	22.2
Linolic (C18:2)	0.1	0.2	0.3	0.1	0.3	0.3	0.1
Linoleic (C18:3)	0.1	0.0	0.1	0.1	0.0	0.1	0.1
Total fatty acids (mg·g^{-1})	31 ± 3	71 ± 4	56 ± 0	36 ± 2	93 ± 3	54 ± 3	69
Unsaturation index (%)	21	62	69	20	56	64	80

* Yeasts were grown anaerobically (Fig. 1) with no supplements (None), Tween 80 alone (T) or Tween 80 + ergosterol (T + E) and harvested at 23.3 h (growth phase) or 48 h (stationary phase). The right hand column shows results for the same yeast strain grown in SC medium at 26°C without exclusion of air. Individual fatty acids are shown as percentages of total fatty acid. For anaerobic yeasts, total fatty acids as mg·[g dry yeast]$^{-1}$ are means ± standard deviation of 5 samples and for the aerobic yeast the mean of duplicate assays is shown. The unsaturation index shows the sum of unsaturated C16 and C18 fatty acids as a percentage of total even chain length C12–C18 fatty acids.

Table II. Squalene, ergosterol, unsaturation index and maltose transport capacity of anaerobically grown yeasts and aerobic control*.

	Anaerobic growth phase			Anaerobic stationary phase			Aerobic
Lipid supplement	**None**	**T**	**T + E**	**None**	**T**	**T + E**	**None**
Ergosterol (mg·g^{-1})	<0.3	<0.3	1.3 ± 0.1	0.2 ± 0.2	<0.3	1.4 ± 0.4	9.2
Squalene (mg·g^{-1})	2.1 ± 0.0	1.8 ± 0.6	0.8 ± 0.0	6.9 ± 0.3	7.2 ± 1.2	1.8 ± 0.0	<0.3
Erg/Squa**	<0.14	<0.17	1.6	<0.03	<0.04	1.3	>31
Unsaturation index (%)	21	62	69	20	56	64	80
Maltose transport at 20°C (U·g^{-1})	11.2 ± 0.5	9.5 ± 0.1	24.5 ± 0.4	9.8 ± 0.3	2.5 ± 0.2	30.4 ± 0.2	57.6 ± 1.1
Maltose transport at 0°C (U·g^{-1})	0.46 ± 0.01	0.29 ± 0.01	1.83 ± 0.03	0.50 ± 0.02	0.055 ± 0.015	3.10 ± 0.21	4.5 ± 0.3

*Yeasts were grown anaerobically (Fig. 1) with no supplements (None), Tween 80 alone (T) or Tween 80 + ergosterol (T + E) and harvested at 23.3 h (growth phase) or 48 h (stationary phase). The aerobic sample was grown in SC containing 40 g maltose · L^{-1} in a shake flask and harvested in stationary phase. Ergosterol and squalene are reported as mg·[g dry yeast]$^{-1}$ and maltose transport as U·[g protein]$^{-1}$. Data are means ± ranges of duplicate assays.

** Erg/squa is the mass ratio of ergosterol to squalene.

gram of yeast as well as decreases in the amounts of C16:1 and C18:1. Supplementation of the anaerobic growth medium with Tween 80 alone caused a large increase in the proportion of oleic acid (to 56% and 50% of total fatty acids in growing yeast and stationary phase yeast, respectively), more than doubled the absolute amount of total fatty acids and specifically decreased the proportions of lauric and palmitoleic acids. The unsaturation indices of these yeasts (62% and 56%) were still lower than that of the aerobically grown yeast. Tween 80 (polyoxyethylene-sorbitan monooleate) is a source of oleic acid. Supplementation with Tween 80 + ergosterol caused similar changes as Tween 80 alone, but the absolute increase in total fatty acids was smaller (probably because the yeast grew more) and the relative increase in oleic acid was somewhat greater.

Table II shows that the ergosterol contents of yeast grown anaerobically without supplements or with Tween 80 alone were low, the contents of squalene (the last intermediate before the steps requiring oxygen in the biosynthetic pathway to sterols) were high and, consequently, the ergosterol/squalene mass ratios were low. The squalene content of the anaerobically grown yeast increased 3- to 4-fold when the yeast entered stationary phase. Supplementation with ergosterol in addition to Tween 80 raised the ergosterol content to about 1.3 mg · [g dry yeast]$^{-1}$, which is still much less than found in the same strain grown aerobically. The ergosterol content did not increase when the yeast entered stationary phase, although only a fraction of the added ergosterol was incorporated into the yeast (added ergosterol was equivalent to about 6 mg · [g dry yeast]$^{-1}$). Ergosterol uptake by yeast is an active process that normally occurs only when sterol biosynthesis is prevented[29]. Possibly the ergosterol taken up from the medium mainly enters the plasma membrane and does not form the sterol esters that accumulate as storage pools in aerobically grown yeast[2].

The standard maltose transport activity measured at 20°C was relatively low in yeast grown anaerobically without supplements or with Tween 80 alone (Table II). For yeast without supplements, maltose transport activity was slightly lower in stationary phase than during growth, but for yeast supplemented with Tween 80 alone, maltose transport activity was much smaller in stationary phase than during growth. Simultaneous supplementation with ergosterol and Tween 80 increased the maltose transport activity (measured at 20°C) of anaerobically grown yeast more than 2-fold during growth phase and about 3-fold in stationary phase. The biggest difference observed was between yeasts grown with supplements of Tween 80 alone or Tween 80 *plus* ergosterol: transport activity was 12-fold higher in stationary phase yeast supplemented with ergosterol and Tween 80 compared to stationary phase yeast supplemented with Tween 80 alone. Maltose transport was also measured at 0°C, where apparent rates were at least 10-fold smaller than at 20°C. However, the rates at 0°C were also linear (over reaction times of 2–4 min; see Materials and Methods) and so represented uptake of labelled sugar rather than mere binding to the cell surface. Greater dependencies on lipid supplementation were observed at this low temperature: supplementation with Tween 80 *plus* ergosterol increased the transport activity at 0°C of growing and stationary phase yeasts by 4-fold and 6-fold, respectively, and this activity was 56-fold greater in stationary phase yeasts supplemented with Tween 80 *plus* ergosterol than yeast supplemented with Tween 80 alone.

Similar results were obtained in replicate experiments (Table III). The large standard deviations for ergosterol and squalene probably represent the varying success with which air was excluded from the growths. The average maltose transport activity (41.8 U · [g protein]$^{-1}$) for stationary phase yeast grown anaerobically with Tween 80 *plus* ergosterol was lower than found in yeast grown aerobically on 40 g maltose · L^{-1} in SC (Table II) or YP (50–60 U · [g protein]$^{-1}$; data not shown) media.

The 40 g · L^{-1} maltose carbon source in these anaerobic growths is expected to induce fully *MALx1, AGT1* and *MTT1* genes. However, we performed Northern analyses to check whether expression was affected by the lipid supplements. RNA from growing cells (23.3 h in Fig. 1) gave strong *MALx1* signals, but, as expected, the messenger was barely detectable in stationary phase cells harvested after maltose was consumed (data not shown). The *MALx1* signals were quantitated by densitometry and normalised in three ways: to the A260 load applied to the gels (equal for all samples), to the amount of ribosomal RNA detected in the gels and to the *ACT1* signal obtained by hybridising blots to an *ACT1* probe (Table IV). The first two methods suggested that Tween 80 alone slightly (ca 25%) increased expression of *MALx1* whereas Tween 80 *plus* ergosterol slightly (4–27%) decreased its expression. Normalisation to *ACT1* suggested changes in the opposite direction, about 40% decrease with Tween 80 alone and 55% increase with Tween 80 *plus* ergosterol. Expression of *AGT1* was not detectable, and this gene has been

Table III. Effect of lipid supplements on ergosterol and squalene levels, fatty acid unsaturation and maltose transport activity in yeast grown anaerobically into stationary phase.*

Lipid supplement	None	T	T + E
Ergosterol (mg · g^{-1})	0.8 ± 0.7	0.4 ± 0.2	1.8 ± 0.5
Squalene (mg · g^{-1})	3.7 ± 2.9	4.7 ± 3.6	1.2 ± 0.6
Unsaturation index (%)	27.3 ± 8.7	58.5 ± 3.5	64.7 ± 1.2
Maltose transport at 20°C (U · g protein^{-1})	13.0 ± 2.9	4.9 ± 3.4	41.8 ± 11.6

* Yeasts were grown into stationary phase without supplements (None) or with Tween 80 alone (T) or Tween 80 *plus* ergosterol (T + E) as described in Materials and Methods. Results are means ± SDs (None, T + E, n = 3; T, n = 2).

Table IV. Relative expression of *MALx1* genes during anaerobic growth with and without lipid supplements.*

	No supplement	Tween 80	Tween 80 + ergosterol
A260 normalised	100	127	96
rRNA normalised	100	123	73
ACT1 normalised	100	62	155

* Equal amounts (based on A260) of RNA from growing cells (23.3 h in Fig. 1) were loaded onto gels. After electrophoresis, gels were photographed and the ribosomal RNA (rRNA) quantitated by densitometry. After blotting, filters were probed for *MALx1* and then for *ACT1*. Hybridisation bands were quantitated by densitometry and the relative amounts of *MALx1* mRNA were calculated after normalisation to the A260 load, rRNA or *ACT1*.

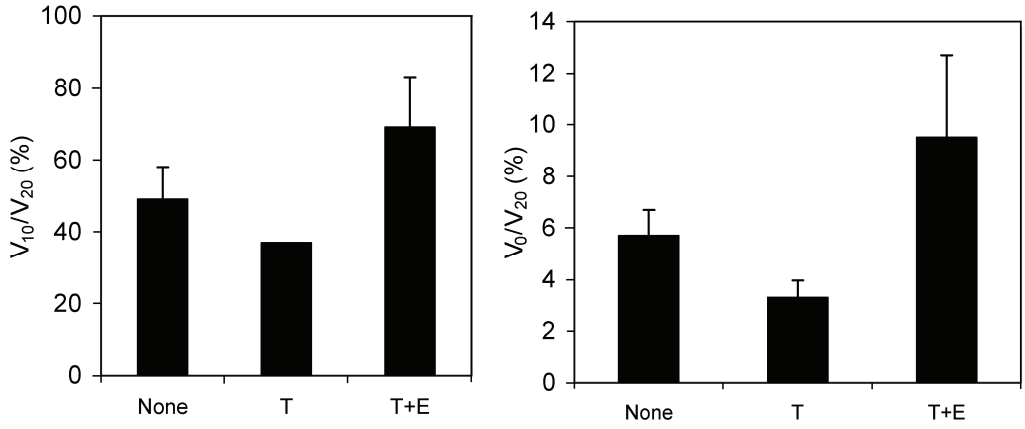

Fig. 2. Different temperature-dependencies of maltose transport by yeast grown anaerobically with no supplement (None), Tween 80 alone (T) or Tween 80 *plus* ergosterol (T + E). The left panel shows the transport activity at 10°C as a percentage of that at 20°C (V_{10}/V_{20}); error bars indicate the range between duplicate experiments. The right panel shows transport activity at 0°C as a percentage of that at 20°C (V_0/V_{20}); results are means ± SDs for experiments with one lot of growing cells and three or (Tween 80 alone) two lots of stationary phase cells. The differences between V_0/V_{20} values were weakly significant ($p = 0.1$) for no supplement *versus* Tween 80 *plus* ergosterol and highly significant ($p = 0.01$) for Tween 80 alone *versus* Tween 80 *plus* ergosterol (matched pair T-test).

previously shown to be non-functional in this brewer's yeast[28].

These Northern results supported the *a priori* assumption that lipid supplements would not change the expression levels of *MALx1* genes, although we cannot rigorously exclude this possibility. More likely, the mechanism of the changes in maltose transport activity involves the altered lipid composition of the plasma membrane. Evidently increasing the unsaturation index of fatty acids by supplementation with Tween 80 alone was not enough to support high maltose transport activity, but supplementation with Tween 80 + ergosterol raised the maltose transport activity towards that found in yeast grown under aerobic conditions, as well as increasing the unsaturation index and ergosterol content. Maltose transport in brewer's yeasts shows a high temperature coefficient below 10°C[21,22], implying that physical changes in membrane lipids at low temperature impinge on the activity of maltose transporters, i.e., the transporters work poorly when the lipid surrounding them becomes more rigid. This was also suggested by the relatively larger changes in transport activity measured at 0°C than 20°C (Table II). We therefore examined in more detail the temperature dependence of maltose transport in yeasts grown anaerobically with or without lipid supplements. Fig. 2 shows combined results from independent experiments with assays at 0, 10 and 20°C. Matched pair T-tests showed that for the ratios of activities at 0°C and 20°C, the differences between no supplement and Tween 80 + ergosterol and between no supplement and Tween alone were only just significant ($p = 0.1$ and 0.05, respectively) whereas the difference between Tween 80 alone and Tween 80 + ergosterol was highly significant ($p = 0.01$).

DISCUSSION

In this brewer's yeast strain the major genes encoding functional maltose transporters belong to the group *MAL11, MAL21, MAL31* and *MAL41*. The *AGT1* gene is present but non-functional[28] and *MTT1* is present but we do not yet know if it is functional. We show here that under growth conditions that were strictly anaerobic but strongly induced expression of *MALx1,* the maltose transport activity of the yeast was markedly increased by supplementation of the medium with Tween 80 *plus* ergosterol (Tables II and III). We conclude that either maltose transporter molecules cannot be properly inserted into plasma membranes that do not contain adequate amounts of sterol or that inserted transporter molecules require sterol molecules in order to fulfil their catalytic role. The results of Fig. 2 show that the temperature-dependencies of maltose transport were greater for non-supplemented cells and cells supplemented with Tween 80 alone than for cells supplemented with Tween 80 *plus* ergosterol. This suggests that the catalytic function of transporter molecules in the plasma membrane was dependent on the lipid composition, in particular the sterol content, of the plasma membrane. Sugar transporter molecules are not simply channels across the membrane. The proteins change shape during each transport cycle[1,13], which involves the surface of the protein moving against the surrounding lipid. The composition of this lipid is thus expected to alter the energetics of the transport process[18]. There is also evidence[3,4] that sterols are required to form "rafts" concerned with the transport of intrinsic membrane proteins from endoplasmic reticulum to the plasma membrane, so that it is also possible that sterol deficiency leads to a decrease in the number of transporter molecules per unit area of membrane.

Supplementation with UFA alone (Tween 80 alone) was not enough to maintain the maltose transport activity of growing cells and actually decreased the transport activity of stationary phase cells and increased the temperature-dependence of the residual activity (Tables II and III, Fig. 2). Supplementation with Tween 80 alone increased the oleic acid content of the yeast but not its sterol content, so that possibly the correct ratio of sterol to oleic

acid is important for transport. It is also possible that Tween 80 alone extracted sterols from the membrane, but our data (Tables II and III) at low ergosterol levels were not accurate enough to show this. We did not carry out the reverse experiment of growing cells under strictly anaerobic conditions with ergosterol supplement but no UFA supplement, because we did not find a convenient way of adding ergosterol to the medium without using Tween 80 as an emulsifier. Thus, we know that efficient maltose transport requires sterols, but we do not know whether or not it also requires UFAs.

Maltose transport capacity is regulated by glucose-repression and maltose-induction of genes encoding maltose transporters and also by glucose-triggered catabolite inactivation of existing maltose transporter proteins[19,27]. The present results emphasise also a third factor, namely the lipid composition, in particular the sterol content of the plasma membrane. What actually happens to the maltose transport capacity when industrially cropped yeast is pitched into aerated wort will depend upon the interaction of these three factors and upon the particular yeast strain and wort sugar composition. The maltose transport capacity of industrially cropped yeast is relatively low, partly at least because it is deficient in sterols. The sterol and UFA contents rise rapidly during the first few hours after pitching[2,8,17]. However, at least in worts with high concentrations of glucose, maltose transport capacity falls during the first 20 h, presumably as a consequence of glucose-triggered catabolite inactivation[22]. Expression of maltose transporter genes is also activated during the first day of fermentation[14], and maltose transport activity recovers and reaches a peak at about the same time as the amount of yeast in suspension reaches its maximum[22]. At this stage, maltose transporter genes are well expressed, glucose has been partially or wholly consumed (bringing an end to catabolite inactivation) and the plasma membranes contain adequate amounts of sterols and UFA. Thereafter, the specific activity of maltose transport declines steadily to the low value found in cropped yeast. During the second half of the fermentation, the remaining maltose and maltotriose must be transported into the yeast cell by transporters that are working suboptimally because the plasma membranes do not contain sufficient sterols. In particular, the daughter cells formed in the last round of cell division may be very deficient in sterols because, although their membranes may receive some sterol from the mother cell, any size increase after separation of mother and daughter occurs in an anaerobic environment where no new sterol can be made.

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