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Role of L-Lysine-α-Ketoglutarate Aminotransferase in Catabolism of Lysine as a Nitrogen Source for *Rhodotorula glutinis*

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Wild-type and saccharopine dehydrogenaseless mutant strains of *Rhodotorula glutinis* grew in minimal medium containing lysine as the sole nitrogen source and simultaneously accumulated, in the culture supernatant, large amounts of a product identified as α-aminoadipic-δ-semialdehyde. The saccharopine dehydrogenase and pipecolic acid oxidase levels remained unchanged in wild-type cells grown in the presence of ammonium or lysine as the nitrogen source. Lysine-α-ketoglutarate aminotransferase activity was demonstrated in ammonium-grown cells. This activity was derepressed in cells grown in the presence of lysine as the sole source of nitrogen.

Catabolism of lysine, an essential amino acid, occurs through diverse pathways in nature (12, 13). Lysine is metabolized in mammals through saccharopine as a result of the reversal of the aminoadipate pathway, which is used for the biosynthesis of lysine in yeasts and other higher fungi (1, 3, 8). Catabolism of lysine through pipecolic acid has been demonstrated in *Pseudomonas putida* (2, 13), plants (6), *Neurospora crassa* (18), and rats (15). In *Flavobacterium lutescence*, the enzyme L-lysine-α-ketoglutarate aminotransferase (EC 2.6.1.36) converts lysine to Δ^1-piperideine-6-carboxylic acid, which remains in equilibrium with α-aminoadipic-δ-semialdehyde (ASA) (16, 19) (Fig. 1).

In yeasts, the catabolism of lysine is complicated by the fact that some yeasts can use lysine as the sole carbon source and others use it as the sole nitrogen source (7, 17). For example, *Saccharomyces cerevisiae* grows in the presence of lysine as the sole source of carbon but not the sole source of nitrogen. Lysine is catabolized via acetylated intermediates in the yeasts *Hansenula saturnus* (14) and *Saccharomycopsis lipolytica* (4), as well as in the mold *Rhizoctonia leguminicola* (5). Our results demonstrate that *Rhodotorula glutinis*, an obligately aerobic yeast, uses lysine as the sole nitrogen source but not as a carbon source. We also present biochemical evidence for the catabolism of lysine as the sole source of nitrogen via a novel pathway not previously reported in any eucaryote (Fig. 1).

Wild-type *R. glutinis* MU-1 and saccharopine dehydrogenaseless mutant strains MNNG6 and MNNG1021 (9) were used in this study. Cells were grown in basal minimal medium (glucose, 10 g; yeast nitrogen base without amino acids and ammonium sulfate, 1.7 g; distilled water to 1 liter) to which 2 to 4 mg of nitrogen source per ml was added (21). Cultures were grown at 28°C with vigorous shaking, and the culture supernatants of cells grown in different nitrogen source media were examined for the accumulation of ASA as a *p*-dimethylaminobenzaldehyde-reactive product (9, 16). The accumulation product was further characterized on the basis of the absorption spectra (9).

Dialyzed extracts were prepared, and pipecolic acid oxidase and saccharopine dehydrogenase (EC 1.5.1.7) activities as well as protein were determined by procedures described previously (9, 11). Lysine-α-ketoglutarate aminotransferase (LKGT) was assayed by determining the amount of ASA formed (9, 19). The reaction mixture consisted of the following: pyridoxal phosphate, 0.125 mM; L-lysine, 2.5 mM; α-ketoglutarate, 2.5 mM; 0.25 M potassium phosphate buffer (pH 7.5); and dialyzed extract equivalent to 0.5 to 1 mg of protein in a final volume of 1.0 ml. After 2 h of incubation at 25°C, 1 ml of a solution of 2% *p*-dimethylaminobenzaldehyde in 2-methoxyethanol was added. The mixture was boiled for 20 min and centrifuged, and the absorbance at 460 nm was determined (9, 16). The control lacked α-ketoglutarate.

Results of growth experiments (Table 1) demonstrate that wild-type and mutant strains of *R. glutinis* used D- or L-lysine as the sole source of nitrogen. As expected, *S. cerevisiae* did not grow in medium containing lysine as the sole nitrogen source. Ammonium provided a better nitrogen source than lysine for *R. glutinis*, as indicated by an increased cell yield. Growth

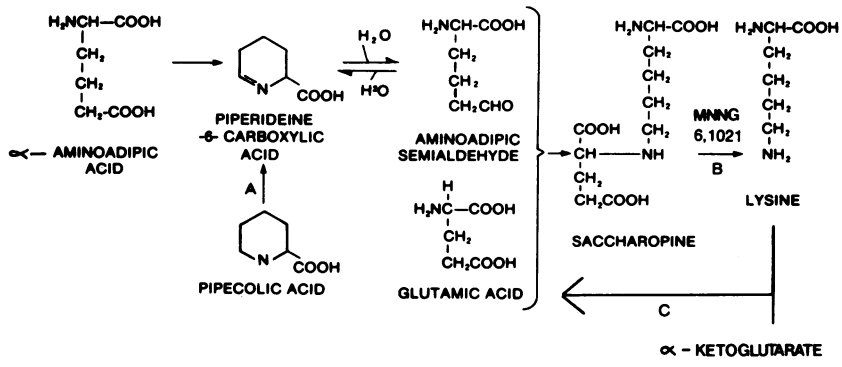

FIG. 1. Pathways for the conversion of α-aminoadipate and pipecolic acid to lysine and catabolism of lysine to glutamate and ASA in *R. glutinis*. A, Pipecolate oxidase; B, saccharopine dehydrogenase; C, LKGT.

with lysine as a nitrogen source was accompanied by accumulation of a product identified by absorption spectra and reaction with *p*-dimethylaminobenzaldehyde as ASA in both the culture supernatant and undialyzed extracts of cells grown in lysine medium (Table 2). The wild-type strain of *R. glutinis* also used arginine, glutamate, histidine, and proline as the sole nitrogen source, but ASA accumulated only when the cells were grown in lysine medium. *R. glutinis* did not use lysine as the sole carbon source, whereas *S. cerevisiae* did (data not shown).

The finding that *R. glutinis* was able to metabolize lysine as the nitrogen source and accumulated large amounts of a product identified as ASA raised the possibility that pipecolate oxidase was involved in the catabolic process. Pipecolic acid is a precursor of lysine in *R. glutinis* but not in *S. cerevisiae* (9, 11). Pipecolate is converted to ASA by pipecolate oxidase (10). Conversion of lysine to ASA could also occur through saccharopine dehydrogenase as a result of reversal of the aminoadipate pathway of lysine biosynthesis (Fig. 1). Levels of both these enzymes remained the same in wild-type cells grown in the presence of ammonium or lysine as the sole nitrogen source (Table 2). In addition, mutant strains MNNG6 and MNNG1021 blocked at the saccharopine dehydrogenase step were able to metabolize lysine through an alternate pathway not involving saccharopine dehydrogenase. Examination of dia-

TABLE 1. Growth of *R. glutinis* and *S. cerevisiae* strains in basal medium with different nitrogen sources

Strain	Optical density at 550 nm of culture grown in the presence of[a]:		
	Ammonium	L-Lysine	D-Lysine
R. glutinis			
MU-1	2.0	0.5	0.4
MNNG6	2.0[b]	0.3	0.2[b]
MNNG1021	2.0[b]	0.3	0.3[b]
S. cerevisiae X2180	2.0	0.0	0.0

[a] D- or L-Lysine or ammonium at 2 mg/ml was used, and the optical density at 550 nm up to an absorbance of 2.0 or at 72 h was determined.

[b] Medium contained 20 μg of L-lysine per ml as supplement.

TABLE 2. Levels of enzymes capable of lysine degradation and in vivo ASA accumulation in wild-type *R. glutinis* MU-1 grown in different nitrogen sources

Nitrogen source[a]	Spec act			ASA accumulation[b]
	LKGT (nmol/min per mg)	Saccharopine dehydrogenase (μmol/min per mg)	Pipecolate oxidase (μmol/min per mg)	
Ammonium	12	0.29	0.11	2.0
Ammonium + L-lysine	22	ND[c]	ND[c]	4.4
L-lysine	112	0.23	0.10	32.6

[a] Ammonium was at 4 mg/ml; ammonium plus L-lysine medium contained 2 mg of each per ml; L-lysine was at a 4 mg/ml concentration.

[b] Expressed as micromoles of ASA formed per milliliter of undialyzed extract after 72 h of growth.

[c] ND, Not determined.

lyzed extract with appropriate controls revealed the existence of LKGT activity, thus providing a novel pathway for the formation of ASA and glutamate from lysine in *R. glutinis* (Fig. 1). Glutamate becomes the amino donor, leaving ASA as the unmetabolized accumulation product. The LKGT activity of cells grown in ammonium and lysine medium was twofold higher than that found in cells grown in the presence of ammonium alone, and the activity was ninefold higher in dialyzed extract from cells grown in lysine as the sole nitrogen source (Table 2).

LKGT activity has not been demonstrated in any other eucaryote. The ability of *R. glutinis* to catabolize lysine may explain its insensitivity to lysine toxicity, a phenomenon which exists in *S. cerevisiae* (20). A significant derepression of LKGT activity in lysine-grown cells suggests that this enzyme plays an important physiological role in the catabolism of lysine in this organism (Fig. 1).

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