

Serine Utilization by *Klebsiella aerogenes*

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Klebsiella aerogenes was found to contain a specific L-serine dehydrase that was induced by threonine, glycine or leucine, but not by its substrate. Cellular concentrations were sensitive to carbon rather than nitrogen sources in the growth medium. A nonspecific isoleucine-sensitive L-threonine dehydrase supplemented the specific L-serine dehydrase activity. *K. aerogenes* also contains a leucine-inducible L-threonine dehydrogenase which probably initiated a threonine-utilization pathway in which the serine-specific dehydrase participated. Strains that were altered in their ability to metabolize serine differed in either L-serine dehydrase or L-threonine dehydrase activity. Thus, *K. aerogenes* growing on L-serine as a sole nitrogen source relies upon two enzymes that metabolize the amino acid as subsidiary functions.

L-Serine supports growth of *Klebsiella aerogenes* at a modest rate and is frequently used as a nitrogen source in diagnostic media during genetic studies of nitrogen utilization. The manner in which the amino acid is catabolized is not known in detail, but failure to support the growth of mutants lacking glutamate synthase indicates that it supplies nitrogen in the form of ammonia (6). Studies in *Escherichia coli* (1, 21, 31) and *Pseudomonas cepacia* (43) implicate a specific L-serine dehydrase as the enzyme responsible. Activity in *P. cepacia* is induced by serine, threonine, or glycine, whereas threonine, glycine, or L-leucine act as inducers in *E. coli*. We have now examined the utilization of L-serine in *K. aerogenes*. This bacterium has two enzymes with serine dehydrase activity, and the action of both was necessary to support the growth rates observed. One of the enzymes was specific for L-serine and probably a component of the pathway for L-threonine metabolism. The other enzyme, L-threonine dehydrase, is presumed to lie in the isoleucine biosynthetic pathway and able to act upon L-serine as well as L-threonine.

In the enteric bacteria, utilization of nitrogen sources is often controlled by the intracellular concentration of glutamine synthetase (25). Enzymes catalyzing the breakdown of L-proline (26), L-arginine (17), L-histidine (32), L-tryptophan (26), L-asparagine (33), and urea (16) are formed in response to a rise in glutamine synthetase concentration which is, in turn, promoted by nitrogen depletion (39). However, L-serine is one of several amino acids the utiliza-

tion of which in *E. coli* was concluded not to be controlled by glutamine synthetase (30). Our results show that, in *K. aerogenes* also, L-serine utilization is not mediated by glutamine synthetase.

MATERIALS AND METHODS

Bacterial strains. *K. aerogenes* strains used in this study are listed in Table 1.

Media and growth conditions. Luria broth (LB) contains 1% tryptone (Difco Laboratories), 0.5% yeast extract, and 1% sodium chloride, adjusted with sodium hydroxide to pH 7.0. Other media were constituted from separately sterilized solutions of W salts (34) and appropriate carbon, nitrogen, and growth factor requirements. The following requirements (with the amount normally added within parentheses) were added: carbon source (0.4%); nitrogen source (0.2%); and amino acid supplement for auxotrophic strains (0.01%). When an amino acid was used as the single source of carbon and nitrogen, it was added at 0.4% concentration; if additional amino acids were included in such media, the concentration was 0.1%. Cultures assayed for β -galactosidase were grown in media to which isopropyl thiogalactoside was added at 1 mM. When included in media, adenosine cyclic AMP was added at 10 mM concentration. Variations from these compositions are noted where they occur.

Media were inoculated with 0.4% (vol/vol) of washed cells from cultures grown overnight in glucose-ammonium sulfate (GN) medium, supplemented with growth factors as needed. Cells were washed twice with a 0.9% sodium chloride solution. Cultures were grown at 30°C in 125-ml side-arm flasks containing 25 ml of medium and shaken at 200 rpm. Growth was measured with a Klett-Summerson colorimeter with a 540-nm filter.

Enzyme assays. Cells were grown to a density of 100 ± 15 Klett units (4×10^8 to 6×10^8 cells per ml), chilled, and pelleted by centrifugation at 4°C. For the

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TABLE 1. *Strains of K. aerogenes*

Strain	Relevant characteristics ^a	Source or reference
MK9000	Wild type	35
MK104	<i>glnA6</i> (GlnA ⁻ CRM ⁻)	32
MK924	<i>glnA51</i> (GlnA ⁻ CRMC)	36 14
MK9512	<i>glnA29</i> (GlnC)	35
MK9770	<i>glnD66</i>	14
CG177	<i>glnG88 ilvA</i>	18
CG191	<i>gln-54</i>	C. M. Gaillardin, this laboratory
CG204	<i>ilvA</i>	18
CG235	<i>glnF82 rha-1</i>	C. M. Gaillardin, this laboratory
FF1011	<i>gltB nadB1</i>	F. Floor, this laboratory
LVS1	SutC	Spontaneous mutant of MK9000, selected for growth on S agar
LV025		EMS ^b mutagenesis of MK9000, selected for poor growth on GS agar
LVG1		EMS mutagenesis of MK9000 selected for fast growth on GS agar

^a All strains carry the *hutC515* allele which confers the ability to metabolize histidine constitutively (10).

^b EMS, Ethyl methane sulfonate.

serine-dehydrase and threonine-dehydrase assays, the pellet was suspended within 2 h in cold saline (1 ml), and portions were diluted to 5.0 ml with cold 0.07 M potassium phosphate (pH 6.0 or 8.0). Serine dehydrase and threonine dehydrase were assayed as described by Pardee and Prestidge (31), but a mixture of cetyl trimethylammonium bromide and sodium deoxycholate was used to render cells permeable. Saturating concentrations of serine and threonine in the incubation mixtures were determined to be 0.15 and 0.12 M, respectively. For maximum activity, pyridoxal phosphate was required.

The procedure used to measure serine dehydrase activity was as follows. A cell suspension (0.3 ml) at pH 6.0 was mixed with a solution (0.1 ml) of L-serine (60 mg/ml), pyridoxal phosphate (25 µg/ml), and any additional supplements used in the assay. After 10 min at 37°C, an aqueous solution (0.05 ml) of cetyl trimethylammonium (1 mg/ml) and sodium deoxycholate (5 mg/ml) was mixed in. Incubation was continued for 15 min and the reaction was then stopped by adding 0.9 ml of 2,4-dinitrophenylhydrazine (0.17 mg/ml in 1.2 N hydrochloric acid, stored in the dark for not longer than 1 month). After a further 20 min at room temperature, 2.5 N sodium hydroxide (1.7 ml) was added, and the absorbance at 520 nm was measured after 15 min. Zero incubation time controls were used as reagent blanks, and the net absorbance was related to values obtained for sodium pyruvate similarly treated. The procedure for measuring threonine dehydrase activity was similar, except that the cell suspension was at pH 8.0 and the substrate solution contained L-threonine at 52 mg/ml.

In experiments in which the influence of pH on enzyme activity was determined, measurements were made in equimolar potassium phosphate, adjusted for

pH values above 8.5 by the addition of potassium hydroxide. To measure serine dehydrase activity in extracts, cells suspended in 0.07 M potassium phosphate (pH 6.0 or 8.0) were disrupted by sonic oscillation for 15 s at 100 W. The homogenate at 4°C was centrifuged (15,000 × *g* for 15 min). The supernatant solution was assayed as for cell suspensions except that the permeabilizing agent was omitted. Threonine dehydrogenase was assayed in extracts of cells washed in 0.07 M potassium phosphate (pH 7.2) and resuspended in the same buffer before sonic disruption. Otherwise, the procedure was that of McGilvray and Morris (27).

Glutamine synthetase activity was determined in cetyl trimethylammonium bromide-treated cells by measuring λ-glutaminy transferase activity as described by Bender and co-workers (5). The assay for β-galactosidase was based on that of Tyler and Magasanik (40).

Protein was extracted from cells with sodium hydroxide (20) and measured as its complex with Coomassie brilliant blue G-250 (9). Bovine serum albumin was used as the standard.

Isolation of mutants. Spontaneous mutations to constitutive serine dehydrase activity were selected by plating cultures on an agar medium containing 0.4% L-serine as the sole carbon and nitrogen source (S agar). Colonies that appeared within 24 h were isolated.

To obtain mutants altered in L-serine utilization, strain MK9000 was mutagenized with ethyl methane-sulfonic acid. Treated cultures were grown to saturation in GN medium and enriched for cells deficient in serine utilization by subjection to two rounds of penicillin treatment in glucose-L-serine (GS) medium. The survivors were grown out in GN medium to select against auxotrophs. Mutagenized cultures were plated on LB medium containing 2% agar (LB agar) and replicated on GS agar. Colonies unable to grow on GS agar were patched to LB agar and grown for 12 h. The cells were transferred to wells of a disposable plastic tray and screened for serine dehydrase activity by a qualitative microadaptation of the standard assay. Keto acid formation was detected by the strong red-brown coloration; negative tests gave a pale yellow color. As an alternative to replica plating, cultures were plated on GS agar supplemented with 1% (vol/vol) of LB medium. After 24 h of incubation, exceptionally small or large colonies were isolated.

Utilization of [¹⁴C]serine. Cells were suspended in 5 ml of W salts solution containing chloramphenicol (300 µg/ml). To 1 ml of the suspension equilibrated at 30°C was added 9 ml of a solution containing 10 µM L-[U-¹⁴C]serine (5 nCi/ml) in W salts solution at 30°C. The mixture was shaken for 10 min and then filtered. The cells were washed and extracted with 70% aqueous ethanol. The solution, concentrated, acidified, and extracted with ether to remove lipid, was separated into basic and nonbasic fractions with a cation exchange resin. The basic fraction was examined by thin-layer chromatography. Radioactivity was measured with either a liquid scintillation counter or a scanner equipped with a Geiger-Mueller detector.

Thin-layer chromatography. Keto acids formed during incubation of L-serine and L-threonine with

permeabilized cells were reacted with 2,4-dinitrophenylhydrazine and extracted into ethyl acetate. Products transferred into aqueous sodium carbonate and recovered, after acidifying, into ethyl acetate were chromatographed on thin layers of silica gel G with benzene-ethyl acetate-formic acid (40:7:1). Samples of pyruvic acid, hydroxypyruvic acid, and α -ketobutyric acid 2,4-dinitrophenyl-hydrazones gave double zones (*syn* and *anti* isomers) at R_f values of 0.35 and 0.50, 0.09 and 0.17, and 0.40 and 0.54, respectively.

The basic fraction from cells exposed to L-[U- 14 C]serine was chromatographed on thin layers of silica gel G using, as solvent systems, *n*-butanol-acetic acid-water (4:1:1), *n*-butanol-*n*-propanol-concentrated ammonia (1:7:3), and phenol-water (3:1).

RESULTS

Properties of L-serine dehydrase. The half-life of the enzyme in intact *K. aerogenes* cells grown in LB medium and stored at 4°C was approximately 48 h. In permeabilized cells, the rate of loss increased sharply; little activity remained 5 min after cetyl trimethylammonium bromide or toluene was added. This rapid loss was prevented by the presence of substrate. In the assay procedure in which permeabilizing agent was added last to the incubation mixture, the rate of keto acid production deviated only 12% from linearity during the first 15 min. The response to increasing substrate concentration was hyperbolic, not sigmoid as found by Alfoldi and co-workers (1) for the serine dehydrase of *E. coli* isolated without protection by its substrate. When *K. aerogenes* cells were disrupted by sonication, activity was retained only if L-serine or L-cysteine was present. Additional protection was afforded by glycerol, and extracts prepared in 0.07 M potassium phosphate (pH 6.0) containing 30% (vol/vol) glycerol and saturated with L-cysteine retained 25% of their activity after 24 h at 4°C.

Permeabilized cells showed dehydrase activity with L-threonine as well as with L-serine. Cysteine was not deaminated and inhibited activity with serine. Cells from the *ilvA* strain CG204 were active only with serine. The response to varying pH of cells grown in GN medium (with isoleucine added for strain CG204) and assayed in 0.07 M potassium phosphate is shown in Fig. 1. The *ilvA* strain gave a broad maximum of activity centered at pH 7.5, but appreciable serine dehydrase activity was present at pH 6.0. In the parent strain MK9000, threonine dehydrase activity was negligible below pH 6.5 and increased with increasing alkalinity. The profile for serine dehydrase activity in strain MK9000 shared features with each of the above but shifted to resemble that of strain CG204 when 0.7 mM isoleucine was included in the incuba-

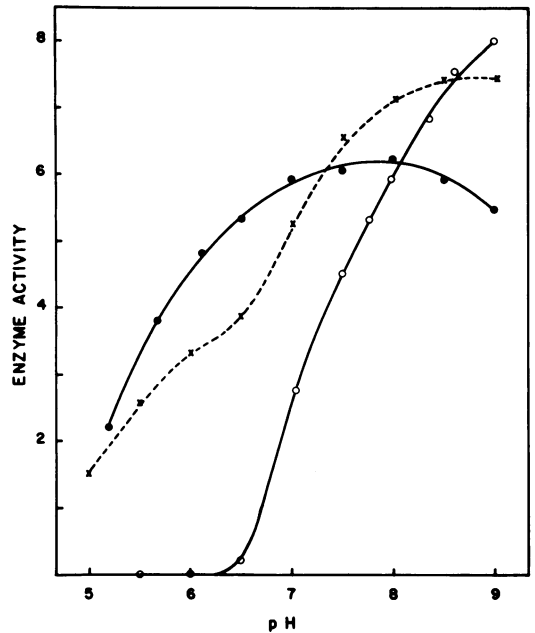


FIG. 1. Enzyme activity in *K. aerogenes* cells assayed at different pH values. Activities of L-serine dehydrase in strain MK9000 (x), L-threonine dehydrase in strain MK9000 (O), and L-serine dehydrase in strain CG204 (●) are shown.

tion mixture. This evidence that serine dehydrase activity in wild-type cells at pH values above 6 is partially due to the nonspecific action of a biosynthetic threonine-deaminating enzyme was supported by measurements of threonine and serine dehydrase activity at increasing isoleucine concentration (Fig. 2). Threonine dehydrase activity was sensitive to low concentrations of the amino acid and was fully inhibited at 0.5 mM. Serine dehydrase activity at pH 8.0 decreased proportionately to 57% of the initial value, then remained constant until the concentration exceeded 10 mM. Isoleucine did not affect serine dehydrase activity at pH 6.0. Assuming that the inhibited activity was due to threonine dehydrase, this enzyme must be 20% as active with L-serine as it is with L-threonine.

Thin-layer chromatography of the 2,4-dinitrophenylhydrazones of products obtained when permeabilized cells were incubated with substrates at pH 6.0 or pH 8.0 under the standard assay conditions or with catalase or 2-ketoglutaric acid added, showed that only pyruvate was formed from serine and that only 2-ketobutyrate was formed from threonine. After 10 min of exposure to L-[U- 14 C]serine, only 20% of the radioactivity taken into strain MK9000 cells was still present as serine. Approximately 40% of the remaining 14 C was in glutamic acid, aspartic acid,

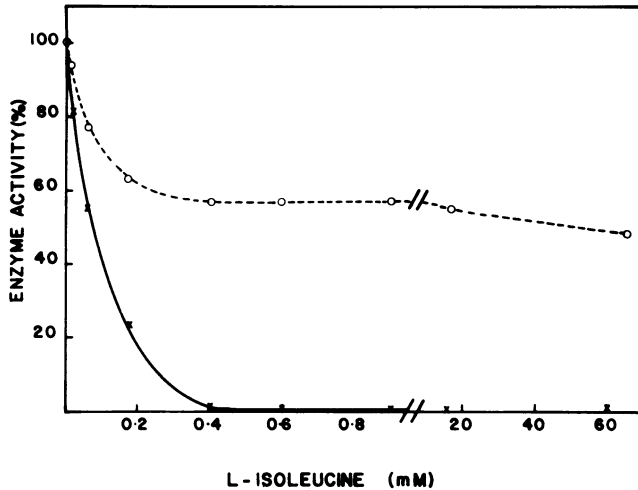


FIG. 2. Effect of L-isoleucine concentration on dehydrase activity at pH 8.0 in cells of strain MK9000. L-Serine (O) and L-threonine (X) were substrates.

alanine, and valine, with smaller amounts in glutamine and leucine; this was as would be expected if serine had been metabolized via pyruvate. Keto acid production was completely inhibited by 2 mM hydroxylamine. Calcium ions inhibited serine dehydrase activity at 0.2 mM; magnesium ions inhibited at 2 mM. Adenosine phosphates, nicotinamide nucleotides flavin nucleotides, coenzyme A, ethylenediaminetetraacetic acid, and mercaptoethanol were without effect. A spectrophotometric assay for a possible L-serine dehydrogenase, based on that described for threonine dehydrogenase (27), showed no activity.

Inducibility of serine dehydrase. Cells of strain MK9000 grown in GN or GS medium contained low serine dehydrase activity (Table 2). A much higher level was present in cells grown in LB medium or in a medium with Casamino Acids (Difco Laboratories) as the sole source of carbon and nitrogen. Casamino Acids could be replaced with a mixture in equal weight proportions of its principal 15 amino acids. The single omission of threonine and isoleucine caused decreases of 56 and 42%, respectively, but no other inducing components could be identified in this way. In media with single amino acids as the sole source of both carbon and nitrogen, *K. aerogenes* grew well on histidine, proline, arginine, aspartic acid, glutamic acid, asparagine, and arginine, but serine dehydrase levels were low. The remaining 13 protein amino acids, alone or combined with ammonium sulfate, did not support appreciable growth and were tested with histidine as a primary source of carbon and nitrogen. Under these conditions,

TABLE 2. Growth rate and serine dehydrase activity of strain MK9000 in various media

Medium ^a	Doubling time (h)	Serine dehydrase activity ^b
GN	0.82	2.12
GS	2.46	2.38
C ₆ A	0.80	81.0
H	1.99	4.36
H-leu	1.16	18.7
H-gly	1.76	21.9
H-thr	1.73	33.7

^a G, D-Glucose; N, ammonium sulfate; S, L-serine; C₆A, Casamino Acids; H, L-histidine; leu, leucine; gly, glycine; thr, threonine.

^b Expressed as micromoles of pyruvic acid per milligram of protein per hour.

threonine, glycine, or leucine induced serine dehydrase activity (Table 2). Isoleucine showed no effect. The increases in activity given by glycine and leucine were not additive. However, each of these amino acids gave an additive increase when combined with threonine.

The inducing effect of threonine and glycine on serine dehydrase activity was also observed with cultures incubated in a medium containing serine as the sole carbon and nitrogen source. With serine alone, strain MK9000 had an initial doubling time of greater than 12 h. The precise value was difficult to measure because selection occurs for a serine-utilizing variant strain (see below). Addition of 0.1% leucine reduced the initial doubling time to 9.25 h but, at this growth rate, selection for the variant was still rapid. Addition of 0.1% threonine or glycine, which gave serine dehydrase activities of 14.1 and 26.8

μmol of keto acid per mg of protein per h, reduced the initial doubling time to 4.38 and 4.22 h, respectively, and little selection occurred.

Threonine dehydrogenase activity. Although threonine and glycine did not support growth of *K. aerogenes* strain MK9000 when offered as the sole source of carbon, they allowed slow growth (doubling times 11.0 and 15.5 h, respectively) when used as nitrogen sources in combination with glucose. Leucine with glucose did not support growth. However, in glucose-threonine medium growth was faster (doubling time, 4.22 h) if 0.1% L-leucine was added. The result suggests that, as in *E. coli* (29), leucine induces enzymes for threonine utilization and threonine dehydrogenase activity occurs in *K. aerogenes* (Table 3). Cells grown in histidine-ammonia medium and in histidine-ammonia medium supplemented with 0.1% threonine contained similar amounts of threonine dehydrogenase, but those grown in HN medium with 0.1% leucine and threonine were more active. Cells from the threonine- and threonine-plus-leucine-supplemented media were progressively induced for serine dehydrase.

Catabolite repression of serine dehydrase. Regardless of the nitrogen source used and whether or not inducers were present, the use of media containing glucose resulted in cells with a serine dehydrase activity lower than that of cells grown on media in which amino acids alone or citrate were the source of carbon. Reduced serine dehydrase activity was accompanied by the expected decrease in isopropyl thio-galactoside-induced β -galactosidase activity (Table 4). Adding cyclic AMP to media containing glucose partially restored both activities.

When cells grown in GN media, in which glutamine synthetase should be strongly repressed, were compared with cells grown in glucose-glutamine or glucose-histidine media, in

TABLE 4. Effect of glucose and cyclic AMP on serine dehydrase (SD) and β -galactosidase (β -Gal) activity in strains MK9000 and LVS1

Strain and medium ^a	Doubling time (h)	Enzyme activity ^b	
		SD	β -Gal
MK9000			
HN-thr	1.68	22.0	245
GHN-thr	0.91	3.62	16.9
GHN-thr + cAMP	0.79	6.40	47.0
LSV1			
HN	1.84	59.6	241
GHN	0.81	36.2	14.0
GHN + cAMP	0.82	41.3	47.5

^a See Table 1, footnote a. cAMP, cyclic AMP.

^b Serine dehydrase activity expressed as micromoles of keto acid formed per milligram of protein per hour; β -galactosidase activity is expressed as micromoles of *o*-nitrophenylgalactoside hydrolyzed per milligram of protein per hour.

which glutamine synthetase activity should be derepressed (25), serine dehydrase activities were similar (2.0 to 2.4 μmol of pyruvic acid per mg of protein per h). With inducers present, the values were higher (3.6 to 6.9 μmol of pyruvic acid per mg of protein per h) but not significantly different from one another. The absence of marked repression by ammonia indicates that glutamine synthetase is unlikely to be a controlling element in L-serine utilization. This was confirmed by comparing serine dehydrase activity in the wild-type strain MK9000 with that of mutant strains defective in glutamine metabolism. No appreciable differences were found, either for strains unable to make glutamine synthetase because of a structural gene defect (strains MK104 and MK924) or for those altered in enzyme level or activity (12, 22) as a result of regulatory mutations (strains MK9512, MK9770, CG191, CG235, and CG242).

Strain CG177 which carries the *ilvA* marker, showed elevated serine dehydrase after growth in GN medium. This is attributed to the isoleucine supplement which caused a similar increase (from 2.12 to 10.4 μmol of pyruvate per mg of protein per h) in strain MK9000, possibly by increasing the intracellular threonine pool. A strain (FF1011) unable to make glutamate synthase showed similar serine dehydrase activity to the wild type.

Serine dehydrase-constitutive strain. Selection of a variant showing enhanced serine dehydrase activity was observed during growth with serine as the sole source of carbon and nitrogen. By plating on S agar and selecting colonies visible after 24 h, the variant was found in strain MK9000 at a frequency of 5:10⁶. A similar selection occurred in media in which

TABLE 3. Serine dehydrase (SD) and threonine dehydrogenase (TDH) activity in strains MK9000 and LVS1

Strain and medium ^a	Doubling time (h)	Enzyme ^b	
		SD	TDH
MK9000			
HN	1.73	4.60	0.92
HN thr	1.85	22.0	0.91
HN thr leu	1.68	54.8	2.54
LVS1			
HN	1.72	53.5	0.93

^a See Table 1, footnote a.

^b Serine dehydrase activity expressed as micromoles of keto acid formed per milligram of protein per hour; threonine dehydrogenase activity expressed as micromoles of NADH per milligram of protein per hour.

serine was the sole source of nitrogen but a supplementary carbon source was present and isoleucine or α -ketobutyric acid was added.

Variant strains contained similar amounts of serine dehydrase as the parent when grown under highly inducing conditions in LB or Casamino Acids medium. In noninducing media, activity in the variants was much higher and was insensitive to supplements of threonine, glycine, or leucine (Table 5). Mutation to serine dehydrase constitutivity (SutC phenotype) was accompanied by reduced sensitivity of the enzyme to catabolite repression by glucose (Table 4). However, the inducibility of β -galactosidase and its cyclic AMP-dependent catabolite repression by glucose were not affected. As with the parent, serine dehydrase levels were not altered by adding ammonia to the medium and glutamine synthetase levels were comparable under varying degrees of nitrogen repression.

Serine dehydrase activity of the SutC mutant grown in GN medium responded to pH changes in the assay mixture in the same manner as that of strain CG204, suggesting that the nonspecific threonine dehydrase activity was swamped by elevated serine dehydrase levels. When grown in histidine-ammonia medium, cells of the SutC and parent strains contained similar amounts of threonine dehydrogenase (Table 3). Like the parent, the variant could not grow on threonine as the sole source of carbon and nitrogen. It grew only marginally faster than the parent in glucose-threonine (doubling times, 9.8 versus 11.0 h) and glucose-glycine medium (doubling times, 15.5 versus 21.0 h).

Mutants with altered serine utilization. Of the mutagenized MK9000 isolates that did

not grow normally on GS agar, none was completely blocked in serine utilization, but several grew very slowly. One such strain, LV025, showed consistently low serine dehydrase activity; the enzyme was not induced by adding threonine to nonrepressive media (Table 5).

Examination of a mutant, LVG1, that formed exceptionally large colonies on GS agar showed normal serine dehydrase but high threonine dehydrase activity in cells grown in GN or GS medium (Table 5). The increased growth rate over that of the parent in GS medium implicated threonine dehydrase in serine utilization *in vivo*. The mutant grew much faster than the parent (initial doubling times, 7.4 versus 17.4 h, respectively) in GS medium containing 0.005% isoleucine. Since assays showed that the enzyme was not relieved of feedback inhibition by isoleucine, the increased growth rate is ascribed to altered control of enzyme synthesis. Additional evidence that threonine dehydrase participates in serine utilization *in vivo* was afforded by the very slow initial growth observed when the *ilvA* strain, CG204, was grown in GS medium supplemented with 0.001% isoleucine or 0.001% α -ketobutyrate. When threonine was added, the resulting induced level of serine dehydrase supported faster growth (doubling time, 2.67 h) and this increased further (doubling time, 1.80 h) when citrate was used in place of glucose as the carbon source.

DISCUSSION

K. aerogenes cells contain a serine-specific dehydrase similar in its stability characteristics and pH optimum to the L-serine deaminase obtained by Alföldi and co-workers (1) from *E. coli* strain K-12 grown in a tryptone medium. The enzyme also has properties in common with the L-serine dehydrases extracted by Wong and Leslie from *P. cepacia* (43) and by Bell and Turner from various bacteria grown on L-threonine (3).

We have not attempted purification and detailed study of the *K. aerogenes* enzyme which, like its *E. coli* counterpart, loses activity rapidly during isolation unless protected by substrate or by cysteine acting, presumably, as a competitive enzyme inhibitor (1). However, we have demonstrated that a specific L-serine dehydrase is present both in the wild-type strain MK9000 and in an *ilvA* mutant lacking threonine dehydrase. As in other bacteria (1, 2, 7, 37), serine dehydrase does not act upon threonine or cysteine. The 20% activity shown by threonine dehydrase with L-serine as substrate is within the range reported for this enzyme in other bacteria (24, 41). Absence of hydroxypyruvate or hydroxybutyrate among the products of the enzyme reaction rules out the participation of amino acid

TABLE 5. Serine dehydrase and threonine dehydrase activities in mutant strains of *K. aerogenes*

Strain ^a	Medium ^b	Doubling time (h)	Enzyme activity ^c	
			SD	TD
LVS1	H	1.78	57.9	11.4
	H thr	2.00	52.7	9.60
	H gly	1.65	58.2	12.7
	H leu	2.17	61.0	20.2
LVO25	GS	3.35	0.65	22.8
	H	1.56	1.57	20.1
	H thr	1.78	2.73	11.5
LVG1	GN	0.86	3.92	31.3
	GS	1.25	5.83	45.8

^a Phenotypes: LVS1 grew on serine as sole carbon and nitrogen source; LVO25 grew slowly on GS agar; LVG1 grew rapidly on GS agar.

^b See Table 1, footnote a.

^c Expressed as micromoles of keto acid per milligram of protein per hour.

oxidases or transferases, and no evidence was found for a serine dehydrogenase comparable to the enzyme that initiates L-threonine catabolism in a number of organisms (3, 8, 19, 27, 29, 42). Thus, the measurements of serine dehydrase and threonine dehydrase activity made during this study reasonably represent the L-serine catabolizing capabilities of *K. aerogenes*.

As in *E. coli* K-12 (21), serine dehydrase is not induced by its substrate but is induced by other amino acids. It is induced by threonine or leucine, and its activity is decreased if glucose is present; in these respects, it resembles the biodegradative threonine deaminase of *E. coli* (44). However there are differences in base levels and inducibility (13, 45). The inducing action of threonine, glycine, and leucine in *K. aerogenes* also links serine dehydrase to a pathway of aerobic threonine degradation (Fig. 3). Evidence for participation of serine dehydrase in threonine metabolism has been reported for a number of microorganisms (3, 27, 42, 43) in which growth on threonine increased the specific activity of the enzyme from 0 or 1 to 15 or 60 μmol of pyruvate formed per mg of protein per h. Taking account of differences in assay procedure, the value agrees well with those reported here. Moreover, *K. aerogenes* can use glycine and threonine as sources of nitrogen, and threonine dehydrogenase, the initial enzyme in the pathway, is present.

The value of induction by glycine as well as by threonine is evident in bacteria in which glycine is converted to serine and subsequently metabolized via pyruvate (11, 23, 28). However, the rationale for induction by leucine is uncertain. The amino acid has been postulated to act as a metabolic signal for the utilization of amino acids (15, 29), bringing about enzyme modification through an amino acid transfer reaction (38). In *E. coli*, serine dehydrase is reported to be influenced by the nitrogen status of cells (21). We have found no evidence that the metabolism of serine in *K. aerogenes* is controlled in this way. Unlike the enzymes for catabolizing a number of amino acids and other nitrogenous compounds, serine dehydrase does not vary with the

level of glutamine synthetase in the cells. The decreased level of serine dehydrase induced when glucose is present and the progressive recovery when glucose is replaced with citrate and histidine suggest control over the utilization of serine as a carbon source.

Selection of a spontaneous mutant constitutive for serine dehydrase occurs whenever the ability to metabolize serine is growth-limiting. Mutation to the SutC phenotype is a selective event and does not alter the level of threonine dehydrogenase or its inducibility by leucine. The insensitivity of serine dehydrase in the constitutive strain to further induction by glycine, leucine, or threonine suggests that these amino acids act by a common mechanism and not, as proposed for leucine induction in *E. coli* (29), through separate control systems.

Rapid selection in *ilvA* strains or in the wild type grown on serine as the source of nitrogen in isoleucine-supplemented inducer-deficient media can be attributed to the inability of uninduced serine dehydrase to supply sufficient ammonia. This implicates threonine dehydrase in the metabolism of serine. The evidence from *in vitro* assays that both enzymes contribute to the serine-deaminating activity in permeabilized cells thus appears to reflect the situation *in vivo*. Further evidence that both serine dehydrase and the serine-deaminating activity of threonine dehydrase sustain the normal growth rate of *K. aerogenes* on serine nitrogen includes the following: (i) cultures in which serine dehydrase activity cannot be induced or in which threonine dehydrase activity is suppressed grew more slowly, (ii) suppression of threonine dehydrase could be compensated for by inducing higher serine dehydrase activity, and (iii) a mutant strain with above-normal threonine dehydrase activity grew faster than its parent. The difficulty encountered in isolating a mutant lacking serine dehydrase may well be due to the participation of two enzymes in serine utilization. Newman and co-workers (28) were unsuccessful in attempts to find serine dehydrase-negative strains of *E. coli*. Although threonine dehydrase has not previously been shown to mediate serine

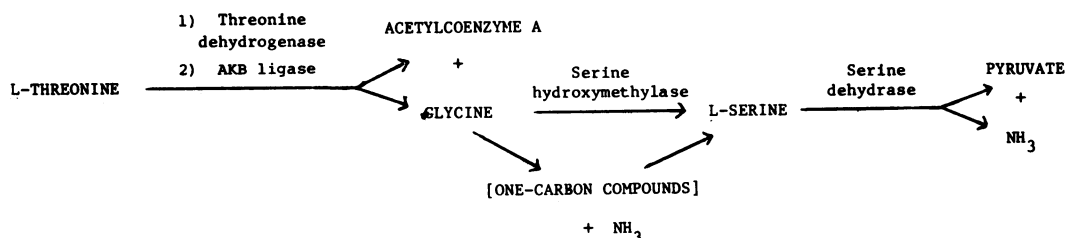


FIG. 3. Pathway for the catabolism of L-threonine.

utilization and, in *P. cepacia*, is incapable of supporting growth of serine-dehydrase-negative mutants (43), it has recently been shown to be responsible for the catabolism of threonine in *Corynebacterium* species (4).

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