0273-2289/88/1901—0027$02.00

Studies on *Saccharomyces cerevisiae* Carrying the Plasmid pCYG4 Related with Ammonia Assimilation

Batch Experiments

J. L. LIMA FILHO*[,1] AND W. M. LEDINGHAM[2]

[1]*Departamento de Bioquimica da Universidade Federal de Parnambuco, Cidade Universitaria, 50.761, Recife-PE, Brasil; and* [2]*Department of Biochemistry and Microbiology, University of St. Andrews, St. Andrews, Fife, KY16 9AL, Scotland*

ABSTRACT

Batch culture experiments of three different strains of *Saccharomyces cerevisiae* have been carried out. The first strain was transformed by a plasmid pCYG4, which carries the glutamate dehydrogenase (NADP-GDH, E.C. 1.4.14) gene conferring an 11-fold increase in activity. The second was transformed by the same plasmid, but without NADP-GDH, and the third was the wild type. The specific growth rates of the two recombinant DNA strains were below that of the wild type, which can be related to extra plasmid protein production.

Index Entries: *Saccharomyces cerevisiae;* plasmid; ammonia; batch.

INTRODUCTION

Ammonia can be assimilated into microorganisms by incorporation into glutamine or glutamate. Glutamine synthetase (GS, E.C. 6.3.12), an ATP-requiring enzyme, catalyses the first of these reactions, and, in many microorganisms, serves together with glutamate synthetase (GOGAT, i.e., glutamine 2-oxoglutarate amino transferase, E.C. 2.6.1.15) as the main ammonia-scavenging pathway, which is strongly induced under

*Author to whom all correspondence and reprint requests should be addressed.

nitrogen limitation (*1–3*). This pathway has been described by Meers et. al. (*4,5*), where it was shown that glutamate is synthesized by a two-step process that involves first the amidation of endogenous glutamate to glutamine and then the reductive transfer of the glutamine amide-nitrogen to the 2-position of 2-oxoglutarate.

The latter step involves GOGAT. Glutamate dehydrogenase (NADP-GDH) catalyzes the second reaction, and is usually regarded as a high capacity/low affinity enzyme, mainly functional under unlimited ammonia supply (*6,7*). In organisms having both aminating pathways, GS is drastically decreased on an increase in ammonia supply, and amination proceeds via NADP-GDH (*3*). The engineered strain used in this work was transformed by a plasmid, which carries the glutamate dehydrogenase (NADP-GDH) gene conferring an 11-fold increase in activity (*8,9*).

MATERIALS AND METHODS

Microorganisms

Three different strains of *Saccharomyces cerevisiae* were used in this work.

1. 1278b (Wild type);
2. BC55 (a gdh^- $leu2^-$ Bla^+) carrying the pYE13 same plasmid as pCYG4, but without NADP-GDH gene encoded; and,
3. BC55 (a gdh^+ $leu2^-$ Bla^+) with plasmid pCYG4, which directs substantial overproduction of NADP-GDH (11-fold greater than wild type cells).

The strains were obtained from A. Racher and J. K. Kinghorn (University of St. Andrews).

Medium and Growth Conditions

These strains were cultured in a 1 L glass vessel, using a culture medium containing 2% glucose, 2 m*M* ammonium sulfate, and 0.17% yeast nitrogen base at 30°C, dissolved oxygen being maintained at 30% of air saturation, with agitation at 200 rpm (sufficient to maintain the level of oxygen constant), pH 5 controlled by addition of ammonia (2 *M*). These were controlled by a Processor Control System (PCS) (*10*), which also measured the concentration of biomass (*11*) and the amount of ammonia used to maintain the pH constant (*12*).

Preparation of Extracts

A 3 mL aliquot of cell culture was centrifuged (Eppendorf: 3 min/13,000 rpm). The pellet was resuspended in 1.5 mL extraction buffer (Tris-HCl, pH 8.0, 100 m*M* with 0.15% triton X-100), and glass beads (0.45–0.50 mm of diameter, Waters, USA) were added up to approximately the same

volume as the pellet. Cells were broken with a vortex mixer for 3 min. Supernatants were removed after centrifugation (Eppendorf, 13,000 rpm) and used for enzyme assay.

Enzyme Assay

The Enzyme activities (NADP-GDH and GOGAT) were determined using the methods described in Bergmeyer (*13*).

RESULTS AND DISCUSSION

Table 1 shows the maximum specific growth rate and the NADP-GDH and the GOGAT activities of three different strains of *Saccharomyces cerevisiae* sampled at the end of the exponential growth phase. The growth curves are shown in Fig. 1. Two of them are recombinant DNA strains and one is a wild type strain used as control. The specific growth rates of the two recombinant DNA strains were below that of the wild type (Table 1). This result is in agreement with Lee et. al. (*14*), who showed that yeast cells without plasmids were able to grow faster than those carrying plasmids. This is also in accordance with previous observations in bacteria (*15,16*). Such behavior might result from the added reproductive and metabolic loads on cells carrying extra plasmids (*14,17*). Furthermore, Srienc et. al. (1986) have described experiments in which plasmid-bearing microorganisms grow more slowly than cells without plasmids in the absence of selective pressure. In addition, this growth rate penalty increases as the number of plasmids per cell increases and as the level of plasmid gene expression increases.

Plasmid-encoded gene products may be proteins that are part of the normal metabolic pathways, such as, NADP-GDH (the major pathway for

Table 1
Batch Culture of Three Strains of *Saccharomyces cerevisiae*[a]

Strain	Vectors	Max, /h	NADP-GDH U/mg protein	GOGAT U/mg protein
W. type 1278b	—	0.286	0.474	0.190
BC55	pCYG4	0.248	5.391	1.090
BC55	YEp13	0.105	<0.01	0.230

[a]Table shows the maximum specific growth rate and the NADP-GDH and the GOGAT activities of three different strains of *Saccharomyces cerevisiae* sampled at the end of the exponential growth phase. These strains were cultured in a 1 L 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, with agitation at 200 rpm and pH 5 controlled by addition of ammonia (2 *M*).

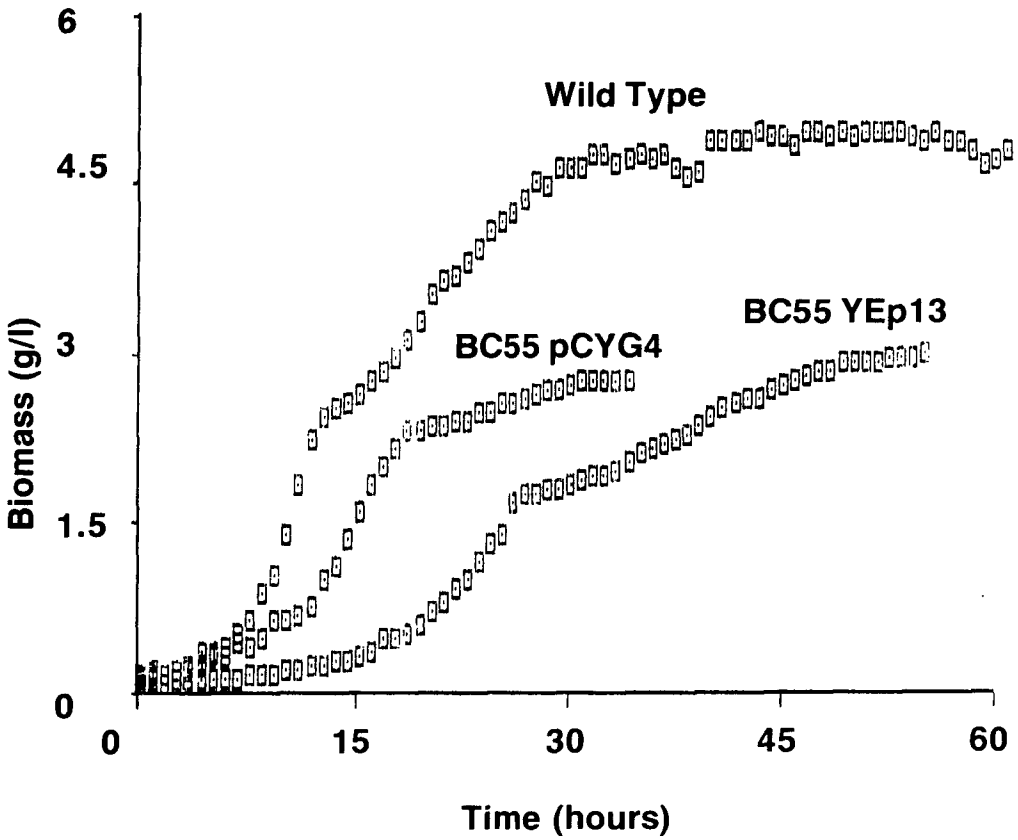

Fig. 1. Batch culture of three strains of *Saccharomyces cerevisiae*. These strains were cultured in a 1 L glass vessel, using a culture medium containing 2% glucose, 2 m*M* ammonium sulfate, 0.17% yeast nitrogen base at 30°C, 30% of air saturation, with agitation at 200 rpm and pH 5 controlled by addition of ammonia (2 *M*).

ammonia assimilation in *Saccharomyces cerevisiae*) (*7,18*). Lack of NADP-GDH in *Saccharomyces cerevisiae* can cause a reduction in growth rate in ammonia, since, in the absence of this pathway, cells start to utilize a more energetically expensive pathway (GOGAT pathway: a pathway with a high affinity for ammonia, but which utilizes more ATP than the NADP-GDH pathway). *Saccharomyces cerevisiae* (strain BC55), lacking NADP-GDH (gdh^-), can overcome this problem using NADP-GDH coded by a NADP-GDH gene on the pCYG4 plasmid (11-fold more activity than NADP-GDH in *Saccharomyces cerevisiae* wild type 1278b). This plasmid also carries the gene for penicillinase (useful marker) and for leucine production (leucine is a selective pressure for BC55 cells (Leu^-)).

The slow growth of BC55 carrying the plamid pCYG4 (gdh^+) compared with wild type cells (1278b) can be related to extra plasmid protein production (e.g., penicillinase, enzymes involved in leucine biosynthesis)

(*14,19*). BC55 cells without NADP-GDH activity carrying the plasmid YEp13 (same plasmid as pCYG4, but without NADP-GDH gene encoded) had a lower growth rate than both gdh$^+$ and wild type 1278b, consequential on the lack of NADP-GDH activity and the extra plasmid protein production (e.g., penicillinase, enzymes involved in leucine biosynthesis) (*20*).

ACKNOWLEDGMENTS

This work was supported by the CNPq (Brazil), O.R.S., University of St. Andrews (Scotland). J. R. Kinghorn and A. Racher (St. Andrews University) are thanked for supplying transformed strains.

REFERENCES

1. Vichido, I., Mora, Y., Quint, C., Palacios, R., and Mora, J. (1978), *J. Gen. Microbiol.* **106,** 251–259.
2. Bogonez, E., Satrastegui, J., and Machado, A. (1985), *J. Gen. Microbiol.* **131,** 1425–1432.
3. Magasaniz, B., Prival, M. J., Brenchley, J. E., Tyler, B. M., De Leo, A. B., Streicher, S. L., Bender, R. A., and Paris, C. G. (1974), *Current Topics in Cellular Regulation.* **8,** 119–138.
4. Meers, J. L. and Tempest, D. W. (1970), *J. Gen. Microbiol.* **64,** 187–194.
5. Tempest, D. W., Meers, J. L., and Brown, C. M. (1970), *Biochemical J.* **117,** 405–407.
6. Burn, V. J., Turner, P. R., and Brown, C. M. (1974), *Antonie van Leeuwenhoek* **40,** 93–102.
7. Tempest, D. W., Meers, J. L., and Brown, C. M. (1973), *The Enzymes of Glutamine Metabolism* (Prusiner, S. and Stadtman, E. R. eds.), Academic, New York, 167–182.
8. Nagasu, T. and Hall, B. D. (1985), *Gene* **37,** 247–253.
9. Moye, S. W., Amuro, N., Rao, J. K. M., and Zalkin, H. (1985), *J. Biol. Chem.* **14,** 8502–8508.
10. Lima Filho, J. L. and Ledingham, W. M. (1987), *Proc. 4th European Congress on Biotechnology, Amsterdam* **3,** 82.
11. Lima Filho, J. L. and Ledingham, W. M. (1987), *Biotechnology Techniques* **1,** 145–150.
12. Lima Filho, J. L. (1987), Ph.D. Thesis, University of St. Andrews, Scotland.
13. Bergmeyer, H. U. (1974), *Methods in Enzymatic Analysis,* 2nd ed., vols. III and IV, Academic, New York.
14. Lee, G. M., Song, K. B., Rhee, S. K., and Han, M. H. (1986), *Biotechnol. Lett.* **6,** 385–390.
15. Helling, R. B., Kinney, T., and Adams, J. (1981), *J. Gen. Microbiol.* **123,** 129–141.
16. Old and Primrose (1985), *Principles of Gene Manipulation,* Blackwell, London.

17. Srienc, F., Campbell, J. L., and Bailey, J. E. (1986), *Biotechnol. Bioeng.* **28,** 996–1006.
18. Burn, V. J., Turner, P. R., and Brown, C. M. (1974), *Antonie van Leeuwenhoek* **40,** 93–102.
19. Caulcott, C. A. (1984), *Biochemical Society Transactions,* 1140–1141.
20. Grenson, M., Dubois, E., and Piotrowska, M. (1974), *Molec. Gen. Genet.* **128,** 73–85.