BASIC AMINO ACID INHIBITION OF GROWTH IN *SACCHAROMYCES CEREVISIAE*

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SUMMARY: Growth of *Saccharomyces cerevisiae* on poor nitrogen sources such as allantoin or proline is totally inhibited by addition of lysine to the medium. The same result is observed with ornithine if its degradation to glutamic semialdehyde is prevented. Since inhibition occurs even when arginine is used as nitrogen source it is not likely that cessation of growth is due to arginine limitation as previously suggested. Preliminary observations suggest that addition of basic amino acids to slowly growing cultures of *Saccharomyces* inhibits some process associated with the cell cycle.

Bourgeois and Thouvenot reported (1,2) that 5 mM lysine inhibited the post-exponential growth of *Saccharomyces cerevisiae*. Concurrent with growth inhibition, they observed a dramatic decrease in the total amount of free arginine in the cell. Since both wild type and arginase defective strains were equally sensitive to growth inhibition, they suggested that decrease in growth might be due to a limitation of available arginine brought about by a negative influence of lysine upon arginine biosynthesis.

We have re-examined this phenomenon and find that it is far more general in scope than previously recognized and is likely brought about by factors other than lysine induced arginine starvation. It appears that whether or not cells are growing exponentially is of little consequence. The critical parameter is how fast they are growing. It is also clear that growth inhibition can occur even under conditions where arginine is present in large amounts.

MATERIALS AND METHODS

The two strains of *Saccharomyces* used in this work were M-25, our wild type diploid strain and M-399, a diploid mutant strain carrying a homozygous lesion in the *car-2* (ornithine transaminase) gene. The phenotype of strain M-399 was verified both physiologically and biochemically. It is able to use urea, arginine and proline as sole nitrogen sources, but cannot use ornithine in this capacity. Cell-free extracts prepared from this strain did not exhibit significant ornithine transaminase activity under conditions where it is easily demonstrable in the wild type organism. The medium used here is

Wickerham's (3) with 0.6% glucose as sole carbon source. The nitrogen source has been indicated for each experiment and was present at a final concentration of 0.1%.

RESULTS AND DISCUSSION

Proximity of the *lys-1* and *dal-2* loci on chromosome IX of *Saccharomyces* prompted us to isolate mutant strains that were simultaneously auxotrophic for lysine and incapable of using allantoin as sole nitrogen source. It was our hope that some of these strains would contain deletions whose ends extended into both loci. However, during characterization of such mutant strains, we observed that wild type cells could not use allantoin as a nitrogen source in the presence of lysine (see Figure 1, strain M-25 growing on allantoin plus lysine). This result was surprising since urea, another intermediate of the allantoin degradative pathway and somewhat better nitrogen source could be used normally as is shown in Figure 1. These facts argued that lysine inhibition of allantoin utilization was not specific regulation of that pathway, but alternatively might be due merely to lysine inhibition of slowly growing cultures. To test this hypothesis, wild type cultures were grown in medium containing proline as sole nitrogen source in the presence and absence of lysine. As can be seen, growth on this poor nitrogen source is completely inhibited by lysine.

To determine whether or not growth inhibition is specific to lysine or characteristic of basic amino acids in general, we repeated the above experiment and added ornithine in place of lysine. Unlike lysine, ornithine may be used as sole nitrogen source. Therefore, the experiment was performed using a mutant strain which could not use ornithine as a source of nitrogen. As shown in Figure 1 no growth of this strain is observed on proline or allantoin. Growth is also somewhat impaired on urea, but remains normal on ammonia. The total absence of growth of the mutant strain on lysine argues that the two basic amino acids may act additively to inhibit growth. A prerequisite of this argument is the fact that arginine must be available for degradation under these conditions to bring about an increase in the intracellular orni-

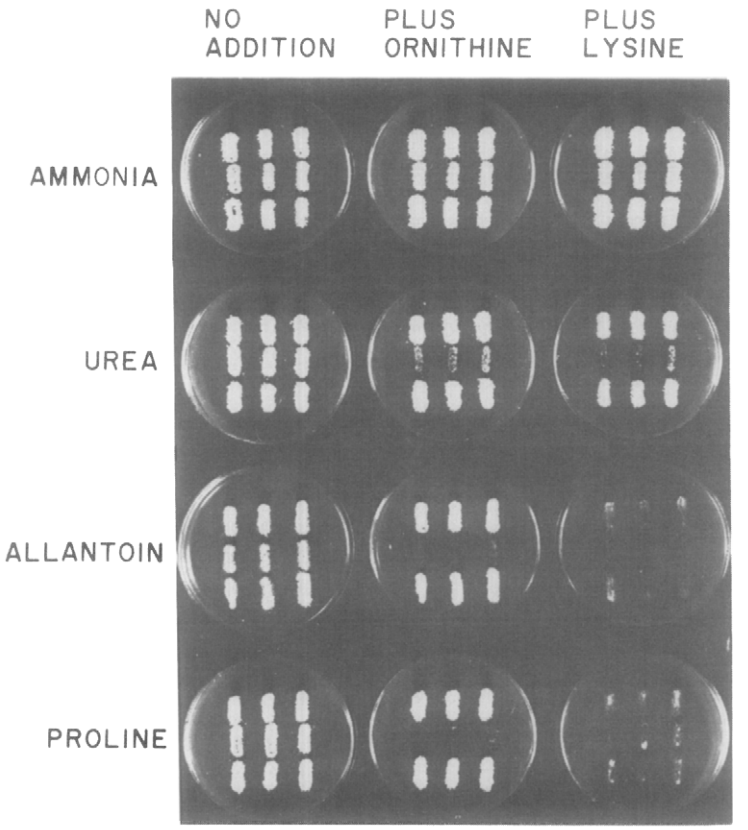

Figure 1: Growth of wild type and ornithine transaminase defective strains on various nitrogen sources in the presence and absence of lysine or ornithine. Each horizontal row of three plates was prepared by replica plating a master plate onto the three plates shown. Each plate in the horizontal rows contained the nitrogen source listed on the left hand margin. Positioning of cells on the plates was the same in all cases. The top and bottom horizontal rows of cells on each plate are M-25, the wild type. The center row of cells on each plate is strain M-399, the ornithine transaminase defective mutant strain. All of the plates in the first vertical row received no additions other than the nitrogen source. All plates in the center and right hand vertical rows received ornithine (4.7 mM) or lysine (1.75 mM) respectively. These additions are indicated at the top of each row. All cultures were incubated at 30° for 48 hours before being photographed. Typical doubling times of M-25 growing on each of these nitrogen sources is: ammonia--115, urea--139, allantoin--165, and proline--280 minutes.

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thine pool. This has been shown to occur previously (4,5) and was suggested to be the reason behind constitutive production of the allantoin degradative enzymes in mutant strains devoid of allophanate hydrolase activity.

It is quite likely that the basic amino acid inhibition we observe when

cells are growing slowly is similar to the lysine inhibition of post-exponential growth reported by Bourgeois. In that case cultures which were growing rapidly on ammonia would not have been inhibited until growth slowed due to limitation of ammonia or some other nutrient. Once the growth rate decreased, however, inhibition would occur and thus decrease the final yield of cells as Bourgeois observed.

It may be suggested that the basic amino acids, lysine and ornithine, limit growth by subverting normal regulation of arginine biosynthesis thus creating conditions of arginine starvation. To test this supposition we determined the growth rates of strain M-399 on urea and arginine. Since this strain is unable to use ornithine, degradation of arginine to urea and ornithine will increase the intracellular concentration of ornithine with urea serving as nitrogen source. If basic amino acid inhibition of growth is the result of arginine starvation then it should not be observed under these conditions because, as sole nitrogen source, arginine would be present at high concentration in the cells. As shown in Table I growth of the mutant strain is dramatically slowed on arginine as compared to urea arguing that basic amino acids inhibit growth by some mechanism other than arginine starvation.

In preliminary visual observation of basic amino acid inhibited cells we have observed abnormal cell morphology which is presently being studied

TABLE I

Growth of Wild Type and Ornithine Transaminase on Arginine and Urea.

Strain	Nitrogen Source	
	Urea	Arginine
	doubling time in minutes	
M-25 (wild type)	128	150
M-399 (ornithine transaminase minus)	165	512

in detail to ascertain whether or not lysine and ornithine addition impairs operation of the cell cycle. This might occur through the intermediary formation of polyamines although there is not yet strong experimental evidence to support this view. Mutant strains resistant to this effect have been isolated and will no doubt aid in elucidating the physiological basis for the basic amino acid inhibition of growth in *Saccharomyces*.

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