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Accumulation of magnesium ions during fermentative metabolism in *Saccharomyces cerevisiae*

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When cells of *Saccharomyces cerevisiae* were grown aerobically under glucose-repressed conditions, ethanol production displayed a hyperbolic relationship over a limited range of magnesium concentrations up to around 0.5 mM. A similar relationship existed between available Mg^{2+} and ethanol yield, but over a narrower range of Mg^{2+} concentrations. Cellular demand for Mg^{2+} during fermentation was reflected in the accumulation patterns of Mg^{2+} by yeast cells from the growth medium. Entry of cells into the stationary growth phase and the time of maximum ethanol and minimum sugar concentration correlated with a period of maximum Mg^{2+} transport by yeast cells. The timing of Mg^{2+} transport fluxes by *S. cerevisiae* is potentially useful when conditioning yeast seed inocula prior to alcohol fermentations.

Keywords: yeast; magnesium ions; fermentation

Introduction

Magnesium ions play essential roles in the growth and metabolism of yeast cells [14]. With regard to carbohydrate catabolism and fermentation, Mg^{2+} ions are required as cofactors for the activity of key glycolytic and alcohologenic enzymes and may also play a regulatory role at the level of pyruvate-metabolizing enzymes when cells are grown respirofermentatively [14,16]. Magnesium also plays roles in protecting yeast cells against environmental stresses during fermentation such as those caused by ethanol [3,4], high temperature, or high osmotic pressure [2]. Dombek and Ingram [4] demonstrated that Mg^{2+} deficiencies in a yeast extract-peptone based fermentation broth were primarily responsible for the decline in yeast fermentative activity. In addition, a general stimulation of ethanol production by yeast is observed when complex organic feedstocks like molasses, wine must or malt wort are supplemented with Mg^{2+}, indicating that such media may be deficient in available Mg^{2+} for optimal fermentation performance [16–18]. Therefore, media bioavailability, cellular uptake and subsequent metabolic utilization of Mg^{2+} ions by yeast cells appear to be prerequisites for achievement of maximum fermentative activity.

This paper investigates the metabolic demands by yeast for Mg^{2+} during fermentation. Knowledge of Mg^{2+} accumulation by yeast cells may be usefully exploited in biotechnologies concerned with production of ethanol.

Materials and methods

Organisms, media and culture conditions

The yeast employed in this study was an industrial bakers' strain of *Saccharomyces cerevisiae* (Meyen ex-Hansen, 1883; University of Abertay Dundee culture collection number YO36) originally provided by Mauri Yeast Products Ltd, Hull, UK. Active colonies of the yeast were grown on Sabouraud Dextrose Agar (SDA) slopes at 30°C for 24 h then maintained at 4°C. Experimental fermentations were carried out by inoculating yeast cells in modified Edinburgh Minimal Medium (EMM) [6] in which the level of glucose was increased to 25 g L^{-1}. The calcium concentration was constant at 0.1 mM but the magnesium concentration was varied as described in the Results. Deionized glassware, AnalaR-grade reagents and ultrapure (18 megaohm conductivity) water were employed in preparation of growth media to control magnesium concentrations during yeast culture. To generate suitable inocula for experimental cultures, 10 ml sterile water was added to an SDA slope of the yeast which was resuspended prior to transfer to 100 ml EMM in an Erlenmeyer flask. This primary inoculum was incubated overnight at 30°C with shaking at 180 rpm prior to transfer to 400 ml fresh EMM. This secondary inoculum was incubated as before for 10 h prior to harvesting 100-ml aliquots by centrifugation ($1500 \times g$, 10 min). Cell pellets were washed by resuspension in warm sterile water before re-centrifugation and a second resuspension to provide the inoculum for the main experimental fermentations which were carried out at 30°C in 2-L fermenters (Life Science Laboratories, Luton, UK) with a working volume of 800 ml. Inocula prepared in this way ensured removal of interstitial and loosely-bound cell surface magnesium.

Cell numbers and biomass determination

Yeast cell numbers and mean cell volumes were determined using a Coulter Counter Model D (Coulter Electronics, Luton, UK). To minimize errors due to cell aggregation, culture samples were ultra-sonicated (4 min at maximum setting in a Camlab T310 sonicator) prior to dilution and triplicate analysis. For yeast biomass determinations, cell pellets from duplicate 10-ml culture samples were washed, dried and weighed.

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Chemical analysis

Ethanol was measured in yeast culture supernatants using gas chromatography (Perkin-Elmer GC 8310) with a Poropaq Q packed column (2 m × 3 mm). The injector column and flame ionization detector temperatures were both 210°C and N_2 was used as carrier gas at a flow rate of 45 ml min^{-1}. Isopropanol was used as an internal standard. Duplicate analyses were undertaken. Residual glucose concentration in yeast culture supernatants was measured by HPLC (Bio-Rad) using an Aminex ion exclusion HPX-87H column and refractive index detector. Calibration was by external standardization. Magnesium concentrations in both cells and growth medium were measured (mean of triplicate analyses) by atomic absorption spectrophotometry (Perkin-Elmer 1100B). Flame atomization was used and Mg detected at the 285.2 nm resonance line. Dried cell pellets were suspended in 2 ml water and then hydrolyzed by the addition of 2 ml concentrated nitric acid at room temperature for 24 h followed by 10 min at 100°C to complete cellular hydrolysis prior to atomic absorption spectrophotometry of diluted hydrolysates.

Results and discussion

The influence of Mg^{2+}-limitation on fermentative metabolism by *S. cerevisiae* was measured at the level of ethanol production. Both ethanol production and glucose consumption were highly dependent on the availablity of Mg^{2+} ions in the yeast growth medium (Figures 1 and 2). Concentrations of Mg^{2+} above 500 μM did not affect fermentation when expressed on a cellular productivity basis (Figure 2). Over the Mg-limited range studied, fermentation increased due to the capacity of individual cells to augment ethanol production, rather than by any increase in the cell number. Figure 2 also shows that final yields of ethanol did not appear to be influenced by Mg^{2+} availability to the same extent as ethanol production.

The fact that yeast cells speed up fermentation of glucose as more Mg^{2+} is available raises the question of cell uptake of Mg^{2+}. Saltukoglu and Slaughter [11] showed that yeast cells absorb a constant amount of Mg^{2+} per cell so long as the medium is sufficient in this ion. Under Mg^{2+}-limited conditions however, yeast cells take up Mg^{2+} in proportion to its availability [11,15]. The Mg^{2+} uptake patterns from the growth medium by yeast growing under Mg^{2+}-limited conditions (up to around 500 μM Mg^{2+}) is shown in Figure 1c. For cultures in the four highest initial Mg^{2+} concentrations, the sequence of uptake was progressive removal of Mg^{2+} from the medium followed by a gradual release. It is noteworthy that the level of Mg^{2+} within the medium never returned to its original value but rather 10–20% was removed by the cells. This is in accordance with known roles of Mg^{2+} as most cellular Mg^{2+} would be

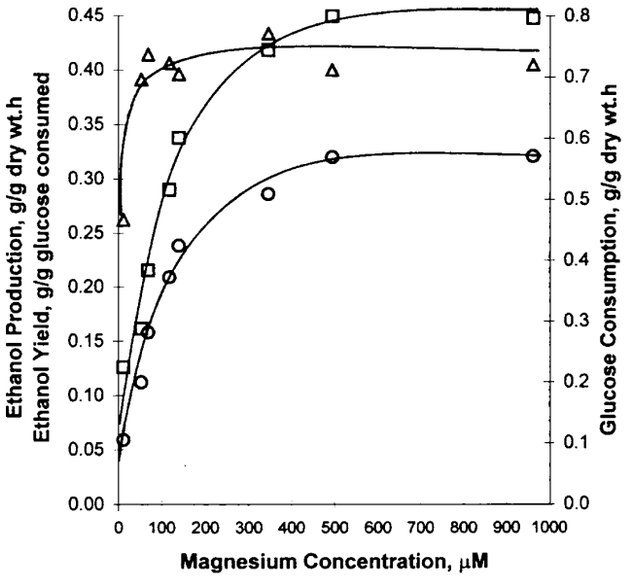

Figure 2 The relationship between ethanol production (❍), glucose consumption (❒) and ethanol yield (Δ) and Mg^{2+} availability during fermentation by *S. cerevisiae*.

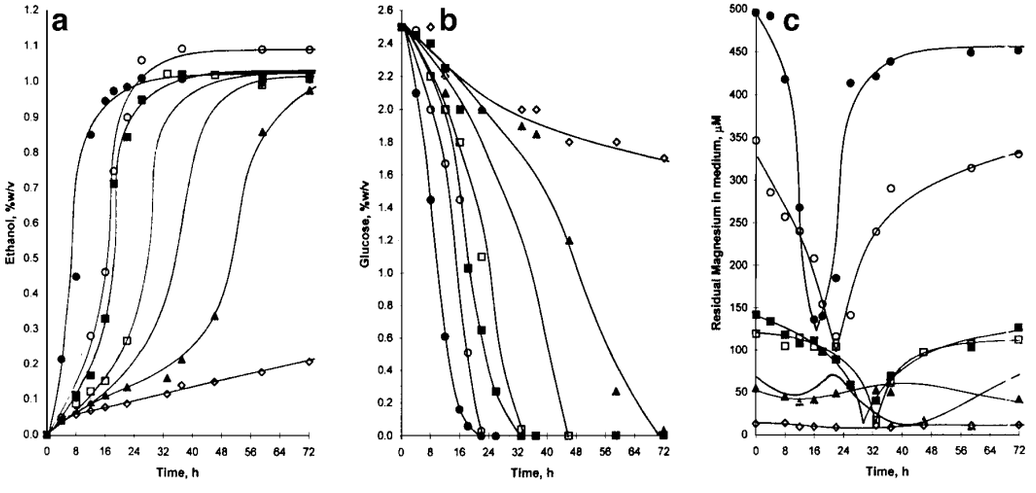

Figure 1 The influence of initial Mg^{2+} concentration on: (a) ethanol accumulation, (b) glucose consumption and (c) Mg^{2+} accumulation during fermentation by *S. cerevisiae* in defined growth media with the following initial concentrations of Mg^{2+}: ❍, 496 μM; ●, 347 μM; ■, 142 μM; ❒, 120 μM; Δ, 71 μM; ▲, 55 μM; and ◇, 13 μM.

Table 1 Correlation of fermentation parameters with Mg accumulation by yeast

Initial Mg in medium (μM)	Time of maximum Mg accumulation (h)	Time for parameter to reach maximum value (h)		
		Ethanol	Glucose uptake	Cell density
55	60	72	72	85
71	39	46	46	42
120	33	36	36	34
142	30	30	30	30
347	22	25	25	24
496	16	20	20	18

employed for non-structural, cofactor roles in glycolytic metabolism. Table 1 shows that the periods of maximal Mg^{2+} accumulation by yeast cells correlate with periods of maximal ethanol accumulation, lending support to the above proposal that Mg^{2+} taken up, but subsequently released by the cells, represents cytoplasmic Mg^{2+}. Such patterns of Mg^{2+} uptake and release which correspond to increases and decreases in metabolic activity reflect the highly regulated nature of Mg^{2+} within the yeast cell [8,14]. Lentini *et al* [9] reported general patterns of Mg^{2+} uptake and release in brewing yeast during fermentation of malt wort which resemble present results in chemically defined media. Recently, we have shown that attention to Mg^{2+} transport in industrial yeast strains is potentially useful as a means of augmenting fermentative activity by physiological adaptation through Mg^{2+}-conditioning of seed inocula prior to alcoholic fermentation [18]. Although the yeast cell wall plays a significant role in divalent metal ion binding in yeast [1,5,7,10,13], it is unlikely that the timing of Mg^{2+} uptake observed in yeast cells during fermentation represents a surface-binding phenomenon. For example, for Mg^{2+} transport by yeast, cell wall binding represents a more immediate, non-specific event [13] whereas uptake patterns observed in the present study (Figure 1c) and by Lentini *et al* [9] are more long-term and associated with active fermentative metabolism. Although Mg-transporters have now been characterized at the molecular level in bacteria [12], the mechanism of active Mg^{2+} transport through the yeast plasma membrane remains to be elucidated. Nevertheless, the current study serves to emphasize the close relationship between fermentation and Mg^{2+} demand in *S. cerevisiae*.

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