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# Ethanol production using nuclear petite yeast mutants

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**Abstract** Two respiratory-deficient nuclear petites, FY23Δ*pet191* and FY23Δ*cox5a*, of the yeast *Saccharomyces cerevisiae* were generated using polymerase-chain-reaction-mediated gene disruption, and their respective ethanol tolerance and productivity assessed and compared to those of the parental grande, FY23WT, and a mitochondrial petite, FY23$\rho^0$. Batch culture studies demonstrated that the parental strain was the most tolerant to exogenously added ethanol with an inhibition constant, $K_i$, of 2.3% (w/v) and a specific rate of ethanol production, $q_p$, of 0.90 g ethanol g dry cells$^{-1}$ h$^{-1}$. FY23$\rho^0$ was the most sensitive to ethanol, exhibiting a $K_i$ of 1.71% (w/v) and $q_p$ of 0.87 g ethanol g dry cells$^{-1}$ h$^{-1}$. Analyses of the ethanol tolerance of the nuclear petites demonstrate that functional mitochondria are essential for maintaining tolerance to the toxin with the 100% respiratory-deficient nuclear petite, FY23Δ*pet191*, having a $K_i$ of 2.14% (w/v) and the 85% respiratory-deficient FY23Δ*cox5a*, having a $K_i$ of 1.94% (w/v). The retention of ethanol tolerance in the nuclear petites as compared to that of FY23$\rho^0$ is mirrored by the ethanol productivities of these nuclear mutants, being respectively 43% and 30% higher than that of the respiratory-sufficient parent strain. This demonstrates that, because of their respiratory deficiency, the nuclear petites are not subject to the Pasteur effect and so exhibit higher rates of fermentation.

## Introduction

Previous work has highlighted the potential of utilising the respiratory-deficient phenotype of cytoplasmic petite mutants for the commercial production of ethanol (Bacila et al. 1978; Bacila and Horii 1979). Lacking functional mitochondrial DNA, cytoplasmic petite mutants are incapable of growth by means of respiration and are not subject to the Pasteur effect, i.e. the oxygen suppression of glycolysis (Slonimski et al. 1968). Therefore, strict control of the oxygen supply to the fermentation is not necessary, in contrast to the situation with fermentations employing respiratory-sufficient (grande) strains. By altering the concentration of oxygen in the feed gas, Brown et al. (1984) demonstrated that the specific rate of ethanol production was always higher in a parental grande strain ($\rho^+$) than in its mitochondrial petite, provided that the supply of oxygen to the $\rho^+$ culture was maintained at a level sufficient for sterol and unsaturated fatty acid biosynthesis. It was suggested that this decrease in productivity was a result of certain deleterious effects of the cytoplasmic petite mutation. These were highlighted by the increased growth-inhibitory characteristics of ethanol added exogenously to the petite compared to the situation in the grande parent. The lack of an intact mitochondrial genome causes several deleterious cell-surface characteristics, such as reduced sugar uptake, altered cell flocculation and agglutination, and an effect on plasma membrane proteins resulting in a decrease in their ethanol tolerance (Wilkie and Evans 1982).

Mitochondria exist in two different, well-defined physiological states depending on the presence or absence of dissolved oxygen. The promitochondrial structure is produced during anaerobic growth and will differentiate into a fully functional mitochondrion when minute quantities of dissolved oxygen are made available (McLean-Bowen and Parks 1981). Over recent years, mitochondrial function in brewers' yeast has been largely ignored as a result of the Crabtree effect, i.e. yeast respiratory activity is repressed by glucose under aerobic conditions. Since brewing yeasts are Crabtree-positive, the role of oxygen is considered to be solely nutritional and the existence of any true respiratory functions in brewing yeast is usually denied. The trans-

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formation of promitochondria into mitochondria is a metabolically expensive undertaking yet this phenomenon has always been observed in brewing yeasts although its significance has never been explained (Garcia et al. 1993). It is suggested, therefore, that fully developed functional mitochondria are, in an as yet unknown manner, essential for maintaining the ethanol tolerance and, consequently, fermentation performance of yeast.

The work described here is concerned with determining whether the increased ethanol sensitivity of mitochondrial petites is a direct consequence of the loss of the mitochondrial genome or is the result of the respiratory-deficient phenotype. The effect of ethanol on the growth and fermentation of nuclear petites has been studied. These mutants have a specific lesion in their respiratory chain owing to the deletion of a chromosomal gene, but their mitochondrial DNA remains unaltered. Such a nuclear petite should exhibit the advantages of oxygen insensitivity, thus avoiding the need for stringent control of oxygen to the fermentation, and lack the damaging pleiotropic effects of the mitochondrial petite mutation.

Two nuclear petites were generated, each having a mutation associated with cytochrome *c* oxidase, which is the final electron-carrier complex in oxidative phosphorylation. The enzyme consists of nine polypeptide subunits, subunit V being encoded by two non-identical nuclear genes, *COX5a* and *COX5b* (Cumsky et al. 1987; Séraphin et al. 1985). Disruption of *COX5a* (located on chromosome XIV) renders the mutant 85%–90% respiratory-deficient. *PET191* (chromosome X) encodes a 14.1-kDa protein required for the assembly of the polypeptide subunits that constitute the active cytochrome *c* oxidase holoenzyme. Disruption of *PET191* causes failure of holoenzyme assembly, resulting in 100% respiratory deficiency (McEwen et al. 1993).

In this paper, we demonstrate that the mitochondrial genome is indeed required for the maintenance of yeast ethanol tolerance. Coupled with an increased tolerance to the toxin, circumvention of the Pasteur effect by means of a chromosomal lesion of the respiratory chain enables the achievement of a fermentation rate 43% higher than that of the respiratory-competent parental yeast.

## Materials and methods

### Strains

The parental *Saccharomyces cerevisiae* FY23, a haploid, grande [$\rho^+$], *MAT***a**, *ura3-52, trp*Δ*63*, *leu2*Δ*1* strain (Winston et al. 1995), and FY23 ($\rho^0$), a mitochondrial petite mutant lacking all detectable mtDNA, as determined by equilibrium centrifugation in CsCl Hoechst 33258 density gradients (Williamson and Fennell 1975), were used. The petite was derived from the grande parent by growth on yeast extract/peptone/glucose (YPD) in the presence of 100 μg/ml ethidium bromide. Cell growth on YPD but not on yeast extract/peptone/glycerol (YEPG) replica plates was indicative of cytoplasmic petites (Slonimski et al. 1968).

The respiratory-deficient nuclear petite mutants were generated using the polymerase-chain-reaction (PCR)-mediated gene disruption technique developed by Wach et al. (1994). A standard 50-μl PCR reaction mix and conditions were used. The reaction mix comprised 0.1 mM dNTP, 0.2 μM primers, 1.5 mM $MgCl_2$, 20 ng template pFA6-kanMX4, 2.5 U *Taq* polymerase, and standard PCR conditions were 1 cycle at 92 °C for 2 min; 30 cycles at 92 °C for 30 s, 55 °C for 30 s, 72 °C for 90 s followed by 1 cycle at 72 °C for 4 min.

PCR was initially used to construct the disruption cassette pet191::kanMX4 (1.3 kb) using two primers having 19–22 nucleotides homologous to the pFA6-MX4 multicloning site and 35-nucleotide extensions, which were homologous either to the region immediately downstream of the start codon or to that upstream of the stop codon of the target open-reading frame (ORF) *PET191*. Following correct amplification of the cassette, 1–2 μg ethanol-precipitated DNA was used for yeast transformation (Gietz et al. 1995). Upon transformation, recombination between the homologous regions of the pet191::kanMX4 cassette and the target gene resulted in the disruption of the ORF. Transformant selection took place on YEPD/agar containing 0.2 mg/ml geneticin (G418).

Generation of the 85% respiratory-deficient FY23Δ*cox5a* was achieved as for FY23Δ*pet191* using the deletion cassette cox5a::kanMX4, which has tails homologous to the *COX5a* ORF. Correct targeting of each of the two disruption cassettes into the $G418^R$ yeast genomic loci was verified by analytical PCR (Wach et al. 1994). This was performed on whole-cell genomic DNA of transformants growing on 0.5 mg/ml G418. Analytical PCR conditions were 1 cycle at 92 °C for 2 min; 30 cycles at 92 °C for 30 s, 56 °C for 30 s (for *PET191*) or 54 °C for 30 s (for *COX5a*), 72 °C for 90 s (*PET191*) or 72 °C for 3 min (*COX5a*) followed by 1 cycle at 72 °C for 4 min. Three primers were used per PCR reaction. Two primers (forward 1, reverse 1) were designed to amplify the wild-type, non-disrupted alleles (542 bp *PET191*, 460 bp *COX5a*) and the third primer, reverse 2, was homologous to the kanMX4 deletion cassette. Correct disruption of the target ORF would result in a deletion fragment between the forward 1 and kanMX4 reverse 2 primers (700 bp *PET191*, 1468 bp *COX5a*).

Further confirmation of correct ORF disruption was achieved by testing for respiratory deficiency on YEPG plates. The putative nuclear petites were also subjected to pulsed-field gel electrophoresis using the contour-clamped homogeneous electric field (CHEF) technique in order to separate the chromosomes (Vollrath and Davis 1987; Gardner et al. 1993). This was followed by Southern hybridisation whereby a $^{32}$P-labelled KanMX4 cassette was used to probe the membrane. Subsequent exposure to a phosphoimager (BioRad, UK) indicated that the Cox5a::kanMX4 and pet191::kanMX4 cassettes had integrated correctly into chromosomes XIV and X respectively.

### Media

Organisms were grown in complete YPD medium (1% w/v yeast extract, 2% w/v peptone, 5% w/v glucose). Respiratory deficiency was determined by growth on YEPG medium, which contained 3% (w/v) glycerol as the sole carbon source.

### Batch fermentations

Strain-specific ethanol productivities were determined in triplicate in batch cultures in 3-l Bioengineering LKF2000 basic fermenters (Bioengineering AC, CH8636-Wald, Switzerland) with a working volume of 2 l. Monitoring and control of fermentations were achieved using a "Pi" computer control system (Process Intelligence Ltd., Warwick, UK). Data from the variables described below were recorded at 2-min intervals throughout the course of the fermentations.

The cultures were agitated at 850 rpm, which was achieved using 2 × 6-blade Rushton impellers mounted on a central shaft and driven by a 250-W VDE electric motor (Lenze, D-4923, Germany). Excessive foaming was prevented by the addition of several drops of a silicone-based anti-foaming agent (Mazu DF8005). Cultures were aerated at 1 l $min^{-1}$ (1 vvm), which was achieved using a rotameter and precision control valve. The fermenters were

equipped with a 405-DPAS-50-K85/200 combination pH electrode and the pH of all fermentations was maintained at 5.0 by the addition 3 M HCl and 3 M NaOH solutions via W/M 501u pumps (Watson and Marlow, UK). Control of pH was implemented via the "Pi" system, using an on/off control loop about the set point of pH 5.0 (±0.005). All fermentations were carried out at 30 °C (±0.005 °C) using the proportional (P) and integral (I) controller of the "Pi" system. Dissolved oxygen in the media was continuously monitored by means of a dissolved oxygen probe (Ingold, UK), as was $CO_2$ in the exhaust gas by an infrared gas analyser (ADC instruments Ltd., Hoddesden, UK). The various control systems of the fermenter were established and then inoculation followed with a 1% overnight dense seed culture. The fermentation was allowed to proceed for 45 h and the $CO_2$ concentration in the exhaust gas was monitored constantly. Samples were taken from the middle of the exponential phase at 1-h intervals right through to the end of the stationary phase and underwent dry-weight and gas chromatography analysis (GC).

Strain-specific ethanol tolerances were determined in batch cultures in specially adapted 100-ml conical flasks (called Klett flasks) in combination with the Klett Summerson colorimeter and a green filter (Klett Summerson, USA). Cultures were grown to early exponential phase overnight and then split into 18-ml aliquots to which 2 ml either sterile distilled water or ethanol solution was added giving final concentrations of 0, 2%, 3%, 4%, 6% and 10% (w/v) ethanol. Each concentration was prepared in triplicate and hourly measurements were made of cell density. A 1-ml sample was taken from the flask, diluted, and plated onto YPD for a viable-cell count. The remainder of the sample was centrifuged (15 000 rpm), 4 °C, 5 min) and the supernatant frozen for subsequent analysis of ethanol concentration by GC.

### Cell dry weight determination

A 20-ml sample from the fermenter was centrifuged (15 000 rpm, 4 °C, 5 min) and the supernatant decanted into a sterile container for the determination of ethanol concentrations. The cell pellet was washed once in 20 ml sterile distilled water; the cells were harvested again and dried at 90 °C until a constant cell dry weight was achieved.

### Measurement of ethanol concentration

Fermenter samples were stored at −20 °C and assayed for ethanol content by gas chromatography using a Pye-Unicam 204 series fitted with a carbowax column. The injector and flame ionisation detector were set at 200 °C and the column maintained at 80 °C. The flame gas was a mixture of air and hydrogen and the carrier gas was oxygen-free nitrogen (BOC Ltd.) running at 16 lb/in$^2$. A propan-1-ol internal standard was used for all samples and ethanol concentrations were calculated by the computer software. Ethanol concentrations were plotted against time and the specific rate of ethanol production ($q_p$, g g dry cells$^{-1}$ h$^{-1}$) was determined at the steepest point [$q_p = r_p/x$, where $r_p$ is the volumetric rate of ethanol production (g h$^{-1}$) and $x$ the concentration of biomass (g l$^{-1}$)].

## Results

### Ethanol tolerance

All four members of the isogenic series (FY23WT, FY23Δ*pet191*, FY23Δ*cox5a* and FY23$\rho^0$) were grown in batch culture at 30 °C and ethanol, at various concentrations, was introduced at the beginning of the exponential phase. Cell growth was followed for 8 h by use of a Klett meter. Upon addition of ethanol to an exponentially growing population of cells, there was an immediate drop in the growth rate. All four strains show this characteristic which is, at least partially, due to a dilution effect since it also occurs in the cultures dosed with only distilled water. Graphs were plotted of $\log_e$ (cell growth/Klett units) against elapsed time, and linear regression analysis was performed to calculate culture-specific growth rates, $\mu$. The effect of ethanol on the growth rate of the four members of the series was analysed by comparing relative growth rates at each ethanol concentration, and calculating these as a percentage of the growth rate in the absence of ethanol.

Ethanol inhibition kinetics are thought to follow that of non-competitive inhibition (Pamment 1989), and can be modelled using a variant of the Monod equation. From the specific growth rate values calculated, it is possible to determine values of $K_i$ by inserting them into the rearranged equation ($\mu_{max}/\mu_i - 1 = i \cdot 1/K_i$, where $i$ is the inhibitor concentration). Earlier work had shown that simply using raw biomass accumulation data did not produce a straight-line relationship (Brown et al. 1981). This was due to an irreversible effect (cell death), which rendered the application of equilibrium kinetics invalid; cell viability must therefore be taken into account. The initial values of $\mu$ calculated are apparent ($\mu_{app}$) values determined from the growth of a culture containing both living and dead cells. For this reason, $\mu_{app}$ at each ethanol concentration, determined solely from Klett values, had to be corrected to $\mu_{true}$ using the viability data collected during each experiment.

### The effect of ethanol on cell viability

Pirt's (1975) equation, which takes into account the death of cells in a batch culture, was used to calculate the true specific growth rates at each ethanol concentration. This assumes that the rate of cell death in a batch culture is proportional to the number of viable cells present.

$$Y_T = y_{T(0)} + \frac{\mu \cdot y_{v(0)}(e^{(\mu-k)t} - 1)}{\mu - k}$$

$\mu$ was calculated by rearranging the above equation, taking $t = 0$ (at the time of ethanol addition) and $t = t_T$ (at the time of the last reading, e.g. 6 h after ethanol addition). Klett values represented total cell numbers ($y_T$ and $y_{T(0)}$, and values of $y_v$ and $y_{v(0)}$ were calculated as the product of total cell number and the proportion of viability, which was determined from the linear regression graph of cell viability against elapsed time).

Corrected values of $\mu$ ($\mu_{true}$) were used, in the adapted Monod equation, to re-calculate $K_i$ values. Plotting [($\mu_{max(true)}/\mu_{i(true)}$) − 1] against inhibitor concentration ($i$) yields a straight line with a gradient $1/K_i$. In order to increase the accuracy of the plot, inhibitor concentrations plotted were the average of the ethanol concentration in each Klett flask over the course of the batch experiment. This was determined by GC analysis.

**Table 1** Comparison of inhibition constants ($K_i$) for the members of the isogenic series

| Strain | $K_i$ (true growth rate) | |
|---|---|---|
| | (% ethanol, w/v) | (M) |
| FY23WT | 2.30 | 0.50 |
| FY23Δ*pet191* | 2.14 | 0.47 |
| FY23Δ*cox5a* | 1.94 | 0.42 |
| FY23$\rho^0$ | 1.71 | 0.37 |

The higher the $K_i$, the more tolerant is the growth of the strain to ethanol inhibition. Table 1 presents the $K_i$ values for the four strains and shows that the parent FY23WT is the most tolerant, and the mitochondrial petite is the most sensitive (76% the tolerance of FY23WT). Analyses of the nuclear petites demonstrates that the 100% respiratory-deficient nuclear petite, FY23Δ*pet191*, had an ethanol tolerance comparable to FY23WT ($K_i$ is 95% of the wild-type value) and the 85% respiratory-deficient FY23Δ*cox5a* had a tolerance 86% of that exhibited by the wild-type strain.

## Ethanol production

Batch fermentation was performed in a stirred vessel of 2 l working volume to compare the ethanol productivities of members of the isogenic series. In order to allow a good comparison of ethanol productivities, high fermentation rates were required and this was achieved by performing all fermentations under conditions of glucose repression: 5% (w/v) glucose. The medium was saturated with sterile air throughout the fermentation. This was maintained so that oxygen would be available to the yeast for non-respiratory functions during fermentation, primarily for the biosynthesis of sterols and unsaturated fatty acids, and also the biosynthesis of porphyrin and haem, the oxidation of wort components, and the differentiation of promitochondria to mitochondria (McLean-Bowen and Parks 1981). Molecular oxygen would also be available for respiration by FY23WT and FY23Δ*cox5a* once the glucose substrate had been exhausted.

Figure 1a–d shows the fermentation data of the four members of the isogenic series. In each case, the concentration of carbon dioxide in the exit gas gave a profile that follows that of a classical batch growth. Numerous terms have been used to describe the growth phase (15–25 h in Fig. 1a–c, and 5–25 h in Fig. 1d), which is invariably characterised by high levels of fermentative activity and substantially repressed respiration. The term "respiro-fermentative" growth is favoured since it reflects the physiological state of the cells and emphasises the predominantly fermentative growth during which the culture is growing at its maximum specific growth rate, $\mu_{max}$ (Lewis et al. 1993). Table 2 shows the maximum specific growth rates of each of the strains

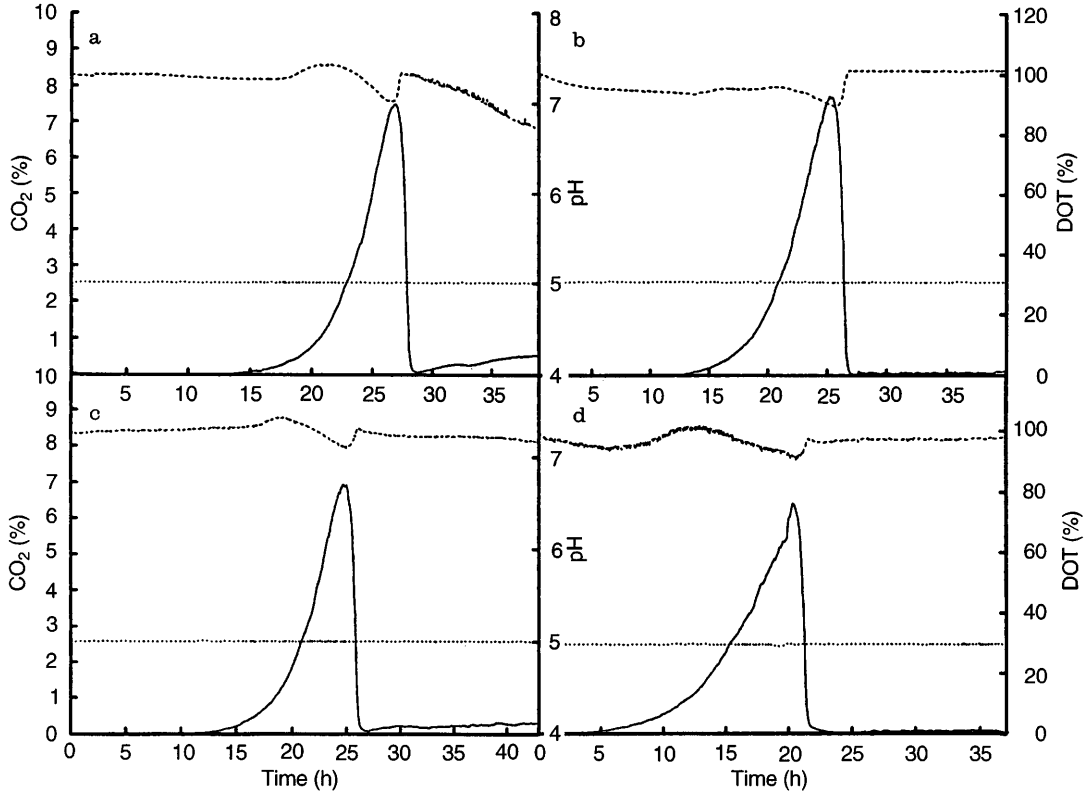


**Fig. 1a–d** A comparison of the fermentation profiles for the four strains of the isogenic series. **a** FY23WT, **b** FY23Δ*pet191*, **c** FY23Δ*cox5a*, **d** FY23$\rho^0$. Carbon dioxide (⊢⊣), dissolved oxygen (*DOT*, - - -) and pH (· · ·) were monitored and datalogged throughout each batch fermentation

**Table 2** Actual values of the specific growth rates ($\mu_{max}$) and ethanol productivities ($q_p$) for the isogenic series

| Fermentation | FY23WT | | FY23Δ*pet191* | | FY23Δ*cox5a* | | FY23ρ$^0$ | |
|---|---|---|---|---|---|---|---|---|
| | $q_p$ (g ethanol g dry biomass$^{-1}$ h$^{-1}$) | $\mu_{max}$ (h$^{-1}$) | $q_p$ (g ethanol g dry biomass$^{-1}$ h$^{-1}$) | $\mu_{max}$ (h$^{-1}$) | $q_p$ (g ethanol g dry biomass$^{-1}$ h$^{-1}$) | $\mu_{max}$ (h$^{-1}$) | $q_p$ (g ethanol g dry biomass$^{-1}$ h$^{-1}$) | $\mu_{max}$ (h$^{-1}$) |
| Batch: 1 | 1.081 | 0.432 | 1.201 | 0.426 | 1.181 | 0.409 | 0.876 | 0.262 |
| Batch: 2 | 0.840 | 0.411 | 1.267 | 0.398 | 1.188 | 0.419 | – | 0.268 |
| Batch: 3 | 0.896 | 0.422 | 1.551 | 0.386 | 1.301 | 0.391 | 0.910 | 0.282 |
| Average ± standard deviation | 0.939 ± 0.10 | 0.422 ± 0.01 | 1.339 ± 0.15 | 0.403 ± 0.02 | 1.223 ± 0.05 | 0.406 ± 0.01 | 0.893 ± 0.02 | 0.271 ± 0.01 |

and, in comparison with FY23WT, shows that disruption of the ORF *PET191* and *COX5a* had no significant effect on this parameter. The specific growth rate of ρ$^0$, on the other hand, is 64.3% that of FY23WT, indicating that loss of the mitochondrial genome has had a considerable effect on growth of the cytoplasmic petite.

The dissolved oxygen concentration was set at 100% saturation at the time of inoculation and also monitored. The decrease in dissolved $O_2$ coinciding with the latter stages of growth represents oxygen requirements for both non-respiratory (all strains) and respiratory functions (FY23WT and FY23Δ*cox5a* only), after which it returned to 100%. For all strains, the growth rate of the culture decreased as the cells entered the diauxic lag phase as the concentration of the initial fermentable substrate, glucose, decreased finally to a minimum. Figure 1a shows that growth occurred following exhaustion of the glucose substrate with a diauxic growth phase, during which time FY23WT resorted to using the product of fermentation, ethanol, as its key metabolite. Cell growth was achieved using respiration whereby ethanol was oxidised to acetate via oxidative phosphorylation. FY23Δ*cox5a* showed evidence of a respiratory growth phase with ethanol being used as the secondary substrate but not to the same extent as for the grande FY23WT. Carbon dioxide levels in the diauxic phase did not reach values as high as those for the grande, and dissolved $O_2$ concentrations did not fall as low, indicating that levels of respiration are not as high for the mutant. This 10%–15% residual respiratory activity is directly attributable to the chromosomal *COX5b* gene (Cumsky et al. 1985). The $CO_2$ profiles for FY23Δ*pet191* and FY23ρ$^0$ for the end of the batch show a concentration of 0% (±0.05%) and dissolved $O_2$ levels were fixed at 100%, indicating that there was no diauxic respiratory growth phase. As expected, these two strains displayed the petite phenotype and consequently were unable to oxidise ethanol as an alternative energy source.

For all fermentations, hourly samples were taken from the middle of the respirofermentative exponential phase through to the end of the "respiratory" growth phase, the cells being used for dry-weight analysis and the supernatant being frozen at −20 °C for GC analysis. Values of specific rates of ethanol production, $q_p$, were subsequently calculated from these data.

Table 2 summarises the values of $q_p$ for each of the four members of the isogenic series. The lowest value of $q_p$ was obtained with the mitochondrial petite FY23ρ$^0$, confirming the results of previous workers. The parental grande, FY23WT, displayed a slightly higher $q_p$ than FY23ρ$^0$ under the conditions tested, probably because of its increased $\mu_{max}$ and the presence of an intact mitochondrial genome maintaining the strain's ethanol tolerance. The partially respiratory-deficient nuclear petite FY23Δ*cox5a* displayed a productivity 30% higher, and the totally respiratory-deficient petite FY23Δ*pet191* 43% higher, than the wild-type. Owing to its inability to respire, FY23Δ*pet191* displayed an increased glycolytic flux from glucose through to ethanol. This was higher than the respiratory-competent wild-type, which preferred oxidative phosphorylation for the production of ATP. The relative productivity of FY23Δ*cox5a* was 9% lower than that of FY23Δ*pet191* and this roughly corresponds to the 10%–15% residual respiratory activity provided by the *COX5b* gene.

## Discussion

Examination of the effect of exogenous ethanol on the four strains demonstrated that functional mitochondria are essential to maintain the tolerance of yeast to ethanol, the cytoplasmic petite exhibiting the lowest tolerance of the series. The mitochondrial genome is thought to play a significant role in certain cell-surface phenomena (Wilkie and Evans 1982) and hence determine, to an undefined extent, the ethanol tolerance of the strain.

From the specific productivity data presented (Table 3), it is suggested that the increased productivity exhibited by the two nuclear petites was a result of their

**Table 3** Change of specific growth rates ($\mu_{max}$) and ethanol productivities ($q_p$) for the isogenic series

| Strain | $\mu_{max}$ (%) | $q_p$ (%) |
|---|---|---|
| FY23WT | 100.0 | 100.0 |
| FY23Δ*pet191* | 95.5 | 142.6 |
| FY23Δ*cox5a* | 96.2 | 130.2 |
| FY23ρ$^0$ | 64.2 | 95.1 |

inability to respire in the "respiro-fermentative" phase of batch growth, and of their retained tolerance to ethanol. Their respiratory-deficient phenotype meant that the Pasteur effect was successfully circumvented, allowing higher fermentation rates to be achieved than with the respiratory-sufficient wild-type. The possession of fully functional mitochondria served to maintain their ethanol tolerance and, hence, a high growth rate. Even though the cytoplasmic petite avoided the Pasteur effect, the lack of the mitochondrial genome prevented high fermentation rates from being achieved.

It is suggested, therefore, that 100% respiratory-deficient nuclear petites will be of use in the commercial production of ethanol in circumstances where the oxygen supply cannot be tightly controlled. Nuclear petites are unable to grow in a diauxic growth phase and so will not metabolise the product of fermentation, ethanol, as their secondary substrate. This would allow higher final titres to be achieved.

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