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Uptake of Ammonia by *Saccharomyces Cerevisiae* Carrying the Plasmid pCYG4 Related with Ammonia Assimilation

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ABSTRACT

Batch culture experiments involving ammonia uptake in *Saccharomyces cerevisiae* BC55 pCYG4 have been carried out. This strain carries the plasmid pCYG4 that directs substantial overproduction of NADP-GDH, conferring an 11-fold increase in activity. The wild type cells had a specific growth rate greater than BC55 pCYG4. The ammonia uptake was practically the same until 15 h of growth. However, the amount of ammonia hydroxide added during growth (60 h) was two and half times greater in the BC55 pCYG4 than wild type cells. The results suggest that the presence of the plasmid pCYG4 can increase the amount of ammonia taken by the cells, but not the amount of biomass.

Index Entries: *Saccharomyces cerevisiae;* ammonium; ammonia hydroxide; plasmid, NADP-GDH.

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INTRODUCTION

Genetic engineering of industrially important strains of *Saccharomyces cerevisiae* with respect to nitrogen assimilation pathways is now of considerable interest, and attention has been directed to the NADP-linked glutamate dehydrogenase (NADP-GDH) (*1*).

Cooper (*2*) pointed out that in *Saccharomyces cerevisiae* the routes of nitrogen catabolism may be conveniently divided on the basis of their end products. Several systems, such as those degrading allantoin, urea, or asparagine, generate ammonia as the final product. In these cases, NADP-GDH is required to convert ammonia to glutamate, the predominant nitrogen donor in many biosynthetic reactions.

This work describes ammonia uptake in *Saccharomyces cerevisiae* BC55 that carries the plasmid pCYG4 that directs substantial overproduction of NADP-GDH, conferring an 11-fold increase in activity. It was developed by Nagasu and Hall (*3*) using the *Neurospora crassa* gene as a probe to screen a lambda library of yeast genomic DNA.

They isolated a gene exhibiting strong homology to the *Neurospora crassa* gene and conferring NADP-GDH activity in yeast. This gene was subsequently cloned into the *Escherichia coli*-yeast shuttle vector CV13 (YEp13) BamHI site in *Saccharomyces cerevisiae* (gdh-leu2 strain BC55) with subsequent selection for LEU2$^+$ transformants. This yeast shuttle vector (CV13) carrying the cloned fragments complements the gdh$^-$ leu2$^-$ strain.

MATERIALS AND METHODS

Saccharomyces cerevisiae strains used in this work were obtained from J. R. Kinghorn (University of St. Andrews). 1—$^{\Sigma}$1278b (wild type); 2—GDH$^-$, BC55 (a, gdh$^-$, leu2$^-$, Bla$^+$) carrying the plasmid pYE13, same as pCYG4, but it does not carry the NADP-GDH gene encoded; 3—GDH$^+$, BC55 (a, gdh$^+$, leu2$^-$, Bla$^+$) carrying the pCYG4 plasmid with overproduction of 11-fold of NADP-GADH.

CULTURE CONDITIONS

The fermenter was designed to operate in batch culture, using a glass vessel with 1.5-L total capacity, but with a working capacity of 0.6 L, dissolved oxygen being maintained at 30% of air saturation; temperature 30°C, and pH 5 controlled by the addition of KOH (2*M*). The experiments were carried out with a microcomputer-controlled fermenter system designed and constructed "in house" (*4*), using the following growth medium: 0.5% (w/v) glucose; 4 g/L ammonium sulfate, and 0.17% Difco yeast nitrogen base (YNB).

Table 1
Uptake of KOH by Three Different *Saccharomyces cerevisiae* Strains

Strain	Vector	NH_4OH uptake, m*M*/h	NADP-GDH, U/mg protein	Generation, time, H
Σ1278b	—	.380	.4740	2.42
BC55	pCYG4	.360	5.391	2.79
BC55	pYE13	.110	<0.01	6.48

The determination of biomass was carried out using an electrode system attached to the laboratory scale fermenter (*5*).

The enzyme activities (NADP-GDH) were determined as described by Bergmeyer (*6*).

AMMONIA UPTAKE

It is based on Wiame et al. (*7*) statement, where they suggested that, during synthesis of a dipeptide from glucose and ammonia, there is a concomitant production of one proton equivalent for each nitrogen atom assimilated. Consequently, during the ammonia uptake process cause the pH to decrease following the equation.

$$C_6H_{12}O_6 + 2NH_4 \longrightarrow C_6O_3N_2H_{12} + 3H_2O + 2H^-$$

The system consists in recording throughout the microcomputer-controlled fermenter system, the duration of operation of the peristaltic pump by the turning a 10-turn digital counter connected with a precision potentiometer. The peristaltic pump has been used to control the value of the pH of the medium, maintaining it at constant value during the growth by addition of alkali (KOH 2*M*).

RESULTS AND DISCUSSION

In the three batch experiments using the same conditions for growth, the wild type cells Σ1278b had a specific growth rate greater than BC55 with plasmid pCYG4, which carries the gene for NADPH-GDH and confers 11-fold increase in the activity of this enzyme, and twice greater than cells without NADP-GDH activity (Table 1, Fig. 1). The reduction in growth rate on plasmid-positive cells comparing with wild type may be attributed to the burden of extra plasmid DNA either owing to plasmid copy number or plasmid size, and also the expression of high levels of foreign proteins (Table 1).

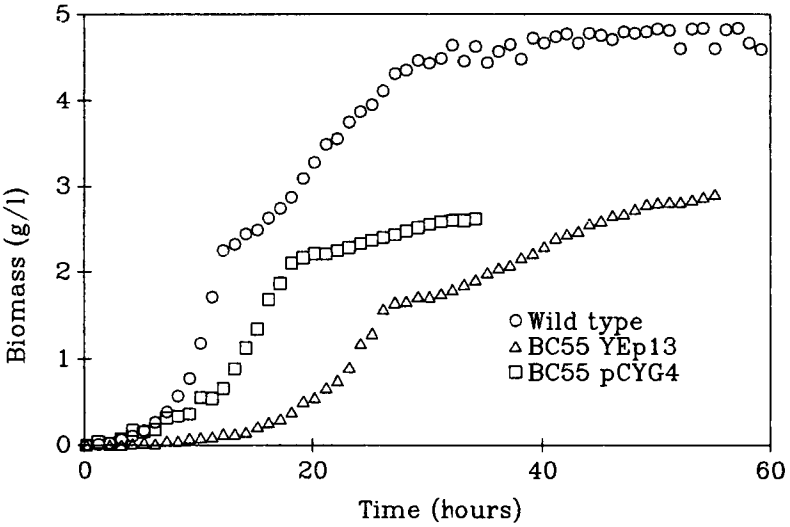

Fig. 1. Growth curves of three strains of *Saccharomyces cerevisiae* in batch culture. The cells were cultured in a 1L glass vessel, using a culture medium containing 2% glucose, 2 m*M* ammonium sulfate, 0.17% yeast nitrogen base at 30°C, 30% of air saturation, under stirring at 200 rpm, and pH, controlled by the addition of KOH (2*M*).

The ammonia uptake in the cells GDH^- was much slower than wild type and the GDH^+ cells. However, the wild type and the GDH^+ cells had practically the same ammonia uptake until the first 15 h (Fig. 2). In the wild type cells, ammonia uptake curve shows four distinct phases, rather than BC55 pCYG4 that had practically only two. The first phase of the wild type cells corresponds to the uptake of glucose and the production of ethanol. The growth rate in this phase is rapid and the metabolism primarily glycolitic, resulting in the accumulation of ethanol in the medium (*8*), then a plateau, which corresponds to the diuxic phase (glucose to ethanol as carbon source). Then another exponential phase occurs that is parallel with utilization of ethanol as carbon source, eventually when the growth stopped the pH raised (Fig. 1), consequently the pump stops, too. The two increased in the pH with the wild type during the growth can be related to K^+ ions diffuse from the cells in exchange for H^+ ions from the medium, which occurs when the substrate was complete utilized (*9*).

With the strain BC55 pCYG4 during the exponential growth and even when the glucose was finished in the medium (the growth rate decreases), and even when cells start to utilize ethanol as a second carbon source, the uptake of ammonia hydroxide was constant, and after 35 h, the amount of ammonia hydroxide added was almost 2.5 times greater in the cells BC55 pCYG4 than with the wild type cells. But, in the cells without NADP-GDH activity, the growth rate was much slower than in both former

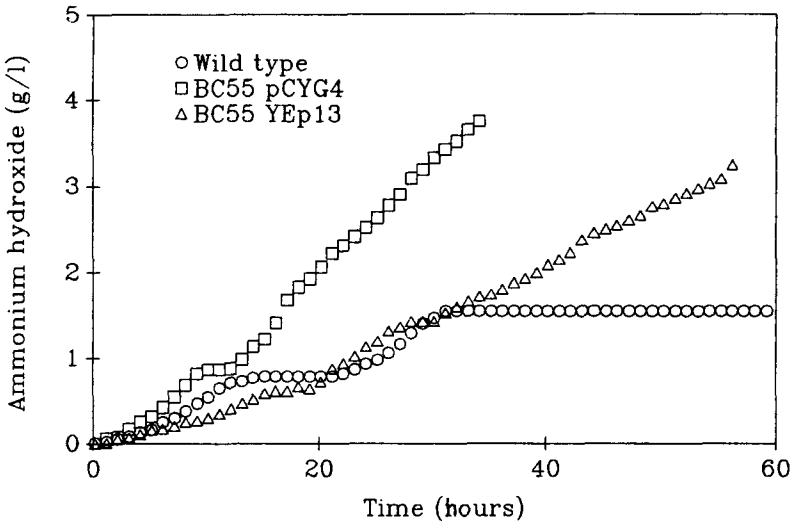

Fig. 2. Ammonia uptake of three strains of *Saccharomyces cerevisiae* in batch culture. The cells were cultured in a 1L glass vessel, using a culture medium containing 2% glucose, 2 m*M* ammonium sulfate, 0.17% yeast nitrogen base at 30°C, 30% of air saturation, under stirring at 200 rpm, and pH, controlled by the addition of KOH (2*M*).

strain, and also the uptake of ammonia hydroxide was approx 3 times slower, however, in contrast with wild type in the BC55 pCYG4 cells the ammonia uptake curve did not presented any similar plateau, which suggest a nontransition period between a utilization of one substrate and another. Similar results were observed with GDH^-. But with an amount of ammonia after 35 h 2.5 times inferior to the GDH^+, it can be expected that these cells do not have the most energetically economical and efficient system of ammonia assimilation working (NADP-GDH), consequently growth rate is much smaller than the former strains (*10*.

It was the observation of San and Stephanopoulos (*11*), that the amount of ammonia added to restore the pH to its set point is exactly equal to the amount taken up by the yeast cells during the growth. The results suggests that the presence of plasmid pCYG4 can increase the amount of ammonia taken by the cells, but not the amount of biomass, comparing the three different strains.

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REFERENCES

1. Brown, C. M. (1976), In *Continuous Culture 6: Application and New Fields.* (Dean, A. C. R., Ellwood, D. C., Evans, C. G., and Melling, J. eds.), Ellis Horwood Ltd., London, pp. 170–180.
2. Cooper, T.G. (1982), In *The Molecular Biology of the Yeast Saccharomyces cerevisiae: Metabolism and Gene Expression.* (Strathern, J. N., Jones, E. W., and Broach, J. R., eds.), Cold Spring Harbor Laboratory, New York, p. 39.
3. Nagasu, T. and Hall, B. D. (1985), *Gene* **37,** 247–253.
4. Lima Filho, J. L. and Ledingham, W. M. (1990), *BioEngineering* **6/86,** 16–18.
5. Lima Filho, J. L. and Ledingham, W. M. (1987), *Biotechnol. Techn.* **1,3,** 145–150.
6. Bergmeyer, H. U. (1974), *Methods in Enzymatic Analysis,* 2nd ed., Vol. III and IV, Academic Press, New York.
7. Wiame, J. L., Greson, M., and Arst, Jr., H. N. (1985), *Adv. Microb. Physiol.* **26,** 1–88.
8. Pamment, N. B., Hall R. J., and Braford, J. P. (1978), *Biotechnol. Bioeng.* **20,** 349–381.
9. Suomalainen, H. and Oura, E. (1971), In *The Yeast* (Rose, A. H. and Harrison, J. S., eds.), Academic Press, London, pp. 3–60.
10. Lima Filho, J. L. and Ledingham, W. M. (1988), *Appl. Biochem. Biotechnol.* **19,** 27–32.
11. San, K. and Stephanopoulos, G. (1984), *Biotechnol. Bioeng.* **26,** 1209–1218.