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Studies on *Saccharomyces cerevisiae* Under Carbon-Limiting Growth Transformed with Plasmid pCYG4 that Carries the Gene for NADP-GDH

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ABSTRACT

The gene (GDH1) coding for the NADP-linked glutamate dehydrogenase system (NADP-GDH) has been cloned from *Saccharomyces cerevisiae* strain. Cells being transformed by the NADP-GDH gene on a 2 μm bared vector (pCYG4) plasmid confering 11-fold higher level on expressed GDH activity over the wild-type cells. The behavior of these cells was investigated under chemostatic growth with a carbon rate-limiting nutrient. Specific growth rates of cells carrying plasmid pCYG4 were found to be slightly slower than wild type cells. Furthermore, the NADP-GDH activity increases proportionally with the dilution rate. In addition, oscillations in the NADP-GDH activity, especially at a dilution rate up to 0.15/h, are probably consequential on the appearance of a changing mixed population (cells with and without plasmids).

Index Entries: *Saccharomyces cerevisiae;* plasmid; NADP-GDH; continuous culture; chemostatic.

INTRODUCTION

The ammonium may be assimilated into the cells following two different pathways (*1–3*). The first is catalyzed by glutamate dehydrogenase

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(NADP-GDH), which is known to be a key enzyme in the metabolism of nitrogen.

The second pathway consists of two reactions (*4,5*), glutamine synthatase (ATP-dependent), which produces a molecule of glutamine from one molecule of ammoniun and one of glutamate, and glutamine 2-oxoglutarate aminotransferase (for convenience GOGAT), which utilizes the glutamine formed in the first reaction plus 2-oxoglutarate and NADPH as cofactor producing two molecules of glutamate according to the equations.

I. (NADP-GDH)

$$NH_4 + \text{2-Oxoglutarate} + NADPH \rightleftharpoons GLU + NADP$$

II. Glutamine synthetase (ATP)

$$GLU + NH_4 + ATP \rightleftharpoons GLN + ADP$$

GOGAT

$$\text{2-Oxoglutarate} + GLN + NADP(H) \rightleftharpoons 2\,GLU + NADP$$

NET

$$NH_4 + \text{2-Oxoglutarate} + ATP + NADP(H) \rightleftharpoons GLU + ADP + NADP$$

In organisms having both aminating pathways, glutamine synthetase is drastically upon an increase in ammonia supply and amination proceeds via glutamate dehydrogenase (*1*).

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) (*2*).

The gene coding for NADP-GDH (GDH1) has been cloned from *Saccharomyces cerevisiae* cells, transformed with the cloned GDH1 gene, and have an 11-fold higher enzyme activity compared to wild-type cells (*5*). We report the NADP-GDH activity of this engineered yeast under carbon rate-limiting growth substrate. Conditions in which the excess of nitrogen inhibits the GOGAT and NAD-GDH activity does not interfere with NADP(H)-GDH activity.

MATERIALS AND METHODS

Host Strain

Saccharomyces cerevisiae wild-type Σ1278b and *Saccharomyces cerevisiae* strain BC55 (a, gdh 1-6, per 1-30, Leu 2-3, and Leu 2-112) were obtained from A. Racher and J. R. Kinghorn (University of St. Andrews).

Plasmids

(1), YEp13 (NADP-GDH−); and 2, pCYG4 (NADP-GDH+YEp13 containing the NADP-GDH gene) (*5*).

Culture Conditions

For batch or continuous culture, experiments were carried out with a microcomputer-controlled fermenter system designed and constructed "in house" (*8*), and maintained under the following conditions: 1 L nominal volume; 0.6 L working volume; dissolved oxygen being maintained at 30% of air saturation; temperature 30°C, and pH 5 controlled by the addition of KOH (2*M*).

Growth Media

Carbon limiting: 0.5% (w/v) glucose; 4 g/L ammonium sulphate, and 0.17% Difco yeast nitrogen base (YNB).

Enzyme and Metabolite Assay

The assay for NADP-GDH has been reported elsewhere (*6*). The GOGAT assay was carried out as previously described (*4*). Protein was determined by Bradford's method (*7*), with bovine serum albumin as the standard. One unit of enzyme activity was defined as the amount of enzyme that transforms 1 μmol of NAD(P)H to NAD (P) per minute.

Preparation of Extracts

A 3 mL aliquot of cell culture was centrifuged (Eppendorf: 3 min/13,000 rpm). The pellet was resuspend in 1.5 ml extraction buffer (Tris-HCl 100 m*M*, pH 8.0, 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.

Determination of Biomass

The determination of biomass was carried out using an electrode system attached to a small scale laboratory fermenter. The system comprises a high intensity light emitting diode (LED) of 500 mcd luminosity coupled with a photodiode. Detector output is amplified and fed to a microprocessor control and logging system (*8*). The curve in the figures were determined by a nonlinear regression method (*9*) using the STATPACK software package for an IBM microcomputer (*10*).

Maximum Specific Growth Rate (μmax)

The determination of Maximum specific growth rate was carried out by Pirt's method (*11*).

Table 1
Batch Culture of Three Strains of *Saccharomyces cerevisiae* Shows the Maximum Specific Growth Rate (μmax) and the NADP-GDH Activity of Three Different Strains of *Saccharomyces cerevisiae* Sampled at the End of the Exponential Growth Phase[a]

Strain	Vectors	μmax/h	NADP-GDH, U/mg protein
W. Type Σ1278b	-	0.286	0.474
BC55	pCYG4	0.248	5.391
BC55	YEp13	0.105	<0.01

[a]These strains were cultured in a 1 L glass vessel, using a culture medium containing 2% glucose, 2 m*M* ammonium sulphate, and a 0.17% yeast nitrogen base at 30°C, 30% of air saturation, with agitation at 200 rpm and pH 5, controlled by the addition of KOH (2*M*).

RESULTS AND DISCUSSION

Saccharomyces cerevisiae BC55 was transformed with the plasmid pCYG4 (GDH-NADP+). The transformed has 11-fold higher GDH-NADP activity than the wild type cells (Table 1). In contrast, in the strain BC55 YEp13, the GDH-NADP activity was practically undetectable owing to the absence of the GDH-NADP gene in the genome of the cells and also in the plasmid. Furthermore, the maximum specific growth rate (μmax) shows to be slightly bigger in the wild type than the other two strains (Table 1). These results is in agreement with Lee et al. (*12*) and Lima Filho et al. (*13*), who showed that yeast cells without plasmid were able to grow faster than those carrying plasmids. This is also in accordance with previous observations in bacteria (*14,15*).

Oscillations in NADP-GDH activity (Fig. 1), especially at dilution rate over 0.15/h is probably consequential on the appearance of a changing mixed population of pCYG4 plasmid-positive cells and pCYG4 plasmid-deficient cells. This may be conferred on the cells by the pCYG4 plasmid (*16*).

The NADP-GDH activity of *Saccharomyces cerevisiae* strain BC55 under carbon-liminting substrate increases proportionally with dilution rates (*16*), which suggests a regulatory effect stimulated by the need for more enzyme activity by the cells at higher dilution rates.

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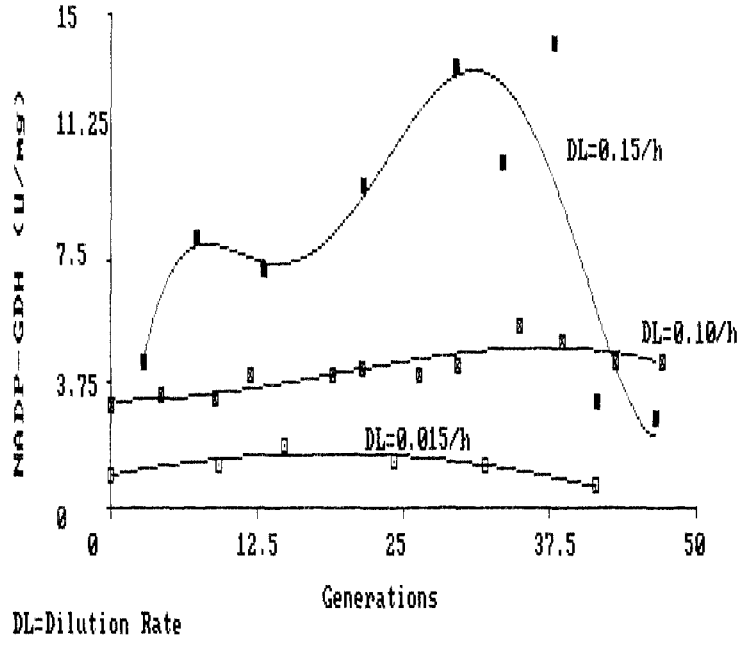

Fig. 1. NADP-GDH activity of BC55+pCYG4 (GDH-NADP+) cells of *Saccharomyces cerevisiae* growing under carbon-limited continuous culture. These strains were cultured in a 1 L glass vessel, using a culture medium containing 2% glucose, 2 m*M* ammonium sulphate, and a 0.17% yeast nitrogen base at 30°C, 30% of air saturation, with agitation at 200 rpm and pH 5, controlled by the addition of KOH (2*M*).

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