0273-2289/99/77–79/0311/$11.50

L-Asparaginase II of *Saccharomyces cerevisiae*

Activity Profile During Growth Using an ure2 Mutant P40-3C and a P40-3C+URE2p Strain

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Abstract

The activity profile of the periplasmic asparaginase of *Saccharomyces cerevisiae* was determined during cell growth in an *ure2* mutant; in an *ure2* transformed with a plasmid containing the gene *URE2* and, for comparison, in the strain D273-10B. Cells were cultivated in media presenting variable quantitative and qualitative nitrogen availability and the enzyme activity was evaluated in fresh and in nitrogen-starved cells. Nitrogen affected the asparaginase II level in fresh and starved cells of all strains. In the best condition, enzyme was produced by the wild-type cells at the late log-phase in the glucose/ammonium medium with a carbon to nitrogen ratio 4.3:1. Upon starvation, the activity doubled. The overall profile of the transformed strain was similar to that of the wild-type strain. In the *ure2* mutant, high-enzyme levels were observed during growth, as expected. However the activity level, upon starvation, in proline grown cells, increased sixfold, suggesting that in addition to the Ure2p-Gln3p system, another system regulates asparaginase II biosynthesis.

Index Entries: *Saccharomyces cerevisiae*; URE2 protein; nitrogen regulation; L-asparaginase II; nitrogen nutrition.

Introduction

The regulation of yeast periplasmic enzymes could be used as a model to study the regulatory system for extracellular enzymes. *Saccharomyces cerevisiae* presents the periplasmic asparaginase II that is produced upon nitrogen starvation. The production of this enzyme is regulated by the Ure2p-Gln3p system *(1–3)* that also regulates some intracellular enzymes such as glutamine synthetase, NAD-linked glutamate dehydrogenase, general amino-acid permease, and high-affinity proline permease *(2)*.

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Table 1
Strains of *Saccharomyces cerevisiae*

Strain	Genotype	Source
D273-10B	Wild-type	Boris Magasanik (MIT)
P40-3C	Mat α; leu 2-3,112; ura3-52; ade2-102; ure2Δ11::Leu2	Boris Magasanik (MIT)
P40-3C +*URE2*	Mat α; leu 2-3,112; ura3-52; ade2-102;ure2Δ11::Leu2 transformed with *URE2*p	This work

Table 2
Nitrogen Composition and C/N Ratio of the Culture Media

Medium	Nitrogen source	C/N ratio
E1	1.32 g/L Ammonium sulfate	25:1
E2	9.9 g/L Ammonium sulfate	4.3:1
E3	17.2 g/L Proline	4.3:1

There are contradictory reports as to the cells growth phase for the L-asparaginase II activity, which is related to the exponential phase by some authors *(4)* and to the beginning of the exponential phase by others *(5)*. The objective of this work was to define the activity profile of asparaginase II of *S. cerevisiae*, during cell growth in the wild-type strain D273-10B, in the *ure2* mutant P40-3C, and in this mutant transformed with a plasmid containing the gene *URE2*. Cells were grown on media presenting variable quantitative and qualitative nitrogen availability and the enzyme activity was evaluated in fresh and nitrogen-starved cells.

Materials and Methods

Strains

S. cerevisiae strains used in this work are listed at Table 1. The P40-3C transformation was performed according to Ito et al. *(6)*.

Growth Conditions

Cells were cultivated at 29°C on a rotatory shaker at 160 rev/min, in 100 mL growth media containing 20 g/L glucose, 2 g/L yeast nitrogen base without amino acids, and ammonium sulfate (Difco, Detroit, MI) and different nitrogen sources, according to Table 2. Cell growth was monitored by the determination of optical density (OD) at 600 nm in a CAM SPEC spectrophotometer (Camspec Ltd, Cambridge, UK). L-Asparaginase activity was determined in cells harvested at different growth phases (lag, mid-log, late-log, and early diauxie).

Nitrogen Starvation Experiments

The effect of nitrogen starvation on L-asparaginase II production was investigated by incubation of fresh cells previously washed in 20 m*M* phosphate buffer, pH 7.0, in a 30 g/L glucose with the same buffer for 2 h.

Enzyme Activity Assay

Asparaginase II activity was assayed in fresh and in starved cells. L-asparagine was added to a cell suspension (OD 600 nm of 0.6) to a final concentration of 500 m*M*. After an incubation period of 30 min, a 0.5-mL sample was withdrawn and filtered to separate the cells and stop the reaction. Ammonia concentration on the supernatant was measured spectrophotometrically by coupling to glutamate dehydrogenase (Boehringer-Mannheim, Indianapolis, IN) *(1)*.

Results

Asparaginase II Production by the Wild-Type Strain D273-10B

This strain presented in medium E1 an overall low enzyme activity with a maximal of 7 U/g cell dry weight (dw) in mid-log phase cells. Upon nitrogen starvation, no activity was observed. A higher enzyme activity, 24 U/g cell dw, was observed in fresh mid-log cells grown in medium E2, which presented a higher ammonium content. This superior nitrogen availability also positively affected the cell response to starvation, because under these conditions asparaginase activity doubled. In the proline medium no activity was observed, either in fresh or starved cells, most probably because proline is a poor nitrogen source (Fig. 1).

Asparaginase II Production by the ure2 Mutant Strain P40-3C

This strain produced L-asparaginase II in all media, independent of the nitrogen composition as shown in Fig. 2. This behavior was already expected, as this strain lacks the protein repressor URE2 that responds to nitrogen availability regulating the cell nitrogen metabolism. In medium E1, enzyme levels of 55, 109, 74, and 35 U/g cell dw were observed at the relevant growth phases and no further increase on enzyme activity was observed after the incubation of these cells in 30 g/L glucose. A different behavior was observed, however, in fresh cells grown in medium E2 as the enzyme activities around 55 U/g cell dw were observed for all growth phases cells and doubled upon starvation. These data, plus the aforementioned response to nitrogen starvation of the wild-type cells grown in medium E2, indicate the importance of an adequate nitrogen nutrition for the correct assessment of the cell response to starvation. Interestingly, both wild-type and *ure2* mutant cells growing in the E2 medium doubled enzyme activity upon nitrogen starvation. Regarding the behavior of the *ure2* mutant grown in proline, the activity in fresh cells increased progressively during growth, and when these cells were nitrogen-starved, the levels of

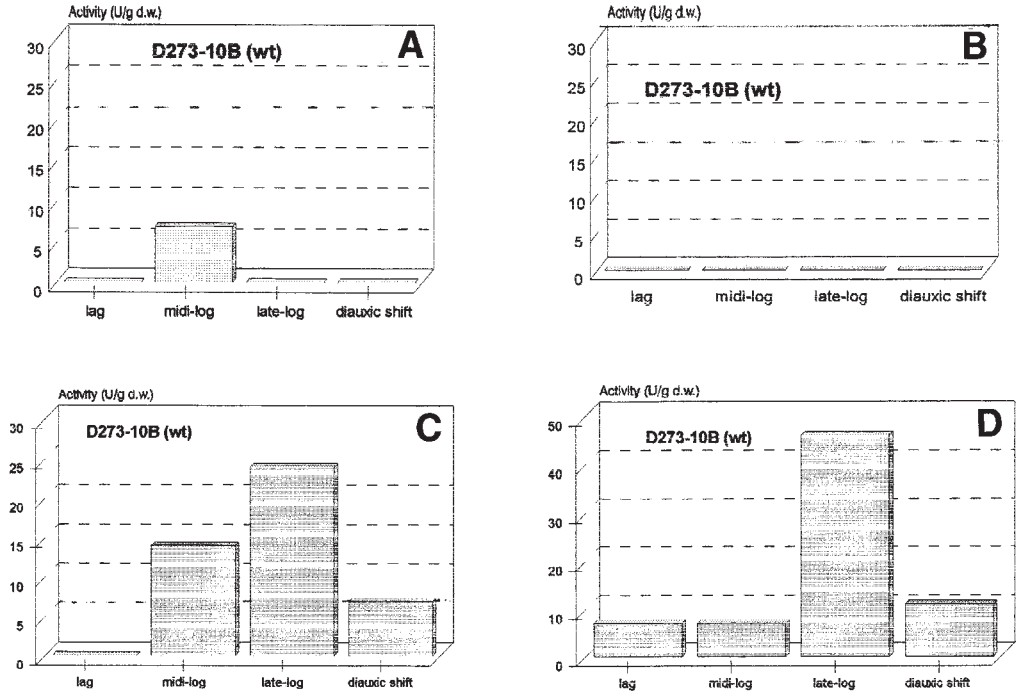
Activity (U/g d.w.)
D273-10B (wt)
A
B
C
D
lag
midi-log
late-log
diauxic shift

Fig. 1.

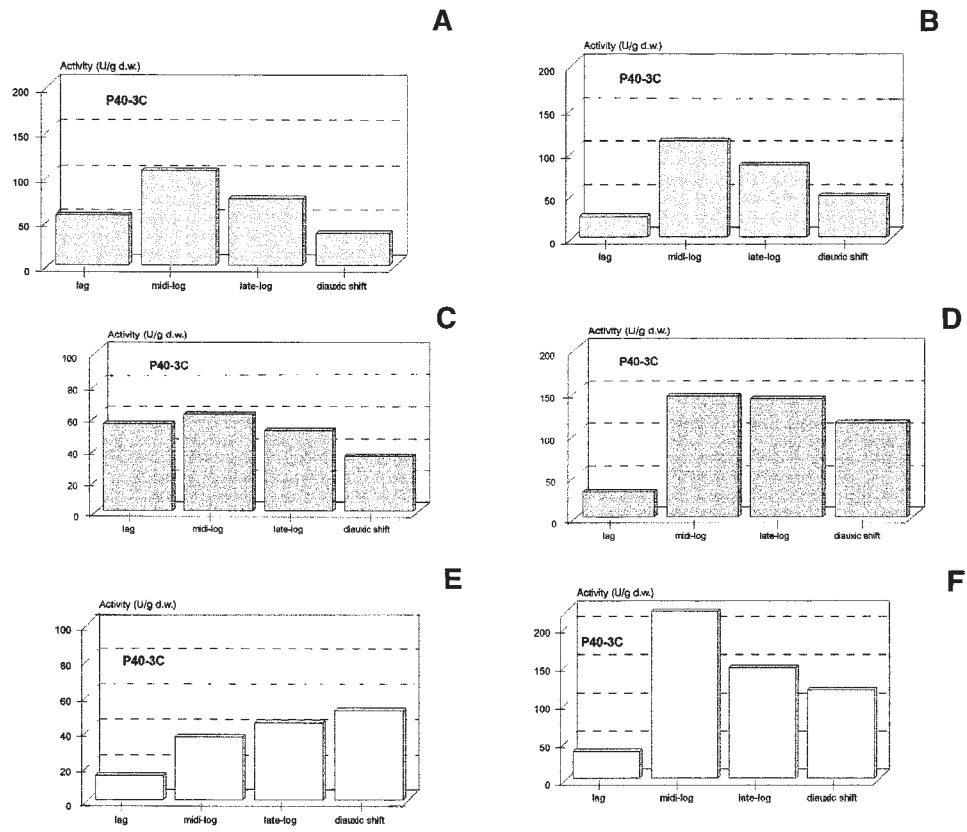
A
B
C
D
E
F
Activity (U/g d.w.)
P40-3C
lag
midi-log
late-log
diauxic shift

Fig. 2.

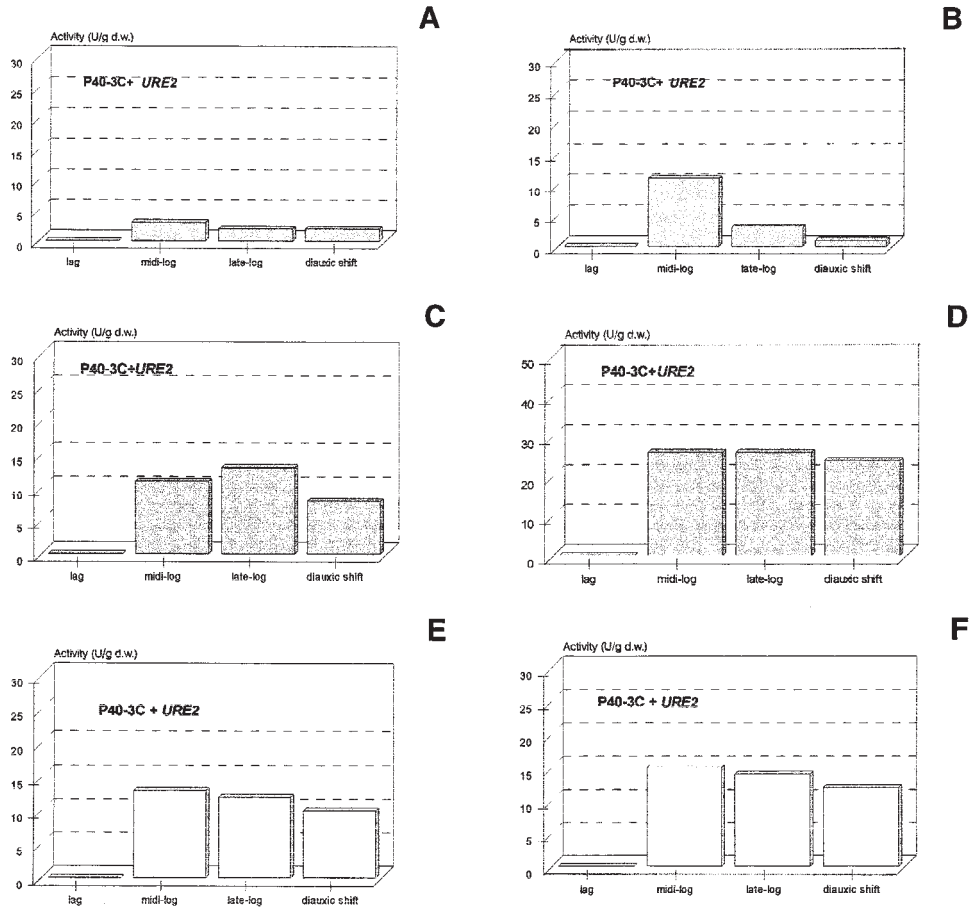

Fig. 3. L-asparaginase II production by transformed strain P40-3C+*URE2* in media E1(1.32 g/L ammonium sulfate) **(A,B)**, medium E2 (9.9 g/L ammonium sulfate) **(C,D)**, medium E3 (17.2 g/L proline) **(E,F)**, during growth (A,C,E), and after nitrogen starvation (B,D,F).

L-asparaginase increased. The starved mid-log cells presented the activity of 219 U/g cell dw, which was sixfold higher than the activity of fresh cells.

*Asparaginase II Production in the Transformed Strain P40-3C+*URE2

The general pattern for L-asparaginase II production shown by the transformed strain was the same as that observed for the wild-type strain (Fig. 3), confirming the role of URE2 protein on L-asparaginase II regulation.

Fig. 1. *(previous page)* L-Asparaginase II activities of wild-type strain D273-10B in media E1(1.32 g/L ammonium sulfate) **(A,B)** and medium E2 (9.9 g/L ammonium sulfate) **(C,D)**, during growth (A,C) and after nitrogen starvation (B,D).

Fig. 2. *(previous page)* L-Asparaginase II activities of *ure2* mutant strain P40-3C in media E1 (1.32 g/L ammonium sulfate) **(A,B)**, medium E2 (9.9 g/L ammonium sulfate) **(C,D)**, medium E3 (17.2 g/L proline) **(E,F)**, during growth (A,C,E), and after nitrogen starvation (B,D,F).

Discussion

The levels of L-asparaginase II in response to nitrogen starvation of the *ure2* mutant cells in medium E3 indicated the existence of a more complex system involved in this enzyme regulation, in addition to the Ure2p-Gln3p system. The gradual increasing enzyme activity presented by the fresh proline-grown cells suggests a progressive derepression of the *ASP3* gene that codes for L-asparaginase II *(7,8)*. Because proline catabolism generates glutamate, its gradual decrease in the metabolic pool would trigger the response of this second hypothetical system that could involve the NIL1 protein. This activator, which responds to glutamate concentration, has been studied in connection with nitrogen-regulated genes *(9,10).*

This information may be quite useful in terms of enzyme production optimization because the enzyme activity in the *ure2* mutant nitrogen starved cells was 30 times higher in comparison to that of wild-type fresh cells. These findings, related to nitrogen regulation of an extracellular enzyme, may have a potential biotechnological application because the same regulatory system may be involved in the regulation of a number of enzymes of industrial interest.

Acknowledgments

This work was partially supported by Brazilian Research Council (CNPq). We thank Prof. Boris Magasanik from the Department of Biology of MIT for providing us with the mutant and the *URE2*p plasmid.

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