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α-Aminoadipate as a Primary Nitrogen Source for *Saccharomyces cerevisiae* Mutants†

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In contrast to wild-type strains of the yeast *Saccharomyces cerevisiae*, *lys2* and *lys5* mutants are able to utilize α-aminoadipate as a primary source of nitrogen. Chattoo et al. (B. B. Chattoo, F. Sherman, D. A. Azubalis, T. A. Fjellstedt, D. Mehnert, and M. Ogur, Genetics 93:51–65, 1979) relied on this difference in the effective utilization of α-aminoadipate to develop a procedure for directly selecting *lys2* and *lys5* mutants. In this study we used a range of mutant strains and various media to determine why normal strains are unable to utilize α-aminoadipate as a nitrogen source. Our results demonstrate that the anabolism of high levels of α-aminoadipate through the biosynthetic pathway of lysine results in the accumulation of a toxic intermediate and, furthermore, that *lys2* and *lys5* mutants contain blocks leading to the formation of this intermediate.

Chattoo et al. (3) previously observed that *lys2* and *lys5* mutants of *Saccharomyces cerevisiae* could grow on medium having α-aminoadipate (αAA) as a primary source of nitrogen, whereas wild-type strains could not. This preferential utilization of αAA was the basis for a means of selecting forward *lys2* and *lys5* mutants. Although the selection procedure has been widely used, the reason why normal *LYS2*$^+$ and *LYS5*$^+$ strains do not use αAA for a nitrogen source was not understood. The present paper addresses this question.

The *LYS2* and *LYS5* genes encode αAA reductase (12), which converts αAA into α-aminoadipate-δ-semialdehyde (αAAδS) (Fig. 1). αAAδS is subsequently converted into saccharopine by what appears to be a multistep process (6) involving the gene *LYS9* and probably *LYS14* (1, 2). Mutations of *lys2* or *lys5* that block the reductase step permit exogenous αAA to serve as a nitrogen donor to α-ketoglutarate in the reverse of the normal transamination reaction (3). The glutamate formed in this manner readily explains the ability of αAA to serve as a primary source of nitrogen in *lys2* and *lys5* mutants. However, it was unclear exactly why exogenous αAA is completely unable to serve as a nitrogen source in wild-type cells, even if the cells are provided with exogenous lysine (which cannot be used by *S. cerevisiae* as a nitrogen source [10]). We have investigated the effect of αAA on a range of *lys* mutants grown in various media and have concluded that high levels of αAA cause the formation of a toxic metabolite that is normally an intermediate in the biosynthetic pathway of lysine. Furthermore, we show that the accumulation of this toxic intermediate is prevented by *lys2* and *lys5* mutations.

MATERIALS AND METHODS

Yeast strains and genetic procedures. The strains used in this investigation are listed in Table 1. The Z-105 and Z-104 mutant strains were directly derived from the wild-type strain D273-10B by selection on solid αAA medium (see below). Conventional yeast genetic procedures of crossing, sporulation, and tetrad analysis were carried out by techniques described by Sherman and Lawrence (11).

Culture media. Routine synthetic and complex nutrient media were as described by Sherman and Lawrence (11). The standard solid αAA medium contained 0.167% yeast nitrogen base (Difco Laboratories, Detroit, Mich.) without amino acids and without ammonium sulfate, 2% glucose, 2 g of DL-αAA per liter, 30 mg of L-lysine per liter, and 2% Bacto-Agar (Difco). Certain solid αAA test media contained one of the following nitrogen sources: 0.5% ammonium sulfate, 0.1% glutamate, and 0.1% proline. Liquid test media contained 0.167% yeast nitrogen base (Difco) without amino acids and without ammonium sulfate, 2% glucose, 30 mg of L-lysine per liter, and, where indicated, 0.2% ammonium sulfate, 0.1% glutamate, and various amounts of DL-αAA as described. The αAA was added aseptically to other autoclaved ingredients of the media by preparing a 6% (final) stock solution of αAA acid, adjusting the pH to 6.0 with 10 N KOH, and filter sterilizing the solution.

Growth curves. Freshly grown cells were used to inoculate 15 ml of liquid nutrient medium in a 50-ml culture tube. After 22 h on a reciprocating shaker at 30°C, the cells were harvested by centrifugation and washed with sterile distilled water. After the cell density was estimated, appropriate inocula were introduced into 100-ml Klett culture flasks containing 20 ml of growth medium. Cultures were shaken at 30°C. The growth rate was estimated by using a Klett-Summerson photoelectric colorimeter equipped with a blue no. 42 filter.

In some cases, *lys2* or *lys5* strains grew in clumps in liquid media; these cultures were sonicated to break up clumps before the Klett readings were taken.

RESULTS

αAA inhibits the growth of wild-type *S. cerevisiae*. Previous studies have shown that wild-type *S. cerevisiae* does not utilize αAA as a primary source of nitrogen (3). Consistent with this finding, the wild-type strain D273-10B did not grow in minimal medium containing αAA as a primary nitrogen source at any of various concentrations of αAA that permit growth of *lys2* and *lys5* mutants (Fig. 2a). To investigate why

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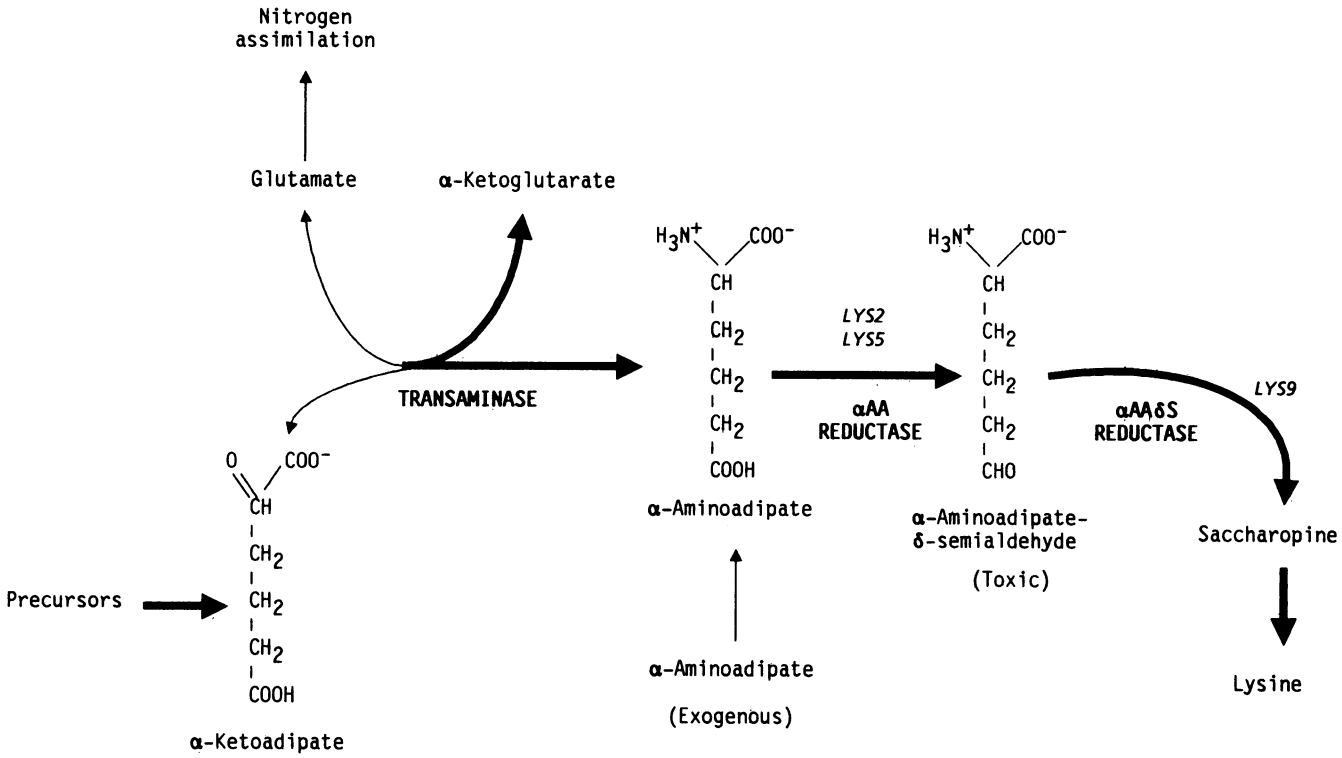

FIG. 1. Metabolic pathways of αAA utilization and lysine biosynthesis and the relationship to *LYS* genes. Some of the intermediates are not shown. (Adapted from Chatoo et al. [3].) The thick arrows indicate the predominant pathway in LYS^+ cells. The thin arrows indicate the predominant pathways in various *lys* mutants.

wild-type *S. cerevisiae* does not use αAA, we studied the effects of various concentrations of αAA in the presence of compounds known to serve as effective nitrogen sources for *S. cerevisiae*. Increasing concentrations of αAA inhibited the growth of strain D273-10B when glutamate was supplied as a primary nitrogen source (Fig. 2a). After 3 days of growth in glutamate plus 2 mg of αAA per ml, the cells grew to only 20% of the density of the cells grown in glutamate alone. Since *lys2* mutants can utilize αAA at 2 mg/ml alone as a primary nitrogen source (3; see below), these results suggest not only that wild-type cells do not utilize αAA as a primary nitrogen source, but also that their growth is inhibited by the presence of αAA in the medium. The data presented in Fig. 2a were obtained with cells grown in liquid medium; similar results have been obtained for cells grown in solid medium containing agar (data not shown). Further experiments with solid medium have shown that αAA also inhibits the growth of wild-type *S. cerevisiae* when proline is supplied as the primary nitrogen source (data not shown). Thus, with different amino acids as primary sources of nitrogen, we observe similar inhibitory effects of αAA on the growth of wild-type *S. cerevisiae*.

Ammonia prevents the growth inhibition caused by αAA. Ammonia serves as an efficient primary nitrogen source for *S. cerevisiae* and is known to repress the activities of amino acid permeases by about 100-fold (4). If αAA, an amino acid, was entering the cells via an amino acid uptake system during growth on glutamate as a primary nitrogen source, we expected that αAA might be excluded from the cells during growth on ammonia. The results in Fig. 2a show that in the presence of ammonia, αAA has little effect upon the growth of the normal D273-10B strain. After 3 days of growth in ammonia plus 2 mg of αAA per ml, strain D273-10B grew to nearly the same density as the cells grown in ammonia alone. However, extremely high concentrations of αAA (10 mg/ml) do inhibit growth. Furthermore, the growth of strain D273-10B in αAA plus ammonia is not due to selection for *lys2* or *lys5* mutants and their subsequent overgrowth, since no such mutants were found in a test of 240 colonies derived from a 5-day culture in 1 mg of αAA per ml plus ammonia (data not shown). Thus, at a concentration of αAA that serves as a primary nitrogen source of *lys2* cells, the growth of wild-type *S. cerevisiae* is not inhibited by αAA in the presence of ammonia. The same results were obtained for wild-type cells grown on solid medium (data not shown).

***lys2* and *lys5* mutations prevent growth inhibition caused by αAA.** To test whether the inhibitory effect of αAA is due to the involvement of αAA in the lysine biosynthetic pathway, we isolated isogenic *lys2* and *lys5* derivatives of the D273-10B LYS^+ strain as described in Materials and Methods. The Z-105 derivative contains the spontaneous *lys2-402* allele (Table 1) and is auxotrophic for lysine. The Z-104 strain contains the UV-induced *lys5-101* allele (Table 1) and exhibits slight growth in normal minimal medium lacking lysine. We often observe that *lys5* mutants are leakier than *lys2* mutants for the Lys^- phenotype.

As shown in Fig. 2b and c, and as expected from previous work (3), both the *lys2* and *lys5* strains grew on αAA as a primary source of nitrogen when the medium was supplemented with only enough lysine to complement the nutritional deficiency. We attribute the weaker growth of the *lys5* strain to the leakiness of the *lys5-101* allelle. As expected, both the *lys2* and *lys5* strains grew well in the presence of αAA when ammonia was utilized as a primary nitrogen source, although some growth inhibition was observed at very high concentrations of αAA (10 mg/ml). In contrast to

the wild-type LYS^+ parent strain, both the *lys2* and *lys5* mutants were not inhibited by the presence of αAA when glutamate was utilized as a primary nitrogen source, although again some inhibition was observed at very high αAA concentrations (10 mg/ml).

Since the αAA uptake system is derepressed in glutamate, the lack of inhibition in *lys2* and *lys5* mutants is most likely not due to improper uptake of αAA. The fact that *lys2* and *lys5* mutants grow slightly better in αAA plus glutamate than in αAA plus ammonia provides additional evidence that the inhibitory effect of αAA is relieved by mutations at the *lys2* or *lys5* locus. These results suggested to us that *lys2* and *lys5* mutants could be blocking the conversion of αAA into a subsequent lysine precursor that is toxic to the cells.

***lys9* mutants are inhibited by αAA to a greater extent than are wild-type *S. cerevisiae*.** Since *lys2* or *lys5* mutations relieve the inhibitory effect of αAA by blocking the reductase step, we considered the possibility that the reductase reaction product, αAAδS, is toxic to wild-type cells. If *lys9* mutants accumulated αAAδS (Fig. 1) and if αAAδS was toxic to *S. cerevisiae*, we predicted that *lys9* cells would be more sensitive to αAA than would wild-type cells. If αAAδS was not toxic but if saccharopine or an intermediate in the saccharopine reductase step was the toxic element, *lys9* cells would be more resistant to αAA than wild-type cells. We tested this hypothesis by examining the growth of *lys9* mutants in the presence of αAA (plus lysine), using glutamate as the primary nitrogen source. Since *lys9* mutations are not selectable directly, we crossed a *lys9* mutant to the *lys2* and *lys5* strains described above and examined various segregants for their growth (Fig. 3).

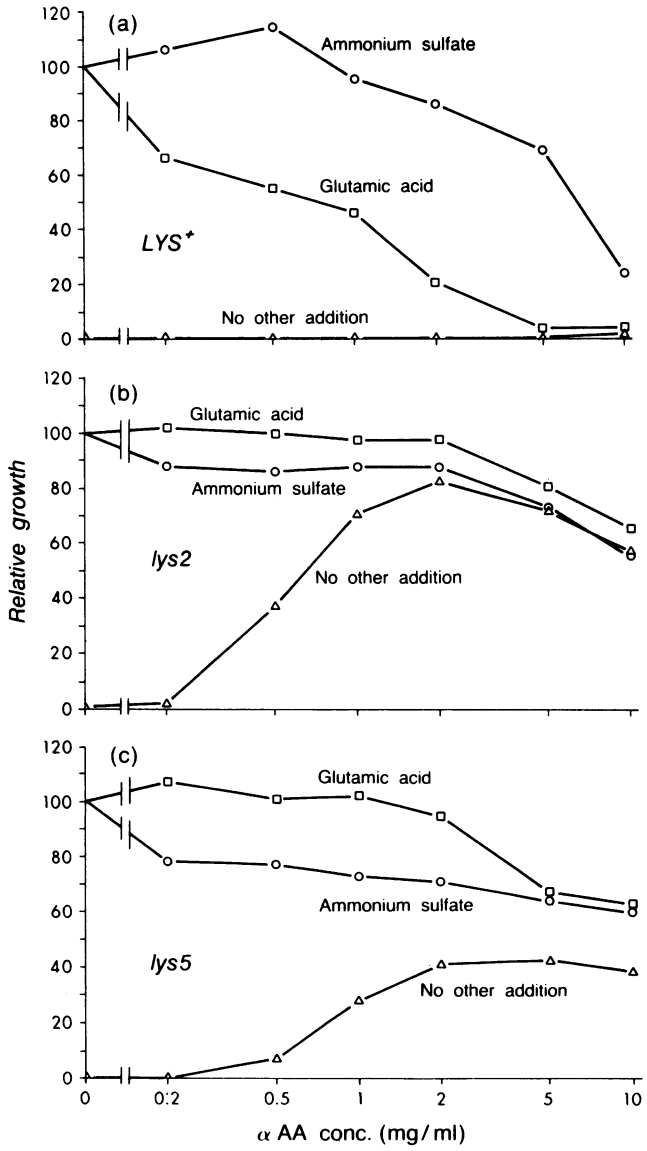

FIG. 2. The relative growth of various strains in liquid medium containing different concentrations of αAA and other additions. The growth has been normalized to the growth of each strain grown in medium lacking αAA. (a) LYS^+ strain D273-10B after 3 days of growth. (b) *lys2* strain Z-105 after 2 days of growth. (c) *lys5* strain Z-104 after 2 days of growth.

Growth of the *lys9* mutant was inhibited by αAA to a greater extent than was growth of LYS^+ cells (Fig. 3). Although the distinction between *lys9* and LYS^+ growth rates in liquid medium was small, it was reproducible and has been observed for several *lys9* segregants. Numerous segregants have been tested on solid medium; LYS^+ cells usually exhibited residual growth on 2 mg of αAA per ml plus glutamate, but *lys9* cells were completely negative (data not shown). In contrast, the *lys2 lys9* and *lys5 lys9* double mutant segregants grow as well in αAA as the *lys2* and *lys5* single mutant segregants, respectively. We therefore conclude that resistance to αAA can be obtained by blocking the lysine biosynthetic pathway before the synthesis of αAAδS, but not after it.

DISCUSSION

The intracellular concentration of αAA is dependent upon its rate of uptake and upon its utilization as a substrate by the reductase and transaminase (see above). One suggested reason for the ability of *lys2* and *lys5* mutants to grow on αAA as a primary nitrogen source is based upon the finding that the reductase activity in *S. cerevisiae* is 10-fold greater than the transaminase activity (3). If the uptake rate is limiting, then wild-type *S. cerevisiae* is more likely to shuttle αAA down the reductase pathway, whereas *lys2* and *lys5* mutants, which are deficient in reductase, would have more αAA available to serve as a nitrogen donor via the prevailing transaminase pathway. However, wild-type *S. cerevisiae* strains are not simply unable to use αAA as a primary nitrogen source; here we have shown that αAA actually inhibits the growth of *S. cerevisiae* when the cells are growing on other amino acids as a primary source of nitrogen. By analyzing the growth of various mutant strains, we have tentatively identified the inhibitory component as αAAδS, the precursor that is subsequent to αAA in the lysine biosynthetic pathway. The formation of saccharopine from αAAδS involves several enzymatic steps (6), and we have only tested a block at the *lys9* step. Because the *lys9* block has not been completely characterized, any one of the αAAδS reductase intermediates could be the toxic compound. Consistent with this view is the finding that *lys9* mutants accumulate αAAδS, although the determinations were carryout on cultures grown with limited amounts of lysine and no αAA (2).

In a study by Winston and Bhattacharjee (13), *lys14* mutants were shown to grow in αAA medium, suggesting that the toxic intermediate could be the metabolic product of *lys14* activity. Furthermore, Borell et al. (2) presented evidence that *lys14* mutants are blocked in αAAδS reductase, similar to *lys9* mutants. However, in contrast to *lys9*

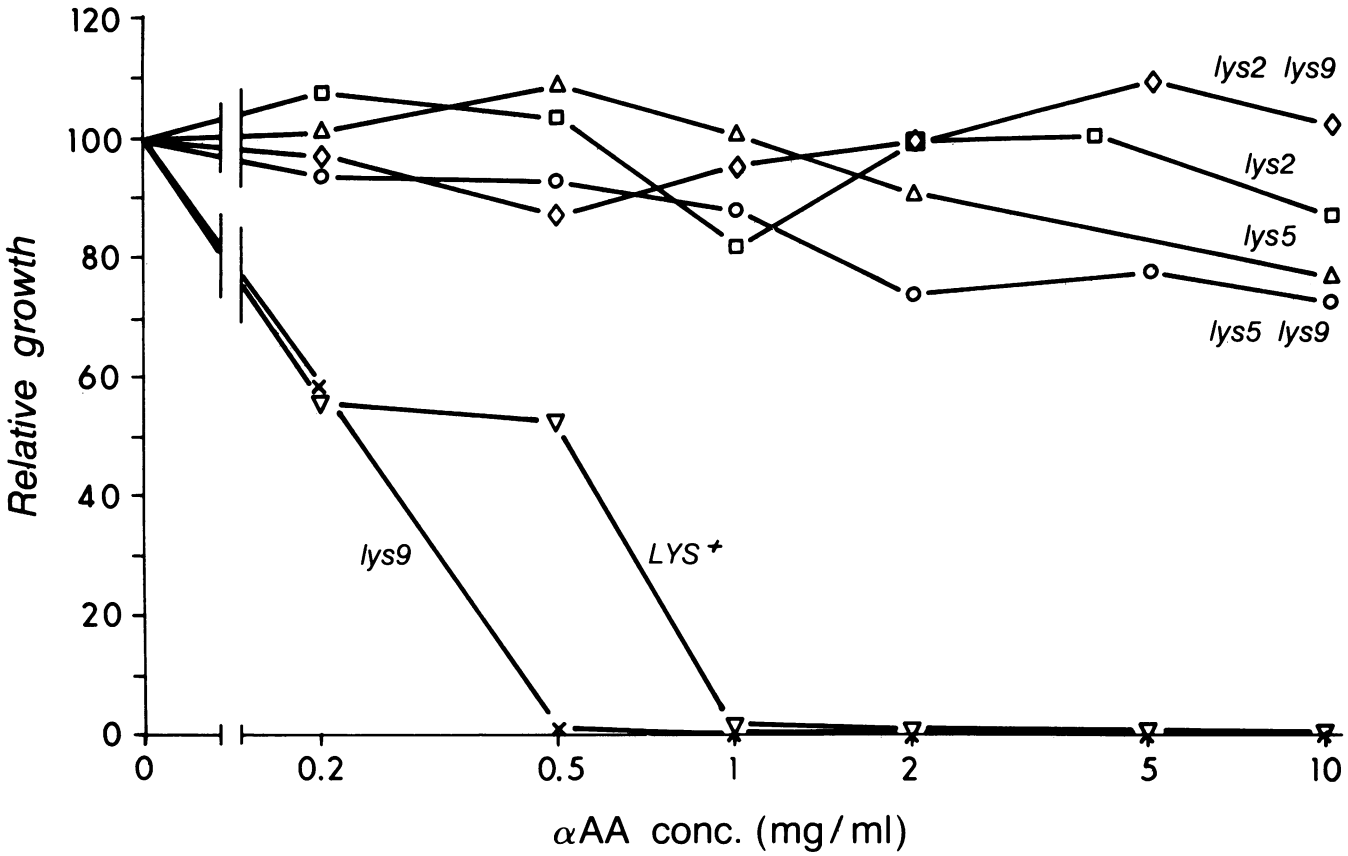

FIG. 3. Relative growth of the following strains in liquid media containing 0.1% glutamate and various concentrations of αAA: KZ186-10A, *LYS*$^+$; KZ186-10B, *lys9*; KZ186-10C, *lys2*; KZ186-10D, *lys2 lys9*; KZ189-4B, *lys5*; and KZ189-4D, *lys5*, *lys9*.

mutants, all *lys14* mutants grew partially on medium lacking lysine, all had 5 to 10% of the normal activity of αAAδS reductase, and all accumulated less αAAδS than *lys9* mutants (2). Because all *lys14* mutants are blocked only partially, *LYS14* probably does not encode a key enzyme in the αAAδS reductase step. Because the formation of saccharopine from αAAδS proceeds by several enzymatic steps (6), and because of the partial block and undefined lesion in *lys14* mutants, it is difficult to relate our results to the results of Winston and Bhattacharjee (13). In addition, the *lys9* mutants used in our study and the *lys14* mutants used in the study by Winston and Bhattacharjee (13) may harbor additional genetic differences modifying responses to the toxic effect of αAAδS.

Also in contrast to our study, Winston and Bhattacharjee (13) reported that αAA inhibits the growth of wild-type *S. cerevisiae* in the presence of ammonia. However, Rytka (9) has shown that certain wild-type *S. cerevisiae* strains carry an allele, designated *amc*, that prevents the repression of amino acid permeases by ammonia. The normal strain used in our study, D273-10B, is relatively resistant to the effects of αAA when grown in the presence of ammonia. The findings of Winston and Bhattacharjee (13) can be reconciled with ours by assuming that their strain, X2180-1A, is *amc*. Thus, αAA could indeed be entering the cell via the ammonia-repressible, general amino acid permease system. In either case, we have demonstrated that *lys2* and *lys5* mutants block the formation of an inhibitory compound and thereby permit αAA to serve as a primary nitrogen source.

TABLE 1. *S. cerevisiae* strains

Strain	Genotype	Source
D273-10B	*MATα LYS*$^+$	F. Sherman
Z-105	*MATα lys2-402*	This study
Z-104	*MATα lys5-101*	This study
552	*MATα lys9 ade2*	J. K. Bhattacharjee
KZ186-10A	*MATα LYS*$^+$	This study
KZ186-10B	*MATα lys9*	This study
KZ186-10C	*MATa lys2-402*	This study
KZ186-10D	*MATa lys2-402 lys9*	This study
KZ189-4B	*MATa lys5-101*	This study
KZ189-4D	*MATα lys5-101 lys9*	This study

Under conditions of αAA uptake, the accumulation of a toxic intermediate and its alleviation by *lys2* and *lys5* mutations is reminiscent of other systems that have been used to select for forward mutations. Mutants of *Escherichia coli* (7, 14) and *S. cerevisiae* (5), blocked in galactose-1-phosphate uridyltransferase or uridine diphosphoglucose 4-epimerase, accumulate the toxic precursor, galactose-1-phosphate, when grown on galactose medium. Because secondary mutations producing deficiency in galactokinase also prevent the formation of galactose-1-phosphate, forward mutations can be simply selected on galactose medium. Yeast *ura4* mutants defective in pyrimidine biosynthesis accumulate the toxic precursor ureidosuccinic acid, causing growth inhibition and allowing selection of *ura2* mutants, which prevent formation of the toxic precursor (8). The advantage of the αAA selection procedure is that *lys2* and *lys5* mutants can be selected from any prototrophic or auxotrophic yeast strain, including those having later blocks in the biosynthetic pathway of lysine.

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