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Ammonia Assimilation in *S. cerevisiae* Under Chemostatic Growth

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ABSTRACT

Glutamate, glutamine, and ammonia pool size have been determined in two *S. cerevisiae* strains (GOGAT$^+$ and GOGAT$^-$) growing under ammonia excess and limitation at a dilution rate of 0.10/h. The biomass levels and glutamate dehydrogenase NADPH-dependent (NADPH-GDH) activities were also measured for both strains. The strain that lacks GOGAT activity showed lower levels of metabolites under both media and lower levels of biomass under carbon limitation (ammonia excess) compared to the GOGAT$^+$ strain. Under nitrogen limitation, the biomass level was the same for both strains, but GOGAT$^-$ changed from rounded to ellipsoidal cells.

Index Entries: *S. cerevisiae;* enzymes of ammonia assimilation.

INTRODUCTION

Most of the nitrogen required for the growth of *S. cerevisiae* is usually supplied as added ammonia. The primary products of ammonia assimilation in this microorganism are L-glutamate and L-glutamine. Although enzymes of the pathways and some of the factors affecting their regulation have been determined, there are still considerable gaps in the understanding of the physiology of ammonia assimilation. There are two main pathways for the assimilation of ammonia by *S. cerevisiae.* In the first one,

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the interconversion between ammonia and glutamate is mediated by two glutamate dehydrogenases. The NAD-dependent enzyme (Reaction I) has been pointed out as a catabolic enzyme and the NADPH-linked enzyme (Reaction II) as a metabolic enzyme (*1,2*).

Reaction I:

$$\text{L-glutamate} + \text{NAD} \overset{\text{GDH}}{\rightleftharpoons} \text{2-oxoglut.} + \text{NADH} + \text{NH}_4^+$$

Reaction II:

$$\text{NH}_4^+ + \text{NADPH} + \text{2-oxoglut.} \overset{\text{GDH}}{\rightleftharpoons} \text{L-glutamate} + \text{NADP}$$

A secondary pathway can be achieved by the coupling of the ATP-dependent glutamine synthetase (GS), which synthesizes glutamine from glutamate and ammonia (Reaction III), with a glutamate synthase (GOGAT). This enzyme catalyses the transfer of the amide group of glutamine to 2-oxoglutarate, a reaction that is NADPH-dependent for bacteria and NADH-dependent for yeasts (Reaction IV).

Reaction III:

$$\text{L-glutamate} + \text{ATP} + \text{NH}_4 \overset{\text{GS}}{\longrightarrow} \text{L-glutamine} + \text{ADP} + \text{Pi}$$

Reaction IV:

$$\text{L-glutamine} + \text{2-oxoglut.} + \text{NAD(P)H} \overset{\text{GOGAT}}{\rightleftharpoons} \text{L-glutamate} + \text{NAD(P)}$$

The accepted view is that NADPH-GDH is the primary route for ammonia assimilation with a minor contribution of GS-GOGAT pathway, which would work mainly in conditions of nitrogen starvation (*1,3*).

In this paper, we report the effects of the elimination of GOGAT activity in *S. cerevisiae* growing under carbon or nitrogen limitation, using chemostatic growth technique.

MATERIALS AND METHODS

Strains

S. cerevisiae strains AR2 and AR5 were provided by Racher and Kinghorn from the University of St. Andrews. The AR2 strain was derived from BC55 (GOGAT$^+$ GDH$^-$ leu 2$^-$). *S. cerevisiae* AR5 was selected from a BC55 mutant that showed very low GOGAT activity. Both strains bear approx 30 copies of the plasmid pCYG4, which confers NADPH-GDH activity (about five-fold higher compared to wild types).

Growth Conditions

Cells were inoculated into 100 mL of YNB medium (0.17% yeast nitrogen base, 2% glucose, and 20 m*M* ammonium sulphate) at 30°C and incubated overnight on an orbital shaker. Subsequently, 50 mL of the inoculum were transferred to 500 mL of fresh YNB medium in a fermenter vessel. When the cells achieved late exponential phase in batch culture, the continuous influx of carbon-limiting medium (0.5% glucose) with excess of ammonia (20 m*M* ammonium sulphate) or nitrogen-limiting medium (2 m*M* ammonium sulphate) was started at a dilution rate of 0.10/h and run for approx 10 d. Samples were collected every 10 or 12 h for biomass, enzymes, and metabolites measurement. The pH was maintained at 5 by addition of 2 *M* KOH, and oxygen saturation was kept at 30%. The biomass concentration was measured by the relationship of absorbance–dry wt of cells.

Metabolites Measurement

The enzymatic determination of glutamate, glutamine, and ammonia were carried out as described by Bergmeyer (*4*).

Enzymatic Activities

Aliquots of cell culture were centrifuged (Eppendorf:3 min:13,000 rpm), and the pellets resuspended in 0.1 *M* potassium phosphate buffer pH 7.5 containing 5 m*M* ethylenediaminetetraacetic acid disodium salt (EDTA), 0.1m*M* phenylmethylsulphonylfluoride (PMFS), and 0.25% 2-mercaptoethanol. Cells were broken with glass beads, and supernatants were used as samples for enzyme assay after centrifugation (Eppendorf:3 min:13,000 rpm). NADPH-GDH and GOGAT activities were determined by recording the decrease of absorbance at 340 nm after addition of appropriate substrate, using a molar extinction coefficient of 6.22 cm/μmol (3.5).

RESULTS AND DISCUSSION

Table 1 shows the average results of three experiments with concentrations for ammonia, glutamate, and glutamine, as well as NADPH-GDH activities for both strains growing under a dilution rate of 0.10/h. The data were evaluated statistically for the difference between two group means supposing H_0: $x_1 = x_2$ for a level of $= 0.01$). The results of the test showed that the true hypothesis was H_1: $x_1 \neq x_2$ for all the groups, except enzymatic activities. GOGAT activities were only 0.1% of the NADPH/GDH activities for AR2 and not detectable for AR5. In wild types of *S. cerevisiae,* the GOGAT activity has been found to be about 10% of that of NADPH-GDH; but the presence of the plasmid pCYG4 in the AR2 and AR5 strains

Table 1
Average Intracellular Metabolite and Biomass Concentrations and NADPH-GDH Activities for *S. cerevisiae* Growing Under Carbon or Nitrogen Limitation*

	Glutamate μmol/mg protein	Glutamine	Ammonia	Biomass mg/mL	NADPH-GDH U/mg
Carbon limitation:					
AR2	0.70	0.20	5.00	3.50	10.00
AR5	0.36	0.04	4.20	2.00	10.00
Nitrogen limitation:					
AR2	1.80	0.25	1.50	1.45	14.00
AR5	0.60	0.15	1.40	1.45	12.00

*At D of 0.10/h, 30°C, 30% of oxygen, and pH 5.0.

directs substantial overproduction of NADPH-GDH, explaining the low percentage of GOGAT activity. The GS-GOGAT pathway has been suggested as a scavenging pathway for ammonia assimilation under nitrogen starvation. In that case, the lack of this pathway would not be expected to affect the primary products of ammonia assimilation in conditions of excess of ammonia. However, even under such conditions, the glutamate and glutamine pools of the strain lacking GOGAT activity were only around 40% of those of the GOGAT$^+$ strain. In GOGAT$^-$ strains of *N. crassa*, an accumulation of glutamine has been reported, but the presence of two different mechanisms of glutamine degradation (glutaminase activity and w-amidase pathway) in *S. cerevisiae* would prevent the accumulation of this metabolite in the microorganism (*6–8*).

The glutamate and glutamine levels were much higher for both strains growing under nitrogen limitation than under excess of ammonia. It has been shown that nitrogen starvation results in a marked increase in general aminoacid permease activity, which could explain the increased aminoacid concentration (*9*). Furthermore, substantial amounts of aminoacids are associated with vacuoles of *S. cerevisiae*, which could provide the necessary amount of aminoacids for the cell to continue its vital cycle (*10*).

As high intracellular ammonia concentration leads to a repression of NADPH-GDH synthesis, the activities for both strains were higher under nitrogen limitation. The lack of the GOGAT pathway also resulted in a decrease in the biomass concentration compared to the GOGAT$^+$ strain growing under ammonia excess. Under nitrogen limitation, the biomass level was the same for both strains, but the cells of the GOGAT$^-$ microorganism changed from rounded to ellipsoidal form (*see* Figs. 1–4). Changes in the shape of yeast cells may be related to different factors, such as membrane composition, aminoacid concentration, or starvation of some

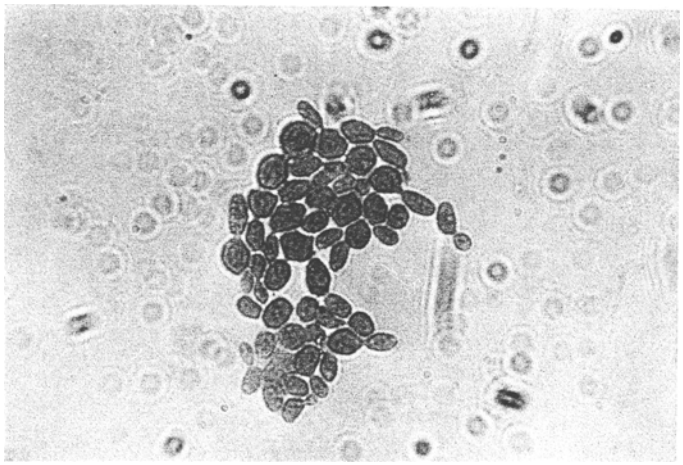

Fig. 1. AR2 cells growing under carbon limitation.

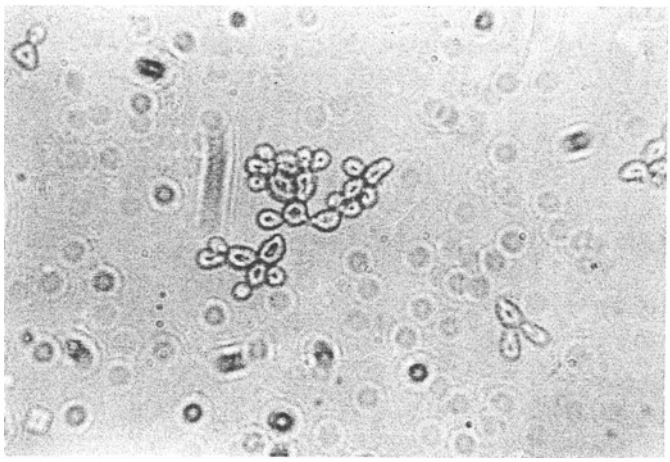

Fig. 2. AR2 cells growing under nitrogen limitation.

nutrient (*11,12*). It has been reported that cells of *T. variabilis* appear in a triangular form when grown in the presence of methionine, but in the absence of this aminoacid, ellipsoidal-shaped cells are produced (*13*).

The lower metabolite concentration together with the biomass effects fore the GOGAT$^-$ strain growing under both media suggest a more important role than just that of a scavenging pathway for the GOGAT system. As the enzymes of ammonia assimilation are in the interface between the carbon and nitrogen metabolism, it may be that the GOGAT pathway is related to the maintenance of any of the intermediates of the Krebs cycle. Further studies are needed to investigate this possibility.

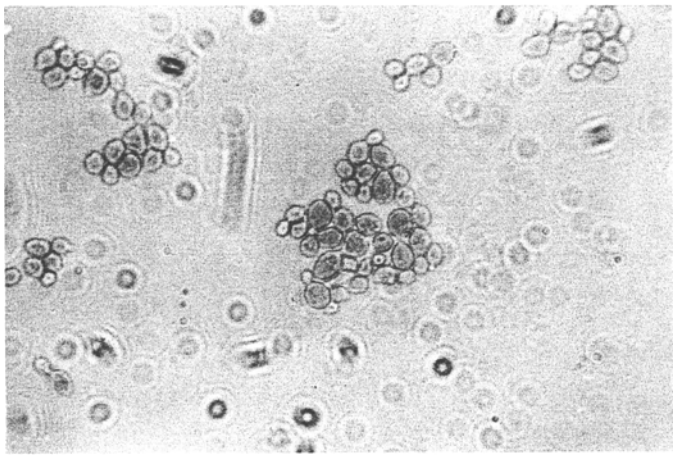

Fig. 3. AR5 cells growing under carbon limitation.

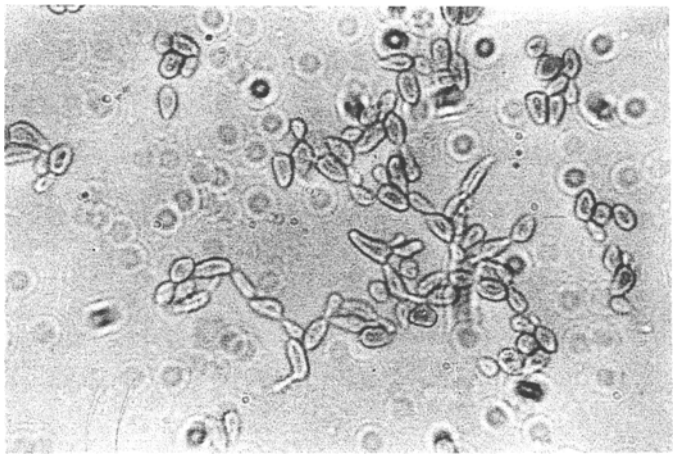

Fig. 4. AR5 cells growing under nitrogen limitation.

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