Pathways for glutamate biosynthesis in the yeast *Kluyveromyces lactis*

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Purified glutamate synthase (GOGAT) from *Kluyveromyces lactis* was characterized as a high-molecular-mass polypeptide, a distinction shared with previously described GOGATs from other eukaryotic micro-organisms. Using degenerate deoxyoligonucleotides, designed from conserved regions of the alfalfa, maize and *Escherichia coli* GOGAT genes, a 300 bp PCR fragment from the *K. lactis* GOGAT gene *KIGLT1* was obtained. This fragment was used to construct null GOGAT mutants of *K. lactis* by gene replacement. These mutants showed no growth defect phenotype and were able to grow on ammonium as sole nitrogen source. Double mutants obtained from a cross between a previously described *KIGDH1* mutant and the *K. lactis* null GOGAT strain were full glutamate auxotrophs. These results indicate that glutamate biosynthesis in *K. lactis* is afforded through the combined action of *KIGDH1* and *KIGLT1* products.

Keywords: glutamate biosynthesis, glutamate synthase, glutamate dehydrogenase

INTRODUCTION

Two pathways for ammonium assimilation and glutamate biosynthesis have been found in a variety of micro-organisms. The first one, described by Holzer & Schneider (1957), is mediated by NADP-glutamate dehydrogenase (NADP-GDH; EC 1.4.1.4), which catalyses the reductive amination of 2-oxoglutarate to form glutamate. In an alternative pathway demonstrated by Tempest *et al.* (1970), glutamate is aminated to form glutamine by glutamine synthetase (GS; EC 6.3.1.2), the amide group of which is then transferred reductively to 2-oxoglutarate by glutamate synthase (GOGAT; EC 1.4.1.13), resulting in the net conversion of ammonium and 2-oxoglutarate to glutamate. The GS-GOGAT pathway has been found in several micro-organisms (Senior, 1975; Hummelt & Mora, 1980; Bravo & Mora, 1988; Marqués *et al.*, 1992) and in higher plants (Mifflin *et al.*, 1980).

Mutants affected in either NADP-GDH or GOGAT have been obtained in a number of fungal species. In the yeast *Saccharomyces cerevisiae*, besides the GS-GOGAT

pathway, there are two NADP-GDH isozymes, NADP-GDH1 and NADP-GDH3, encoded by *GDH1* and *GDH3*, respectively (Avendaño *et al.*, 1997). In this yeast, only the triple mutant lacking GOGAT, GDH1 and GDH3 shows a tight glutamate auxotrophy (Avendaño *et al.*, 1997). In *Neurospora crassa* and *Aspergillus nidulans*, the simultaneous lack of GOGAT and NADP-GDH leads to full auxotrophy, indicating that in this species, only two pathways for glutamate biosynthesis are functional (Romero & Dávila, 1986; Macheda *et al.*, 1999). Thus, *S. cerevisiae* is so far the only example of a micro-organism possessing three pathways for glutamate biosynthesis.

In *Kluyveromyces lactis*, both NADP-GDH and GOGAT activities have been detected (Valenzuela *et al.*, 1995). Mutants devoid of NADP-GDH showed a wild-type phenotype, suggesting that either the GS-GOGAT pathway plays a major role in glutamate biosynthesis or that, as found for *S. cerevisiae* *gdh1* mutants, glutamate biosynthesis could be maintained by the combined action of the GS-GOGAT pathway and a third biosynthetic route. To analyse this matter, we isolated a *K. lactis* single mutant impaired in GOGAT activity and a double mutant lacking both GOGAT and NADP-GDH. The first mutant strain did not display any growth defect phenotype whilst the double mutant was a full glutamate auxotroph. This implies that, as in *N. crassa* and *A.*

Abbreviations: GDH, glutamate dehydrogenase; GOGAT, glutamate synthase; GS, glutamine synthetase.

midulans, *K. lactis* has only two pathways for glutamate biosynthesis.

METHODS

Growth conditions. *K. lactis* MD2/1 (*MAT α argA lysA uraA*) and WM37 (*MAT α his3*) were grown on minimal medium (MM) containing salts, trace elements and vitamins following the formula of the yeast nitrogen base (Difco). Glucose (2%, w/v) was used as the carbon source and 38 mM (NH₄)₂SO₄ as the nitrogen source. Auxotrophic requirements were added at 0.01% (w/v) and cells were incubated at 30 °C with agitation. Growth was monitored by measuring optical density at 600 nm. (Hewlett Packard model 8452A spectrophotometer). Yeast extract/peptone/dextrose (YPD) plates contained 2% glucose, 1% yeast extract (Difco), 2% peptone (Difco) and 2% agar (Difco).

Determination of GOGAT and NADP-GDH activities. Soluble extracts for enzyme assays were prepared as previously described (Valenzuela *et al.*, 1995). NADP-GDH and GOGAT were assayed by the methods of Doherty (1970) and Roon *et al.* (1974), respectively. Protein was measured by the Lowry method with bovine serum albumin as a standard.

Purification of GOGAT from *K. lactis*. *K. lactis* MD2/1 was grown in 12 l MM, harvested at an OD₆₀₀ of 0.5 and stored at -70 °C until used. All purification steps were carried out at 4 °C.

Step 1: crude extract. Cells were thawed and resuspended in 2 ml buffer A [0.1 M potassium phosphate (pH 7.5), 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 50 µg *N*- α -tosyl-L-lysine chloromethyl ketone (TLCK) ml⁻¹ and 100 µg *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK) ml⁻¹] (g cells)⁻¹. Crude extracts were obtained after mechanical disruption of cells with a Bead-Beater (10 cycles of 1 min), using 0.5 g 710–180 µm diameter glass beads (ml cell suspension)⁻¹ and centrifugation at 35 000 g for 30 min.

Step 2: ammonium sulphate precipitation. Crude extract was treated with a saturated solution of ammonium sulphate (pH 7.5). Proteins precipitating at 30% saturation were discarded and the supernatant was dialysed four times (30 min each) against 2 l buffer A but lacking DTT and protease inhibitors (buffer B).

Step 3: affinity chromatography. The dialysed fraction was applied to a 40 ml blue Sepharose column (23 × 1.7 cm) previously equilibrated with buffer A plus 0.2 mM 2-oxoglutarate and 5% glycerol (buffer C). After sample application, the column was washed with 20 column volumes of buffer C and GOGAT activity was eluted with buffer C that also contained 0.1 mM NADH. Fractions with GOGAT activity were pooled and dialysed three times against buffer B for 30 min; protease inhibitors (0.5 mM DTT, 25 µg ml⁻¹ TLCK, 50 µg ml⁻¹ TPCK and 0.05 mM PMSF) were added and protein was concentrated by ultrafiltration using an Amicon YM30 membrane.

Molecular mass determination. The apparent molecular mass of the GOGAT polypeptide was determined by SDS-PAGE (Schägger *et al.*, 1986). Molecular mass standards were obtained from Sigma.

Western blot analysis. Immunoblotting was carried out as described by Towbin *et al.* (1979), with the modifications of González-Halphen *et al.* (1988). *S. cerevisiae* GOGAT anti-serum, obtained as described by Cogoni *et al.* (1995), was

diluted 1:50 000 and goat anti-rabbit immunoglobulin G-alkaline phosphatase conjugate was used as the secondary antibody.

Northern analysis. Northern analysis was carried out as previously described (González *et al.*, 1992). Total RNA of the MD2/1 wild-type strain was prepared from 50 ml of overnight cultures in MM, as described by Struhl & Davis (1981). Prehybridization was carried out at 65 °C for 60 min and hybridization was carried out at 65 °C for 12 h. Filters were first washed with a tenfold dilution of 20 × SSC (Maniatis *et al.*, 1982) containing 0.10% SDS at 65 °C for 30 min and then with a 130-fold dilution of 20 × SSC containing 0.10% SDS at 65 °C for 30 min.

Southern analysis. Southern analysis was carried out as described by Nasmyth & Reed (1980). Total *K. lactis* DNA was prepared from 50 ml of overnight cultures of the MD2/1 wild-type strain or of the WM37 *glt1* mutant in MM. When a heterologous probe was used, prehybridization was carried out at 45 °C for 45 min and hybridization was carried out at 45 °C for 24 h. Filters were washed twice with a tenfold dilution of 20 × SSC containing 0.10% SDS at 45 °C for 35 min. When an homologous probe was used, prehybridization and hybridization conditions were those described for the Northern analysis.

PCR amplification. *K. lactis* *GLT1* (GOGAT) PCR amplification was conducted using the deoxyoligonucleotides and the programme described by Cogoni *et al.* (1995). Total DNA from *K. lactis* strain WM37 was used as template for amplification by PCR in a Stratagene Robocycler 40. The amplification product (300 bp as judged on agarose gel) was cloned in the *EcoRI* restriction site of the Stratagene SK plasmid and sequenced with an ABI PRISM Genetic Analyzer, using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit from Applied Biosystems-Perkin Elmer. To obtain a PCR product suitable for gene disruption, the PCR-based ORF replacement protocol described by Wach *et al.* (1994) was used with kanMX as a marker. Two deoxyoligonucleotides were designed based on the nucleotide sequence of the *K. lactis* GOGAT fragment obtained by PCR and on the sequence of the multiple cloning site present in the pFA6a vector (Wach *et al.*, 1994). The deoxyoligonucleotide S1 (5'-GAG ACT CAT CAA ACA TTA GTT TTG AAT GAT TTG AGA GGA AAC GTC CGT ACG CTG CAG GTC GAC-3') comprises 45 bp from the most 5' end of the *K. lactis* PCR GOGAT fragment and 18 bp (indicated above in bold lettering) of the pFA6a multiple cloning site. The deoxyoligonucleotide S2 (5'-GAA TTT GTC TCT CAA AAC GGG GTC TTG AGT GGC AAT ACC AAC TGC ATC GAT GAA TTC GAG CTC G-3') contains 45 bp from the 3' end of the PCR GOGAT fragment and 19 bp (bold) from the pFA6a multiple cloning site. Qiagen purified pFA6a DNA was used as template for amplification by PCR, carried out in a Stratagene Robocycler 40 with the following programme: one denaturing cycle for 30 s at 94 °C, followed by 26 cycles of 30 s denaturation at 94 °C, 1 min annealing at 50 °C and 3 min extension at 72 °C. A 1500 bp PCR product obtained was gel-purified and used to transform strain WM37.

RESULTS AND DISCUSSION

K. lactis GOGAT purification

GOGAT was purified from *K. lactis* strain MD2/1 by classical chromatographic methods. A 0.6% yield and a 1000-fold increase in specific activity were attained

Table 1. Purification of *K. lactis* NADH-GOGAT

From 6 g wet weight cells.

Purification step	Total protein (mg)	Total activity (nmol min ⁻¹)	Specific activity (nmol min ⁻¹ mg ⁻¹)	Yield (%)	Purification factor*
Crude extract	170	1340	8	100	1
30% (NH ₄) ₂ SO ₄ supernatant	121	950	8	71	1
Blue Sepharose	0.001	8	8000	0.6	1000

* Fold increase in specific activity.

Fig. 1. (a) SDS-6% PAGE of the main fractions obtained during purification of *K. lactis* GOGAT. Lanes: 1, crude extract (50 µg); 2, supernatant obtained after ammonium sulphate precipitation (30%) (50 µg); 3, fraction from blue Sepharose (40 ng); 4, molecular mass standards (myosin, 205 kDa; β -galactosidase, 116 kDa; phosphorylase *b*, 97.4 kDa; bovine serum albumin, 66 kDa; ovalbumin, 45 kDa; and carbonic anhydrase, 29 kDa); 5, pure GOGAT from *S. cerevisiae* (Cogoni *et al.*, 1995). Proteins were visualized by Coomassie blue staining. (b) Western blot with antibodies raised against GOGAT from *S. cerevisiae*. GOGAT antiserum was probed against 40 ng purified *K. lactis* GOGAT (lane 1) and 250 ng purified *S. cerevisiae* GOGAT (lane 2).

(Table 1). The yields and final specific activities are comparable with those obtained for GOGAT purification from *S. cerevisiae* (Cogoni *et al.*, 1995). SDS-PAGE analysis of the fractions obtained throughout purification is shown in Fig. 1(a). Purification of the *K. lactis* GOGAT followed the procedure used to purify the *S. cerevisiae* enzyme (Cogoni *et al.*, 1995) except that

ion-exchange using DEAE-Biogel A and the hydrophobic chromatography steps were omitted. The *K. lactis* protein so obtained was readily degradable. To avoid degradation, the mixture of protease inhibitors described in Methods was included in all steps. A homogeneous fraction was obtained by applying the supernatant from the ammonium sulphate precipitation to a blue Sepharose column (Fig. 1a). This result suggests that *K. lactis* GOGAT comprises a single high-molecular-mass polypeptide, the possible multimeric association of which remains to be established.

Being a high-molecular-mass polypeptide, the NADH-GOGAT from *K. lactis* is similar to the GOGAT enzymes of other fungi and of higher plants (Hummelt & Mora, 1980; Anderson *et al.*, 1989; Cogoni *et al.*, 1995), and different to the dimeric enzymes composed of small and large subunits found in all the prokaryotic micro-organisms so far analysed (Miller & Stadtman, 1972; Trotta *et al.*, 1974; Adachi & Suzuki, 1977; Schreier & Bernlohr, 1984; Ratti *et al.*, 1985; Vanoni *et al.*, 1990; Carlberg & Nordlund, 1991).

The apparent molecular mass of the pure polypeptide was 199 kDa, as estimated by SDS-PAGE. This value is comparable to those of *S. cerevisiae*, *N. crassa* and plant NADH-GOGAT (Hummelt & Mora, 1980; Benny & Bolland, 1977; Chen & Cullimore, 1988; Hayakawa *et al.*, 1992; Anderson *et al.*, 1989; Cogoni *et al.*, 1995).

Western blot analysis was carried out with antibodies raised against the *S. cerevisiae* GOGAT protein (Cogoni *et al.*, 1995). These antibodies cross-reacted with crude extracts (data not shown) and pure GOGAT protein from *K. lactis* (Fig. 1b), suggesting that these two proteins bear similar antigenic epitopes. The same antibodies did not react with *Escherichia coli* NADPH-GOGAT (Cogoni *et al.*, 1995).

Since the immunoblotting results suggested similarity between the *S. cerevisiae* and *K. lactis* GOGAT enzymes, we decided to use an internal *EcoRI*–*EcoRI* 4.3 kb fragment of the *S. cerevisiae* GOGAT gene (Cogoni *et al.*, 1995) as a hybridization probe against total DNA from *K. lactis* strain MD2/1. Using low-stringency hybridizing conditions we were able to detect an *EcoRI*–*EcoRI* 4.3 kb band in total DNA of *K. lactis*

Fig. 2. (a) Southern blot of *K. lactis* total DNA digested with *EcoRI*. A 4.3 kb *EcoRI*–*EcoRI* fragment of *S. cerevisiae* GOGAT (Cogoni *et al.*, 1995) labelled with [α - 32 P]CTP was used as probe. Lanes: 1, *S. cerevisiae* DNA; 2, *K. lactis* DNA. Pointers on the left indicate the migration positions of lambda *HindIII*-digested DNA. (b) Northern blot of total RNA from *K. lactis*. RNA sample (10 mg) was separated by electrophoresis on a denaturing 1% agarose gel and transferred to a Hybond-N filter (see Methods). The filter was probed with a 300 bp internal PCR fragment of the *K. lactis* GOGAT gene. Arrows on the right indicate the migration positions of GOGAT mRNA, 25S rRNA and 16S rRNA from top to bottom, respectively. Pointers on the left indicate the migration positions of a 1.35–9.49 kb RNA ladder from Gibco-BRL.

digested with *EcoRI* (Fig. 2a). This result suggested that the *K. lactis* genome contained a sequence homologous to the *S. cerevisiae* GOGAT probe used.

To obtain an homologous *K. lactis* GOGAT fragment, PCR amplification of *K. lactis* genomic DNA was performed as described in Methods, using two deoxy-oligonucleotides derived from the central region of the alfalfa GOGAT gene, those previously utilized to amplify a GOGAT fragment from *S. cerevisiae* (Cogoni *et al.*, 1995). The PCR product was subcloned into the SK vector and sequenced. The deduced amino acid sequence showed 92% identity with the corresponding region of the GOGAT protein of *S. cerevisiae* (data not shown).

To determine the size of the mRNA that encodes the *K. lactis* GOGAT, the 300 bp PCR product was hybridized against total RNA extracted from the *K. lactis* wild-type strain (MD2/1). As shown in Fig. 2(b), a major band of around 7 or 8 kb was identified. These results indicate that the GOGAT enzyme from *K. lactis* should be constituted by a high-molecular-mass polypeptide. In

Fig. 3. Southern analysis of *K. lactis* DNA digested with *BglII*. Lanes: 1, wild-type strain WM37; 2, mutant strain WM37a. The blot was probed with a 300 bp internal PCR fragment of the *K. lactis* GOGAT gene. Pointers on the right indicate the migration positions of lambda *HindIII*-digested DNA.

order to analyse whether the 199 kDa polypeptide was the only GOGAT in *K. lactis* we decided to disrupt the *KIGLT1* gene that encodes this protein.

To obtain a null GOGAT mutant, the PCR product flanked by *GLT1* sequences, obtained as described in Methods, was used to transform strain WM37, following the lithium acetate protocol described by Gietz & Woods (1994). After transformation, cells were allowed to grow for 3 h in liquid YPD (see Methods). Transformation mix was then plated on YPD plates containing 200 mg G418 l⁻¹. After 3 d incubation, four colonies appeared on the transformation plates. These were streaked out on YPD/G418 plates and three of them (WM37a, WM37b and WM37c) grew out to form single colonies. Chromosomal DNA was isolated from the three transformants and digested with *BglII*. Southern analysis was carried out with the 300 bp PCR fragment as the probe. The pattern obtained from the transformants clearly indicated the insertion of the construct in the wild-type genomic sequence of *KIGLT1* (Fig. 3). The null mutants were completely devoid of GOGAT activity (Table 2).

Isolation and characterization of a double mutant devoid of NADP-GDH and GOGAT activities

To isolate a double mutant impaired both in *KIGDH1* and in *KIGLT1*, strains JR1 (*MAT α lysA argA gdh1::uraA*) and WM37a (*MAT α his3 glt1::kanMX*)

Table 2. NADP-GDH and GOGAT specific activities

Cells were grown to an OD₆₀₀ of 0.7 with 38 mM (NH₄)₂SO₄ except for strain C4A, which was grown on 7 mM glutamate without (NH₄)₂SO₄.

Strain (relevant genotype)	NADP-GDH*	GOGAT†
WM37 (<i>GDH1 GLT1</i>)	500	12
WM37a (<i>GDH1 glt1</i>)	450	ND
MD2/1 (<i>GDH1 GLT1</i>)	530	13
JR1 (<i>gdh1 GLT1</i>)	ND	39
C4A (<i>gdh1 glt1</i>)	ND	ND

ND, Not detected.

* Expressed as nmol NADPH oxidized min⁻¹ (mg protein)⁻¹. The reported values are the means of three experiments; variation was 5–10%.

† Expressed as nmol NADH oxidized min⁻¹ (mg protein)⁻¹. The reported values are the means of three experiments; variation was 5–10%.

were crossed and random spore analysis was performed. One quarter of the analysed population was unable to grow on MM in the presence of ammonium as sole nitrogen source, but recovered wild-type growth when 0.05% glutamate was added to the medium.

Table 2 shows that when grown on glutamate, the double mutant C4A showed neither NADP-GDH nor GOGAT activity. As can be seen in Fig. 4, single GOGAT null mutants grew as well as the wild-type strain on ammonium as sole nitrogen source. However the double mutant (C4A) was completely unable to grow in the absence of glutamate. These results show that in *K. lactis* two pathways exist for glutamate biosynthesis and that both of them have to be knocked out in order to obtain a glutamate auxotroph.

The presence of two pathways for glutamate biosynthesis in several micro-organisms has stimulated discussion on the need for two routes for the biosynthesis of the same end product. The role of these two pathways has been clearly elucidated in *Klebsiella aerogenes*. In this bacterium, the GS-GOGAT pathway functions to assimilate ammonium and synthesize glutamate when the ammonium concentration is low, whilst GDH plays this role when the cells are cultivated under ammonium excess (Senior, 1975). In *E. coli*, GDH and GOGAT are simultaneously present and this raises the issue of their relative contributions to glutamate synthesis. Neither GDH- nor GOGAT-deficient strains have an observable growth defect when these strains are grown under ammonium excess or ammonium limitation, nor in energy-rich media (Tyler, 1978). However, it has been shown that *E. coli* *gdhA* mutant strains are at a competitive disadvantage relative to wild-type strains in carbon- and energy-limited media. These results suggest that GDH is important for glutamate synthesis during energy-limited growth and that GOGAT is the primary

enzyme for glutamate biosynthesis during growth in energy-rich medium (Helling, 1994). In *N. crassa*, GOGAT has an important role recycling organic nitrogen from glutamine and glutamate; however, both GDH and GOGAT are involved in glutamate synthesis under ammonium excess or limitation (Hummelt & Mora, 1980). The roles of GDH and GOGAT have been studied in some yeasts. In *Schizosaccharomyces pombe* and *Candida albicans*, evidence indicates that the GS-GOGAT pathway is the major pathway for ammonium assimilation (Barel & MacDonald, 1993; Holmes *et al.*, 1989). *S. cerevisiae* constitutes the only example in which three pathways take over glutamate biosynthesis (NADP-GDH1, NADP-GDH3 and GOGAT) (Avendaño *et al.*, 1997), and it has been proposed that GOGAT plays an important role furnishing glutamate during amino acid deprivation; however, when this organism is grown under either ammonium excess or limitation, glutamate biosynthesis is mainly achieved through GDH1 and GOGAT (Valenzuela *et al.*, 1998). The role of NADP-GDH3 in glutamate biosynthesis is yet to be determined.

K. lactis mutants devoid of NADP-GDH, showed no growth defect phenotype. Since these mutant strains have increased GS and GOGAT activities, it has been suggested that in this yeast the GS-GOGAT pathway could constitute either the major pathway for ammonium assimilation or a compensatory mechanism for the lack of NADP-GDH (Valenzuela *et al.*, 1995). The isolation of *K. lactis* null GOGAT mutants has allowed us to show that, similar to the situation occurring in *E. coli*, neither null NADP-GDH mutants nor GOGAT null mutants exhibit a growth defect phenotype. Further experiments will have to be conducted in order to determine whether there are particular physiological conditions in which each of the two pathways present in *K. lactis* plays a major biosynthetic role. As has been pointed out previously, *Schizosaccharomyces pombe* mutants devoid of GOGAT activity grow slowly and show a fivefold increase in their NADP-GDH levels, implying that the GS-GOGAT pathway is the primary route of glutamate biosynthesis (Barel & MacDonald, 1993). Thus, the primary route for glutamate biosynthesis is not the same in all yeast species, although so far all the species studied have both GDH and GOGAT enzymes.

As mentioned earlier, besides GOGAT, *S. cerevisiae* has two non-allelic genes encoding two different NADP-GDH isozymes, one of which most probably evolved as the result of duplication of a primary gene. In this regard, it has been proposed (Wolfe & Shields, 1997), that *S. cerevisiae* is a degenerate tetraploid resulting from whole-genome duplication, and that *Kluyveromyces* and *Saccharomyces* diverged before this proposed genome duplication of *S. cerevisiae* occurred. Accordingly, it has been found that *Kluyveromyces* homologues of many *S. cerevisiae* genes are only found in single copy. This study shows that *K. lactis* has a single copy of the NADP-GDH-encoding genes, thus constituting another example of genes that were duplicated sometime during

Fig. 4. Growth of strains WM37 (*GDH1 GLT1*) (●), WM37a (*GDH1 glt1*) (○), C4A (*gdh1 glt1*) with 38 mM $(\text{NH}_4)_2\text{SO}_4$ (■) and C4A (*gdh1 glt1*) with 0.05% glutamate without $(\text{NH}_4)_2\text{SO}_4$ (□).

the evolutionary history of *S. cerevisiae*, but which evolved as a single allele in *K. lactis*. In addition, it has been proposed, that *S. cerevisiae* genome duplication may have played a role in the evolutionary adaptation of *S. cerevisiae* to fermentative growth (Wolfe & Shields, 1997). In this regard, it could be speculated that the presence of two NADP-GDH isozymes in *S. cerevisiae* provides a mechanism to equilibrate utilization of 2-oxoglutarate and energy production under fermentative or respiratory conditions. Since *K. lactis* is a strict aerobe, only able to grow under respiratory conditions, it could be proposed that in this yeast, a single NADP-GDH enzyme allows glutamate biosynthesis and thus 2-oxoglutarate utilization without compromising energy production.

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