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Hydroxy Amino Acid Metabolism in *Pseudomonas cepacia*: Role of L-Serine Deaminase in Dissimilation of Serine, Glycine, and Threonine

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Growth of *Pseudomonas cepacia* (*P. multivorans*) on serine depended upon induction of a previously undescribed L-serine deaminase distinct from threonine deaminase. Formation of the enzyme was induced during growth on serine, glycine, or threonine. The induction pattern reflected a role of the enzyme in catabolism of these three amino acids. Both threonine and glycine supported growth of serine auxotrophs and were presumably converted to serine and pyruvate in the course of their degradation. Mutant strains deficient in serine deaminase, or unable to use pyruvate as a carbon source, failed to utilize serine or glycine and grew poorly with threonine, whereas strains deficient in threonine dehydrogenase or α-amino β-ketobutyrate:coenzyme A ligase (which together convert threonine to glycine and acetyl coenzyme A) failed to utilize threonine or derepress serine deaminase in the presence of this amino acid. The results confirm for the first time the role of α-amino β-ketobutyrate:coenzyme A ligase in threonine degradation and indicate that threonine does not mimic serine as an inducer of serine deaminase.

Pseudomonas cepacia (*P. multivorans*) is the most nutritionally versatile of the pseudomonads (1, 9, 11). We have examined the possibility that the exceptional versatility of this bacterium might be related to the evolution of broad-specificity enzymes. Our experiments have focused on enzymes related to metabolism of branched-chain and hydroxy amino acids (4, 13). In the case reported here, we have considered whether the atypical properties of the isoleucine-sensitive threonine (serine) deaminase of *P. cepacia* (4, 12, 14) reflect a role of this enzyme in utilization of hydroxy amino acids as well as in biosynthesis of isoleucine. The results indicate clearly that threonine deaminase does not participate in hydroxy amino acid catabolism. Our experiments define the role of a serine deaminase in degradation of both serine and threonine. The present analysis of enzymes involved in hydroxy amino acid utilization and related studies of branched-chain amino acid degradation indicate that the biochemical diversity of *P. cepacia* depends upon its extensive repertoire of enzymes rather than the use of broad-specificity enzymes.

MATERIALS AND METHODS

Bacterial strains. *P. cepacia* 249 was originally obtained from R. Y. Stanier (11). All other strains used in this investigation were derived in our laboratory from strain 249 and are listed in Table 1. The indicated auxotrophic derivatives and strains unable to utilize particular organic compounds as the carbon source were obtained by the D-cycloserine selection procedure described earlier (4).

Preparation of bacterial extracts. Bacteria were grown in 1-liter flasks which contained 200 ml of minimal salts medium consisting of 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.01 mM $FeSO_4$, and pH 6.5 phosphate buffer (33 mM Na_2HPO_4, and 67 mM KH_2PO_4) supplemented with the indicated carbon source. The bacteria were collected by centrifugation, suspended in 5 ml of 20 mM phosphate buffer (pH 6.8) containing 20 μg of pyridoxal phosphate per ml and 10 mM 2-mercaptoethanol, and disrupted by sonic treatment as reported earlier (4). The disrupted-cell suspensions were centrifuged for 10 min at 12,000 $\times$ g, and the supernatant fractions were assayed for enzyme activity.

Enzyme assays. All enzyme activities are expressed in terms of nanomoles of product formed per minute per milligram of protein. Protein was determined by the procedure of Lowry et al. (5).

L-Serine and L-threonine deaminase activities were determined at 37°C. The assay mixtures contained in a volume of 0.5 ml: 200 mM Tris-hydrochloride buffer (pH 8.5), 0.2 mM pyridoxal phosphate, enzyme preparation, and 100 mM L-serine or L-threonine (adjusted to pH 8.5 with NaOH). After 20 to 30 min of incubation, the reactions were terminated by addition of 0.5 ml of 15% (wt/vol) trichloroacetic acid, and pyruvate or α-ketobutyrate formed in the course of the reaction was determined as the dinitrophenylhydrazone (4).

L-Threonine dehydrogenase activity was determined at 24°C by monitoring NAD reduction which occurred during the conversion of L-threonine to α-

TABLE 1. *List of strains*

Strain designation	Characteristics
P. cepacia 249 (ATCC 17616)	Wild type, obtained from R. Y. Stanier (11)
249-13	Isoleucine auxotroph derived from strain 249; deficient in threonine deaminase (4)
13-5	Lysine auxotroph derived from strain 13; fails to use diaminopimelic acid in place of L-lysine
13-8	Serine auxotroph derived from strain 13; deficient in phosphoserine aminotransferase
13-5-1	Serine auxotroph derived from strain 13-5, possesses normal levels of phosphoserine aminotransferase and phosphatase; presumably blocked early in serine biosynthesis
249-16	Threonine dehydrogenase-deficient derivative of strain 249; unable to utilize threonine as the sole carbon source
249-18	Serine deaminase-deficient derivative of strain 249; unable to utilize L-serine as the sole carbon source
249-23	α-Amino β-ketobutyrate:coenzyme A ligase-deficient derivative of strain 249; unable to utilize L-threonine as the sole carbon source
249-30	Unable to utilize pyruvate or glucose as the sole carbon source; grows normally on acetate or citrate; presumably blocked at level of pyruvate dehydrogenase

amino β-ketobutyrate (4). The assay mixtures contained, in 1 ml: 100 mM Tris-hydrochloride buffer (pH 8.5), 0.5 mM NAD, enzyme preparation, and 100 mM L-threonine (adjusted to pH 8.5 with NaOH).

α-Amino β-ketobutyrate:coenzyme A (CoA) ligase-dependent condensation of acetyl-CoA and glycine to form α-amino β-ketobutyrate was determined by measuring aminoacetone produced from α-amino β-ketobutyrate (4, 7). The α-amino β-ketobutyrate:CoA ligase (aminoacetone synthase) assay mixtures contained, in 0.5 ml: 200 mM Tris-hydrochloride buffer (pH 8.5), 1 mM acetyl-CoA, enzyme preparation, and 300 mM glycine. After 20 min of incubation at 37°C, the reactions were terminated by addition of 0.5 ml of 15% (wt/vol) trichloroacetic acid, and aminoacetone was determined as reported earlier (4).

Resolution of the catabolic serine deaminase and threonine deaminase. To obtain preparations of catabolic serine deaminase and threonine deaminase suitable for comparison of pH optima and kinetic and other properties of the two enzymes, bacteria from 10 200-ml cultures of strain 249 grown with 0.4% (wt/vol) L-serine were collected by centrifugation, suspended in 30 ml of 20 mM phosphate buffer (pH 6.8) containing 20 μg of pyridoxal phosphate per ml and 10 mM 2-mercaptoethanol, and disrupted by sonic treatment. Cell debris and unbroken cells were removed by centrifugation of the disrupted-cell suspension for 15 min at 12,000 × *g*. An equal volume of 2% (wt/vol) streptomycin sulfate dissolved in the above-mentioned buffer was added to the supernatant to precipitate nucleic acids, and the precipitated material was removed by centrifugation. Solid ammonium sulfate (25 g/100 ml) was added to the supernatant fraction, and the precipitate, which contained the bulk of the serine and threonine deaminases present in the crude bacterial extract, was suspended in ca. 10 ml of pH 6.8 phosphate buffer containing pyridoxal phosphate and mercaptoethanol. The preparation was dialyzed against 200 volumes of the same buffer and supplemented with 10% (wt/vol) glycerol, and between 3 and 4 ml (100 mg of protein) was applied to a column (2 by 20 cm) of hydroxylapatite equilibrated with the same buffer. The column was eluted with 280 ml of a linear gradient of 20 to 200 mM phosphate buffer (pH 6.8) containing 10% (vol/vol) glycerol, 20 μg of pyridoxal phosphate per ml, and 10 mM 2-mercaptoethanol. Fractions (3.5 ml each) were collected and assayed for L-threonine and L-serine deaminase activities. The catabolic serine deaminase eluted first at a phosphate concentration of 70 mM; L-threonine deaminase eluted at a phosphate concentration of 150 mM. Fractions containing the catabolic serine deaminase were pooled, as were those containing L-threonine deaminase activity, and used for studies of kinetic and other properties of the two enzymes. The recovery of threonine deaminase activity was about 90% of the activity present in the crude bacterial extracts; about 40% of the catabolic serine deaminase activity was recovered. If glycerol was omitted during chromatographic resolution of the two enzymes, the recovery of catabolic serine deaminase activity was only 10%. The presence of glycerol did not influence the recovery of threonine deaminase. The extent of purification of the catabolic serine deaminase and of threonine deaminase was, respectively, 28- and 40-fold.

The catabolic serine deaminase was sufficiently unstable to preclude its further purification. The preparations obtained after hydroxylapatite chromatography lost approximately 50% of their activity after 2 days in storage at 0°C. Less-purified preparations such as crude bacterial extracts or the ammonium sulfate fractions used for the chromatographic step were completely stable for at least 2 weeks when stored under similar conditions.

Estimation of enzyme molecular weight. The apparent molecular weights of serine and threonine deaminases were determined by comparing their sedimentations in sucrose gradients with that of bovine hemoglobin as described by Martin and Ames (6). The gradients contained 20 mM phosphate buffer (pH 6.8) and 20 μg of pyridoxal phosphate per ml.

Chemicals. All chemicals were obtained from Sigma Chemical Co., St. Louis, Mo.

RESULTS

P. cepacia 249 utilizes the L-forms of serine and threonine but not the D-forms of these amino acids as the sole carbon source. In previous experiments, we investigated whether the

atypical threonine deaminase of *P. cepacia* played a role in threonine catabolism and concluded on the basis of mutant studies that it did not (4). *P. cepacia* grows more rapidly on L-serine (generation time, 2.5 h) than on L-threonine (generation time, 5.5 h). To determine whether the serine deaminase activity associated with threonine deaminase was involved in serine catabolism, we tested whether *P. cepacia* 249-13, an isoleucine auxotroph devoid of threonine deaminase activity (4), could utilize serine. This strain grew normally in medium containing 40 μg of L-isoleucine per ml and 0.4% L-serine as the sole carbon source, indicating that threonine deaminase is not essential for serine utilization. This led us to investigate further the enzymatic basis of serine utilization.

Growth of *P. cepacia* 249 in medium containing 0.4% L-serine as the carbon source resulted in an approximate sevenfold increase in serine deaminase-specific activity compared to bacteria grown on alternate carbon sources such as citrate or glucose or phthalate (Table 2). The increase in specific activity was due to induction of a previously undescribed catabolic L-serine deaminase distinct from threonine deaminase. The catabolic enzyme was readily resolved from threonine deaminase on the basis of its slower sedimentation in sucrose gradients and its relatively weak binding to hydroxylapatite columns (see above). Its molecular weight was estimated to be 60,000 by comparison of its sedimentation behavior with that of bovine hemoglobin (64,500 molecular weight) and threonine deaminase (190,000 molecular weight). Extracts of the threonine deaminase-deficient strain 249-13 (prepared from serine-grown bacteria provided with isoleucine) contained only the catabolic serine deaminase. This enzyme was inactive with L-threonine. (Under our conditions of assay the limit of detection for activity with L-threonine was equivalent to about 0.5% of the activity observed with L-serine.) In this respect, it differed from threonine (serine) deaminase, the activity of which with L-threonine was sevenfold greater than with serine. There was also a marked difference in response of the two enzymes to the threonine deaminase feedback inhibitor L-isoleucine. Threonine deaminase exhibited no significant serine deaminase activity in the presence of 10^{-2} M L-isoleucine. The activity of the catabolic serine deaminase was unaffected by 10^{-2} M L-isoleucine.

The distinguishing features of *P. cepacia* serine and threonine deaminases, including kinetic properties, pH optima, and responses to isoleucine, are summarized in Table 3. The finding that the activity of the catabolic serine deaminase was not influenced by L-isoleucine afforded

TABLE 2. *Induction of L-serine deaminase in strain 249*

Carbon source[a]	Generation time (min)	Enzyme sp act[b]	
		L-Serine deaminase[c]	L-Threonine deaminase
Glucose	70	229	694
Citrate	60	235	704
Phthalate	65	215	685
L-Threonine	300	1,482	512
L-Serine	150	2,319	510
Glycine	540	1,733	493
Casamino acids	60	235	535
Ile + Val + Leu	70	202	615

[a] The bacteria were grown at 37°C in minimal salts medium supplemented with the following amounts of each carbon source: glucose, citrate, and phthalate, 0.5% (wt/vol); glycine, threonine, and serine, each at 0.4% (wt/vol); Casamino Acids at 1% (wt/vol); and branched-chain amino acids, each at 0.2%.

[b] Nanomoles of pyruvate or α-ketobutyrate formed per minute per milligram of protein at 37°C.

[c] Determined in the presence of 10^{-2} M L-isoleucine and therefore does not include serine deaminase activity associated with threonine deaminase.

TABLE 3. *Properties of L-threonine and L-serine deaminases*

Enzyme[a]	Apparent mol wt	pH optimum	Extent of inhibition by 10^{-2} M L-isoleucine	L-Serine K_m (M)	L-Threonine K_m (M)
Serine deaminase	60,000	Broad region of optimal activity between pH 6.5 and 9.5[b]	None	2×10^{-2}[c]	
Threonine deaminase	190,000	With serine, optimal activity was between pH 8.5 and 9.5[d]	Complete	6×10^{-2}	2×10^{-2}

[a] The preparations used in these determinations were resolved on hydroxylapatite columns as described in the text.

[b] Activity at pH 7 was equivalent to 105% of that at pH 8.5.

[c] The K_m value at pH 7.0 was identical to that at pH 8.5.

[d] Activity with L-serine at pH 7 was only 3% of that at pH 8.5.

a rapid means of determining the amount of this enzyme in crude bacterial extracts. About 95% of the serine deaminase activity of serine-grown bacteria and 65% of the activity of glucose- or citrate-grown bacteria was insensitive to inhibition by 10^{-2} M L-isoleucine. Thus, even the basal level of catabolic serine deaminase found in citrate- or glucose-grown bacteria contributed more to the total serine deaminase of *P. cepacia* extracts than did the serine deaminase associated with threonine deaminase, and in the case of fully induced cells, the contribution of threonine deaminase was relatively insignificant.

Induction of L-serine deaminase during growth on L-threonine or glycine. As noted above, the catabolic L-serine deaminase was inactive with L-threonine. It was therefore surprising to discover that the enzyme was induced during growth on threonine (Table 2). This paradox was resolved when we were able to confirm by the mutant studies described below that catabolism of threonine proceeds by its stepwise conversion to α-amino β-ketobutyrate and thence to acetyl CoA and glycine. Metabolism of the glycine derived by cleavage of α-amino β-ketobutyrate appears to require its conversion to serine, which is further metabolized to pyruvate by the action of serine deaminase. This explains the observed induction of serine deaminase when glycine served as the carbon source (Table 2). The proposed route of threonine catabolism (Fig. 1) is consistent with the observed pattern of induction of serine deaminase during growth on threonine, serine, or glycine. The mutant studies outlined below rule out the possibility that threonine directly mimics serine as an inducer of serine deaminase and support the interpretation that the physiological inducer during growth on threonine is serine formed via glycine.

Enzyme deficiencies affecting utilization of L-serine and L-threonine. We have used a D-cycloserine selection procedure (4) to obtain mutants blocked in the utilization of L-serine or of L-threonine. One such strain, 249-18, which proved to be deficient in serine deaminase, failed to grow on serine or glycine and grew slowly on threonine (27-h generation time compared with 5.5 h for the wild type). The level of serine deaminase activity in extracts of bacteria grown on threonine or combinations of threonine and alternate carbon sources such as citrate or glucose was less than 2% of that found in the wild type grown under similar conditions (Table 4). Strain 249-18 had normal levels of threonine deaminase and of the isoleucine-sensitive serine deaminase activity associated with this enzyme (data not shown). The results confirm that the catabolic serine deaminase is essential for serine degradation and that the serine deaminase associated with threonine deaminase cannot substitute for loss of this enzyme.

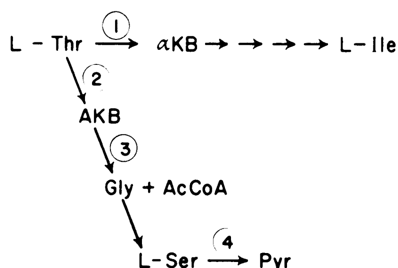

FIG. 1. *Pathways of threonine metabolism in P. cepacia. Threonine deaminase (enzyme 1) and enzymes required to convert α-ketobutyrate to isoleucine were formed constitutively. Enzymes of the threonine degradation pathway, which included L-threonine dehydrogenase (enzyme 2), α-amino β-ketobutyrate: CoA ligase (enzyme 3), and L-serine deaminase (enzyme 4), were induced during growth on L-threonine.*

We obtained two classes of mutants unable to utilize L-threonine as the carbon source. The first was deficient in threonine dehydrogenase, the NAD-specific enzyme which converts threonine to α-amino β-ketobutyrate (2, 4, 7, 8). The second class was deficient in α-amino β-ketobutyrate:CoA ligase, which converts α-amino β-ketobutyrate to glycine and acetyl-CoA. Data indicating the levels of these two enzymes in wild-type and mutant bacteria are presented in Table 4. Strain 249-16 is representative of the first class; strain 249-23 is a member of the second class. Both the threonine dehydrogenase and α-amino β-ketobutyrate:CoA ligase-deficient strains failed to form more than basal levels of catabolic serine deaminase when grown in medium containing threonine in combination with citrate as a carbon source. Growth of the wild type in this medium resulted in formation of elevated levels of serine deaminase. Members of both classes of mutants grew normally with L-serine as the carbon source and produced wild-type levels of catabolic serine deaminase under these conditions. We interpret the results as indicating that threonine degradation must proceed at least to the level of glycine and acetyl-CoA formation for threonine to influence formation of serine deaminase. The simplest interpretation is that in this case the physiological inducer of the catabolic serine deaminase is serine formed from glycine.

It should be noted that the threonine dehydrogenase-deficient strain 249-16 formed wild-type levels of α-amino β-ketobutyrate:CoA ligase when grown on a combination of threonine and citrate (Table 4) and that the α-amino β-ketobutyrate:CoA ligase-deficient strain 249-23

TABLE 4. *Levels of enzymes related to serine and threonine degradation in wild-type and mutant strains of P. cepacia*

Strain	Enzyme deficiency	Carbon[a] source	L-Serine deaminase[b]	Enzyme sp act[c]	
				L-Threonine dehydrogenase	α-Amino β-ketobutyrate: CoA ligase
249 (wild type)	None	L-Thr	1,482	61	31
		L-Thr + Cit	642	29	17
		L-Ser	2,319	3	<1
249-18	Serine deaminase	L-Thr	13	61	33
		L-Thr + Cit	14	31	19
249-16	Threonine dehydrogenase	L-Thr + Cit	156	1.5	25
		L-Ser	2,262	1.5	<1
249-23	α-Amino β-ketobutyrate:CoA ligase	L-Thr + Cit	162	43	<1
		L-Ser	2,451	3	<1

[a] L-Threonine was supplied at 0.4% (wt/vol) when it was the sole carbon source and at 0.2% (wt/vol) in combination with 0.3% citrate in experiments in which threonine was not the primary carbon source.
[b] Determined in the presence of 10^{-2} M L-isoleucine and therefore does not include serine deaminase activity associated with threonine deaminase.
[c] Nanomoles of product formed per minute per milligram of protein at 37°C.

formed wild-type levels of threonine dehydrogenase in the same medium. These results indicate that α-amino β-ketobutyrate:CoA ligase is induced directly by L-threonine and that threonine dehydrogenase is induced by threonine (or α-amino β-ketobutyrate).

In the course of examining the threonine dehydrogenase and α-amino β-ketobutyrate:CoA ligase activities of *P. cepacia*, we examined the molecular weights of these two enzymes by comparing their sedimentation in sucrose gradients with that of bovine hemoglobin and serine and threonine deaminases. The apparent molecular weight values were 140,000 for threonine dehydrogenase and 75,000 for α-amino β-ketobutyrate:CoA ligase.

Two other types of mutant strains provide further insight into the route of degradation of serine, threonine, and glycine. One such strain is 13-8, a serine auxotroph which is deficient in phosphoserine aminotransferase activity. The serine requirement of this strain was spared when it was grown on L-threonine or when bacteria induced to form threonine dehydrogenase and α-amino β-ketobutyrate:CoA ligase by prior growth on threonine were transferred to 0.5% glucose medium supplemented with 100 mg of L-threonine per ml. Threonine also spared the serine requirement of another serine auxotroph, strain 13-5-1, which appeared to be blocked early in the pathway of serine biosynthesis. The behavior of these two auxotrophic strains confirms that threonine was readily converted to serine in *P. cepacia*, presumably via threonine dehydrogenase and α-amino β-ketobutyrate:CoA ligase-dependent formation of glycine followed by serine hydroxymethyl transferase-mediated conversion of glycine to serine.

A second type of mutant the behavior of which is consistent with a role of serine deaminase in the degradation of serine, threonine, and glycine is strain 249-30, which appeared to lack pyruvate dehydrogenase activity. This mutant, which was selected on the basis of its failure to utilize glucose as a carbon source, grew normally on acetate or tricarboxylic acid cycle intermediates such as citrate. It failed to utilize pyruvate, serine, or glycine and grew poorly on threonine. The failure of this strain to utilize glycine as the sole carbon source is consistent with the proposed pathway of glycine degradation in which serine and pyruvate are key intermediates.

DISCUSSION

We undertook the present study to determine whether the atypical properties of *P. cepacia* threonine deaminase reflected a role of this enzyme in serine degradation. The fact that the threonine deaminase-deficient strain 249-13 grew normally on L-serine indicated that threonine deaminase was not essential for serine dissimilation. The induction of a separate catabolic L-serine deaminase during growth on L-serine and the failure of strain 249-18, which is deficient in this enzyme, to utilize serine clearly define the role of this enzyme in serine degradation. A similar induction of serine deaminase was reported for *E. coli*. However, serine deaminase was not directly shown to be distinct from threonine deaminase (3, 10). Threonine deaminase was unable to substitute for the loss of the catabolic serine deaminase in strain 249-18, in-

dicating that it does not play a secondary role in serine catabolism.

The inability of strain 249-18 to utilize glycine or grow normally on L-threonine implicates the catabolic serine deaminase in the degradation of both of these amino acids. The mutant studies reported here indicate that threonine and glycine are readily converted to serine, which appears to be the physiological inducer of serine deaminase. The results confirm for the first time the role of α-amino β-ketobutyrate:CoA ligase in threonine degradation, and indicate that threonine degradation proceeds by cleavage of α-amino β-ketobutyrate to glycine and acetyl-CoA and not by decarboxylation of this compound to produce aminoacetone as postulated earlier (2, 4, 7). To our knowledge, neither α-amino β-ketobutyrate:CoA ligase nor threonine dehydrogenase-deficient strains have been described for other bacteria presumed to metabolize threonine via glycine and acetyl-CoA (2, 7, 8).

At the onset of these studies, our working hypothesis was that the use of broad-specificity enzymes such as threonine deaminase and branched-chain amino acid aminotransferase for both the biosynthesis and catabolism of amino acids might underlie the extreme nutritional versatility of *P. cepacia*. Such roles would be entirely consistent with the in vitro properties of these enzymes (4, 13). Thus, the failure of threonine deaminase to substitute for the catabolic serine deaminase in serine degradation or for threonine dehydrogenase in threonine degradation was unexpected.

The apparent inability of threonine deaminase to participate in threonine or serine degradation might be due to the operation of feedback controls or, in the case of threonine, degradation to an imbalance in branched-chain amino acid biosynthesis rather than any inherent inability of this enzyme to rapidly convert serine and threonine to pyruvate and α-ketobutyrate. For example, the strong inhibition of threonine deaminase by isoleucine might preclude its participation in amino acid degradation, or the toxicity of α-ketobutyrate might contribute to poor growth on threonine. In the presence of excess α-ketobutyrate, the interaction of this compound with acetohydroxyacid synthase to produce acetohydroxybutyrate, a precursor of isoleucine, presumably competes with interaction of the same enzyme with pyruvate to produce acetolactate, a precursor of valine. *P. cepacia* utilizes α-ketobutyrate as the sole carbon source, if the bacteria are supplemented with valine. However, threonine dehydrogenase-deficient bacteria still fail to utilize threonine even if they are supplied with valine. We are examining whether mutant strains deficient in catabolic serine deaminase or in threonine dehydrogenase can mutate to utilize threonine deaminase as a catabolic enzyme. We expect that selection of bacteria able to utilize serine or threonine from serine deaminase- or threonine dehydrogenase-deficient strains might yield mutants uncoupled with respect to isoleucine inhibition of threonine deaminase. In the case of growth on threonine, we anticipate a possible requirement for valine.

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ADDENDUM

We have reexamined the growth behavior of the threonine dehydrogenase-deficient strain 249-16 and find that it can grow slowly in medium containing 0.5% L-threonine if the medium is supplemented with 100 μg of L-valine per ml (14-h generation time versus 3.5 h for the wild-type strain 249). The results indicate that the failure of *P. cepacia* threonine deaminase to play a secondary role in threonine degradation is related to the toxicity of α-ketobutyrate produced by this enzyme from threonine.

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