Evidence for the Existence of Two Homoserine Dehydrogenases in *Serratia marcescens*

By S. KOMATSUBARA,* M. KISUMI AND I. CHIBATA

Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co. Ltd., Kashima,
Yodogawa-ku, Osaka 532, Japan

(Received 26 May 1983)

Strain D-315 was isolated from a wild-type strain of *Serratia marcescens* as a methionine-sensitive mutant. In this mutant, methionine-mediated growth inhibition was reversed by threonine or homoserine. The homoserine dehydrogenase activity of strain D-315 was about 20% lower than that of the wild-type and was not inhibited by threonine. The methionine-sensitive mutation was located in the *thr* region on the chromosome, indicating that strain D-315 lacked homoserine dehydrogenase I, whose activity is inhibited by threonine and whose synthesis is multivalently repressed by threonine plus isoleucine. In a methionine bradytroph derived from strain D-315, homoserine dehydrogenase activity was high during growth in the absence of methionine and low during growth in the presence of excess methionine. Strain D-413, a homoserine auxotroph derived from strain D-315, had no detectable homoserine dehydrogenase activity, indicating that *S. marcescens* had homoserine dehydrogenase II, whose synthesis is controlled by methionine-mediated repression. The gene coding for homoserine dehydrogenase II was located in the *metB-argE* region. Strain TA-191, having homoserine dehydrogenase I and lacking the other isoenzyme, was constructed by transduction. This strain was sensitive to threonine-mediated growth inhibition.

INTRODUCTION

In micro-organisms, homoserine dehydrogenase (EC 1.1.1.3) catalyses the formation of homoserine from aspartate- β -semialdehyde and is one of the rate-limiting enzymes for the biosynthesis of threonine, isoleucine and methionine (Fig. 1) (Umbarger, 1978). *Escherichia coli* K12 and *Salmonella typhimurium* have two homoserine dehydrogenase isoenzymes (Cohen, 1969). The synthesis of homoserine dehydrogenase I is multivalently repressed by threonine plus isoleucine and its activity is subject to feedback inhibition by threonine. The synthesis of homoserine dehydrogenase II is repressed by methionine but it is not subject to feedback inhibition by any related end-product. Therefore, these enzymes are often called threonine-sensitive homoserine dehydrogenase and methionine-repressible homoserine dehydrogenase, respectively.

We have been studying threonine production by various regulatory mutants of *Serratia marcescens* Sr41 and have shown alterations in feedback controls of threonine biosynthesis in these mutants (Komatsubara *et al.*, 1978*a, b*, 1979*a, b*). However, it has not been clear whether *S. marcescens* might have two homoserine dehydrogenase isoenzymes. In this paper, we give genetic evidence for the existence of the two isoenzymes in *S. marcescens*.

METHODS

Bacterial strains. Derivatives of *S. marcescens* Sr41 (Matsumoto *et al.*, 1975) were used (Table 1). The genotype designations used are generally based on those for *E. coli* K12 (Bachmann & Low, 1980; Thze *et al.*, 1974; Thze & Saint-Girons, 1974).

Fig. 1. Pathways for biosynthesis of amino acids of the aspartate family in bacteria. Enzymes: (1) aspartokinase, (2) homoserine dehydrogenase, (3) homoserine kinase, (4) threonine synthase, (5) γ -cystathionine synthase, (6) γ -cystathionase, (7) threonine deaminase.

Table 1. *S. marcescens* Sr41 strains used

Strain*	Relevant genotype†	Origin	Reference
Mu-910	Wild-type	Mutagenesis of 8000	Komatsubara <i>et al.</i> (1978 <i>b</i>)
D-43	<i>thrB/C21</i>	Mutagenesis of Mu-910	This paper
D-44	<i>thrB/C22</i>	Mutagenesis of Mu-910	This paper
D-60	<i>ilvA1</i>	Mutagenesis of Mu-910	Komatsubara <i>et al.</i> (1978 <i>b</i>)
D-315	<i>thrA₂3</i>	Mutagenesis of Mu-910	This paper
D-403	<i>thrA₂3 metB11</i>	Mutagenesis of D-315	This paper
D-408	<i>thrA₂3 metC12</i>	Mutagenesis of D-315	This paper
D-413	<i>thrA₂3 metM13</i>	Mutagenesis of D-315	This paper
D-443	<i>thrA₂3 argE11</i>	Mutagenesis of D-315	This paper
TA-191	<i>metM13</i>	Mu-910 phage $\times$ D-413	This paper

* Strain Mu-910 was derived from strain 8000 (wild-type) as a threonine dehydrogenase-deficient strain. Therefore, all strains listed here were deficient in threonine dehydrogenase.

† In *E. coli* K12, the *thrA* gene specifies a single polypeptide chain (Cohen, 1969) but is composed of two cistrons, *thrA₁* and *thrA₂*, which correspond to aspartokinase I and homoserine dehydrogenase I, respectively (Thze & Saint-Girons, 1974). Also the *metL* gene codes for a bifunctional enzyme, aspartokinase II-homoserine dehydrogenase II (Dautry-Varsat *et al.*, 1977; Krueger *et al.*, 1981). But here we designate a cistron carrying homoserine dehydrogenase II activity as *metM*, according to the classical usage (Thze *et al.*, 1974; Bachmann & Low, 1980).

Genotype designations: *thrA₂3*, lack of homoserine dehydrogenase I (methionine sensitivity); *thrB/C21*, lack of homoserine kinase or threonine synthase (threonine auxotrophy); *thrB/C22*, lesion of homoserine kinase or threonine synthase (threonine bradytroph); *metB11*, lack of γ -cystathionine synthase (methionine auxotrophy); *metC12*, lesion of γ -cystathionase (methionine bradytroph); *metM13*, lack of homoserine dehydrogenase II (homoserine auxotrophy in a *thrA₂3* strain or threonine sensitivity in a *thrA₂⁺* strain); *argE11*, lack of acetylornithine deacetylase (arginine auxotrophy); *ilvA1*, lack of threonine deaminase (isoleucine auxotrophy).

Media. The medium of Davis & Mingioli (1950) was modified by omitting citrate and increasing glucose to a final concentration of 0.5% (w/v). The solution of glucose was autoclaved separately and mixed with the other components.

Isolation of mutants. Cells of parent strains were mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine by the method of Adelberg *et al.* (1965), modified as described previously (Kisumi *et al.*, 1977). Enrichment of auxotrophs or bradytrophs was achieved by nalidixic acid treatment by the procedure of Weiner *et al.* (1974). Methionine-sensitive mutants were enriched in the presence of 10 mM-L-methionine. The survivors were grown on nutrient agar plates overnight at 30 °C. Subsequently, colonies were replica-plated on to minimal plates with or without the addition of necessary supplements. Colonies showing the expected phenotype were purified by single colony isolation, and characterized for genetic lesions.

Transductional analysis. Phage PS20-mediated transduction was carried out by the method of Matsumoto *et al.* (1973), modified as described previously (Komatsubara *et al.*, 1979*b*). In the cross strain D-315 $\times$ strain D-43,

Thr⁺ transductants were selected on minimal plates and tested for methionine-sensitive phenotype on minimal plates containing 1 mM-L-methionine. In the crosses strain D-413 × strain D-443 and strain D-413 × strain D-403, Arg⁺ and Met⁺ transductants were selected on minimal plates containing 0.5 mM-L-homoserine. In the cross strain D-443 × strain D-403, Met⁺ transductants were selected on minimal plates containing 1 mM-L-arginine. For construction of strain TA-191 from strains Mu-910 and D-413, Thr⁺ transductants were selected on minimal plates containing 1 mM-L-methionine. Plates for transductional crosses were incubated at 30 °C for 2 or 3 d. Plates for testing phenotypes were incubated at the same temperature overnight.

Growth study. Cell growth at 30 °C was estimated using a Hitachi automated recording incubator system (Morimoto *et al.*, 1980) as described previously (Komatsubara *et al.*, 1979a).

Enzyme assay. To a 500 ml Sakaguchi shaking flask was added 150 ml of a minimal medium modified by decreasing the glucose concentration to 0.02% (w/v). When required, amino acids were added to the medium at 0.05 mM. The medium was inoculated with a loopful of cells grown on a nutrient agar slant overnight and incubated at 30 °C on a reciprocal shaker (140 r.p.m., 7 cm stroke). After 16 h, growth ceased at an OD₆₆₀ of 0.05 to 0.08 (measured in a Hitachi electric photometer, EPO-B type). More glucose and amino acids were then added to give final concentrations of 0.5% (w/v) and 1 mM, respectively. Incubation was continued until the OD₆₆₀ reached about 0.5 (late exponential phase). Cells were then centrifuged, washed twice with 50 mM-potassium phosphate buffer (pH 8.0) and suspended in the same buffer containing 30% (w/v) glycerol for enzyme stabilization. Cells were disrupted at below 10 °C for 4 min with a sonic oscillator (Kubota Insonator, model 200M, 9 KHz, 200 W). The sonically treated suspension was centrifuged at 30 000 *g* for 40 min at below 5 °C. The supernatant was used for enzyme assays.

The activities of homoserine dehydrogenase and aspartokinase were determined at 30 °C as described previously (Kisumi *et al.*, 1977). Protein was measured by the Lowry method. Specific activities are expressed as μmol product formed (mg protein)⁻¹ min⁻¹.

Chemicals. L-Threonine hydroxamate was prepared from L-threonine methylester and hydroxylamine by the synthetic method for serine hydroxamate (Tosa & Pizer, 1971). L-Isoleucine hydroxamate was obtained from Sigma. L-Homoserine was purchased from Kyowa Hakko Kogyo. The other amino acids, including D-threonine, were products of our company. The other reagents were of reagent grade commercially available.

RESULTS

Control of homoserine dehydrogenase I synthesis. We have previously shown that *S. marcescens* has homoserine dehydrogenase activity sensitive to threonine-mediated inhibition (Komatsubara *et al.*, 1978a). We have now examined the possibility of multivalent repression by threonine and isoleucine in the control of synthesis of this enzyme.

Strain D-44, a threonine bradytroph, had a higher homoserine dehydrogenase activity than that observed for the wild-type strain when grown in a medium containing excess isoleucine but no threonine (Table 2). Similarly, D-60, an isoleucine auxotroph, had an increased activity when

Table 2. Derepression of homoserine dehydrogenase I by limiting threonine or isoleucine

Strain	Medium*	Homoserine dehydrogenase	
		Specific activity†	Inhibition‡ (%)
D-44 (<i>thrB</i> /C22)	No threonine	0.042	76
	Excess	0.010	61
D-60 (<i>ilvA</i> 1)	Isoleucine-limiting	0.046	78
	Excess	0.008	63
Mu-910 (wild-type)	Minimal	0.008	58
	Containing isoleucine hydroxamate	0.051	80
	Containing threonine hydroxamate	0.058	78

* The media contained the following additions to minimal medium: no threonine medium, 1 mM-L-isoleucine and no L-threonine; excess medium, 1 mM-L-threonine and 1 mM-L-isoleucine; isoleucine-limiting medium, 1 mM-L-threonine and 0.5 mM-D-threonine (a limiting source of isoleucine; Kisumi *et al.*, 1971); media containing isoleucine hydroxamate or threonine hydroxamate, 2 mM-L-isoleucine hydroxamate and 1 mM-L-threonine, or 10 mM-L-threonine hydroxamate and 1 mM-L-isoleucine, respectively.

† Expressed as μmol product (mg protein)⁻¹ min⁻¹.

‡ By 10 mM-L-threonine.

Table 3. *Effect of methionine and related amino acids on the growth rates of strains Mu-910, D-315 and D413*

Addition to minimal medium (mM)	Specific growth rate* (h ⁻¹)		
	Mu-910	D-315	D-413
None	0.66	0.39	<0.02
L-Homoserine (0.5)	0.51	0.60	0.53
L-Threonine (1)	0.65	0.63	<0.02
L-Isoleucine (1)	0.66	0.58	NT
L-Methionine (0.01)	NT	0.20–0.39†	NT
(0.1)	NT	0.19	NT
(1)	0.63	0.18	<0.02
L-Methionine (1) + L-homoserine (0.5)	NT	0.69	NT
L-Methionine (1) + L-threonine (1)	NT	0.63	0.69
L-Methionine (1) + L-isoleucine (1)	NT	0.33	NT
L-Cysteine (0.1)	0.66	0.19–0.28†	NT
(1)	0.33–0.50†	<0.02	NT
L-Cysteine (1) + L-homoserine (0.5)	0.28–0.50†	0.36–0.50†	NT
L-Cysteine (1) + L-threonine (1)	0.30–0.53†	0.35–0.53†	NT

NT, Not tested.

* Defined as $\ln 2 \times (\text{mass doubling time, h})^{-1}$.

† The strain grew slowly during the early growth phase and faster thereafter.

cultured in a medium containing excess threonine and a limiting source of isoleucine. The addition of an excess of both threonine and isoleucine lowered the homoserine dehydrogenase activity in the above two strains. In strain Mu-910, the wild-type, the activity was increased by the addition of either threonine hydroxamate (a threonine analogue) or isoleucine hydroxamate (an isoleucine analogue, Kisumi *et al.*, 1971). When an increased activity was obtained, one could consistently observe a higher percentage of inhibition by threonine. On the basis of these results, we concluded that in *S. marcescens*, threonine-sensitive homoserine dehydrogenase was multivalently repressed by threonine and isoleucine. Accordingly, we adopted this enzyme homoserine dehydrogenase I (see Introduction).

Isolation of a methionine-sensitive mutant. In order to show the existence of an isoenzyme distinct from homoserine dehydrogenase I, we intended to isolate a mutant lacking homoserine dehydrogenase I. If *S. marcescens* had no separate isoenzyme, such a mutant would be a homoserine auxotroph. If it had homoserine dehydrogenase II, controlled by methionine-mediated repression as in *E. coli* K12 (Cohen, 1969), the growth of such a mutant would be inhibited by the presence of methionine in the growth medium. Considering these two possibilities, we isolated mutants that did not grow on methionine but did grow on methionine plus threonine. Most of 96 mutants tested grew on threonine but not on homoserine. Three mutants grew slowly in unsupplemented minimal medium and at the normal rate in minimal medium plus homoserine, threonine or isoleucine, as shown for strain D-315 in Table 3. Addition of methionine decreased the growth rate of strain D-315 to half that observed for growth in unsupplemented medium; such inhibition was completely antagonized by homoserine or threonine and partially by isoleucine. Cysteine at 1 mM inhibited the growth of strain D-315 more strongly than methionine.

Homoserine dehydrogenase in a methionine-sensitive mutant. Strain D-315 had a decreased activity of homoserine dehydrogenase (about 20% of that observed for the wild-type) when grown in minimal medium (Table 4). The aspartokinase activity in strain D-315 was higher than that in the wild-type when both strains were grown in minimal medium. Addition of threonine to the medium lowered the aspartokinase activity in strain D-315. These results indicated that this strain lacked homoserine dehydrogenase I but retained aspartokinase activity that was sensitive to threonine-mediated inhibition.

Mapping of the methionine-sensitive mutation. Transductional analysis was performed to confirm that strain D-315 might have a mutation in the *thrA*₂ gene, specifying homoserine

Table 4. Homoserine dehydrogenase and aspartokinase activities in strain D-315

Strain	Addition to minimal medium (mM)	Homoserine dehydrogenase		Aspartokinase	
		Specific activity*	Inhibition† (%)	Specific activity*	Inhibition‡ (%)
Mu-910 (wild-type)	None	0.0078	56	0.06	10
	L-Threonine (1)	0.0065	65	0.06	8
	L-Threonine (1) + L-methionine (1)	0.0068	59	NT	NT
D-315 (<i>thrA₂₃</i>)	None	0.0015	3	0.11	47
	L-Threonine (1)	0.0016	5	0.05	7
	L-Threonine (1) + L-methionine (1)	0.0010	5	NT	NT

NT, Not tested.

* Expressed as $\mu\text{mol product (mg protein)}^{-1} \text{ min}^{-1}$.

† By 10 mM-L-threonine.

‡ By 50 mM-L-threonine.

Table 5. Homoserine dehydrogenase activity in derivatives of strain D-315

Strain	Addition to minimal medium (mM)	Homoserine dehydrogenase	
		Specific activity*	Inhibition† (%)
D-408 (<i>metC12</i>)	L-Threonine (1)	0.0053	3
	L-Threonine (1) + L-methionine (1)	0.0010	-2
D-413 (<i>metM13</i>)	L-Homoserine (0.5)	0.0002	NT
	L-Threonine (1) + L-methionine (1)	0.0002	NT

NT, Not tested.

* Expressed as $\mu\text{mol product (mg protein)}^{-1} \text{ min}^{-1}$.

† By 10 mM-L-threonine.

dehydrogenase I. (For designation of *thrA₂*, see footnote to Table 1.) In the cross between strains D-315 (donor) and D-43 (*thrB/C21*, recipient), Thr⁺ transductants were obtained. Of 128 Thr⁺ strains, 122 were sensitive to methionine-mediated growth inhibition. This indicated that the mutation leading to loss of homoserine dehydrogenase I activity was located in the *thr* region on the chromosome. Therefore, we denoted this mutation as *thrA₂₃*.

Evidence for the existence of homoserine dehydrogenase II. The methionine-mediated growth inhibition indicated that *S. marcescens* might have homoserine dehydrogenase II. However, we could not clarify whether the homoserine dehydrogenase remaining in strain D-315 was subject to repression by methionine (Table 4). Therefore, strain D-408 was derived from strain D-315, as a methionine bradytroph in which the intracellular methionine pool would be limited. This bradytroph had a high homoserine dehydrogenase activity when cultured in minimal medium without methionine and had a lower activity when cultured in minimal medium plus methionine (Table 5). These results indicated that the homoserine dehydrogenase activity remaining in strain D-315 was sensitive to methionine-mediated repression, that is, *S. marcescens* possesses homoserine dehydrogenase II.

Effect of amino acids on the homoserine dehydrogenase II activity. The effect of methionine and related amino acids on the homoserine dehydrogenase II activity was studied with crude extracts of strain D-408 (Table 6; for comparison, data on homoserine dehydrogenase I are given). Methionine, threonine or isoleucine had no effect on the homoserine dehydrogenase II activity at a high concentration. Cysteine inhibited both the homoserine dehydrogenase activities.

Isolation of a mutant lacking both homoserine dehydrogenases. To confirm that homoserine dehydrogenase II might participate in the synthesis of homoserine, we isolated methionine

Table 6. *Effect of amino acids on the activities of the two homoserine dehydrogenases*

Addition to reaction mixture (mM)	Relative activity (%)	
	Homoserine dehydrogenase II*	Homoserine dehydrogenase I†
None	100 (0.0055)‡	100 (0.031)‡
L-Threonine (1)	NT	37
(10)	NT	25
(30)	98	19
L-Methionine (30)	96	96
L-Isoleucine (30)	98	98
L-Cysteine (1)	85	70
(10)	40	24
(30)	32	8

NT, Not tested.

* Crude extracts were prepared from cells of strain D-408, grown in a minimal medium and derepressed for homoserine dehydrogenase II.

† Crude extracts were prepared from cells of strain TA-191, grown in a minimal medium and derepressed for homoserine dehydrogenase I.

‡ Numbers in parentheses represent specific activities [$\mu\text{mol product (mg protein)}^{-1} \text{ min}^{-1}$].

Table 7. *Transductional analysis of the metM13 mutation*

Cross*	Selected marker†	Unselected marker‡	Co-transduction frequency (%)
D-413 (<i>metM13</i>) phage × D-403 (<i>metB11</i>)	Met ⁺ (146)	Hse ⁻ (141)	<i>metB11-metM13</i> (97)
D-413 (<i>metM13</i>) phage × D-443 (<i>argE11</i>)	Arg ⁺ (192)	Hse ⁻ (143)	<i>metM13-argE11</i> (74)
D-443 (<i>argE11</i>) phage × D-403 (<i>metB11</i>)	Met ⁺ (160)	Arg ⁻ (117)	<i>metB11-argE11</i> (73)

* All strains carried the *thrA*₃ mutation.

† Values in parentheses are the numbers of colonies tested.

‡ Values in parentheses are the numbers of colonies found.

auxotrophs from strain D-315 under conditions where the minimal plates contained threonine. Of 23 auxotrophs, two strains, including strain D-413, grew on threonine plus methionine or on homoserine (Table 3). Strain D-413 had no detectable homoserine dehydrogenase activity (Table 5). Accordingly, this strain was considered to lack both homoserine dehydrogenases I and II. The mutation for homoserine dehydrogenase II carried by strain D-413 was designated *metM13*.

Mapping of the gene coding for homoserine dehydrogenase II. In *E. coli* K12, the *metM* coding for homoserine dehydrogenase II has been mapped between *metB* and *argE* (Thèze *et al.*, 1974). (For designation of *metM*, see footnote to Table 1.) Therefore, we examined whether the *metM13* mutation might be linked to *metB* and *argE* (Table 7). Strains D-403 and D-443, carrying the *metB11* and *argE11* mutations, respectively, were isolated from strain D-315. In the cross between strains D-413 (donor) and D-403 (recipient), Met⁺ transductants were selected on minimal plates containing homoserine. Tests of these Met⁺ strains for homoserine auxotrophy revealed that the *metM13* mutation was 97% cotransducible with *metB*⁺. In the cross between strains D-443 and D-403, *metB*⁺ was 73% cotransducible with *argE11*. These results demonstrated close linkage between the three genes *metB*, *metM* and *argE*.

Construction of a strain having homoserine dehydrogenase I and lacking homoserine dehydrogenase II. To determine the physiological significance of homoserine dehydrogenase II, a strain having homoserine dehydrogenase I and lacking homoserine dehydrogenase II was constructed by transduction. In the cross between strains Mu-910 (*thrA*₂⁺ *metM*⁺, donor) and D-413 (*thrA*₂⁺ *metM13*, recipient), Thr⁺ transductants were selected on minimal plates containing methionine to prevent the appearance of the *metM*⁺ colonies. Twenty were tested for growth; all, including

Table 8. *Effect of threonine and related amino acids on the growth rate of strain TA-191*

Addition to minimal medium (mM)	Specific growth rate* (h ⁻¹)
None	0.63
L-Threonine (0.1)	0.33–0.46†
(1)	0.22
(10)	0.12
L-Threonine (10) + L-homoserine (0.5)	0.65
L-Threonine (10) + L-methionine (1)	0.69
L-Threonine (10) + L-isoleucine (1)	0.11
L-Homoserine (0.5)	0.53
L-Methionine (1)	0.63
L-Isoleucine (1)	0.66

* Defined as $\ln 2 \times (\text{mass doubling time, h})^{-1}$.

† The strain grew slowly during the early growth phase and faster thereafter.

strain TA-191, were sensitive to threonine-mediated growth inhibition (Table 8). The growth inhibition was reversed by methionine or homoserine.

The homoserine dehydrogenase activity of strain TA-191 was shown to be inhibited by threonine (Table 6). To confirm the genotype of this strain, a transductional cross was performed between strain TA-191 (donor) and strain D-443 (recipient). About 70% of Arg⁺ transductants selected on minimal plates containing homoserine were found to show homoserine auxotrophy. Therefore, the genotype of strain TA-191 is *thrA*₂⁺ *metM13*; that is, this strain has homoserine dehydrogenase I and lacks homoserine dehydrogenase II.

DISCUSSION

In bacteria, the common pathway for biosynthesis of amino acids of the aspartate family has two branched points; aspartate- β -semialdehyde and homoserine. Homoserine leads to the three end-products threonine, isoleucine and methionine. The regulation of homoserine synthesis depends mainly on feedback controls of homoserine dehydrogenase and aspartokinase activities. On the basis of the results obtained for *E. coli* and *Salmonella typhimurium* (Cohen, 1969; Umbarger, 1978), enteric bacteria have been thought to possess two homoserine dehydrogenase isoenzymes (Cohen *et al.*, 1969). However, there have been few reports on the existence of the two isoenzymes in enteric bacteria other than the above two species. In this paper, we have demonstrated that *Serratia marcescens* Sr41 has homoserine dehydrogenases I and II and that the genetic organization of these regions of the genome of *S. marcescens* is very similar to that in *E. coli* K12 and *Salmonella typhimurium*.

In *E. coli* K12, the growth of the wild-type was reported to be inhibited by threonine in the presence of isoleucine, due to limitation of methionine synthesis by feedback controls of homoserine dehydrogenase I (Patte *et al.*, 1966). This indicates that, in this bacterium, homoserine dehydrogenase II plays a minor role in homoserine synthesis under ordinary conditions. In *S. marcescens*, threonine did not inhibit the growth of the wild-type in the presence of isoleucine but it markedly inhibited that of a strain lacking homoserine dehydrogenase II. This indicates that homoserine dehydrogenase II of *S. marcescens* is more involved in homoserine synthesis than that of *E. coli* K12.

Salmonella typhimurium also has both isoenzymes (Umbarger, 1978); in the wild-type the homoserine dehydrogenase II activity is 20 to 30% of the total homoserine dehydrogenase activity (Cafferata & Freundlich, 1970). A mutation causing a lack of homoserine dehydrogenase I was reported to lead to an absolute requirement for threonine (Stuttard, 1973), presumably due to preferential utilization of homoserine produced by homoserine dehydrogenase II for methionine biosynthesis (channelling of homoserine; Cafferata & Freundlich, 1970). In *S. marcescens*, homoserine dehydrogenase II activity was inferred to be about 20% of the total activity. However, in contrast with the case of *Salmonella typhimurium*, a mutation causing a lack of homoserine dehydrogenase I led to only a slight threonine requirement in the

absence of methionine and to an increased threonine requirement in the presence of methionine. An *S. marcescens* strain carrying such a mutation did not require methionine for growth in the presence of threonine and its homoserine dehydrogenase II was repressed, indicating that this mutant synthesizes sufficient methionine for growth. Thus, *S. marcescens* differs from *Salmonella typhimurium* in the channelling of homoserine.

In *S. marcescens*, strain D-315, having only homoserine dehydrogenase II, was more sensitive to cysteine-mediated growth inhibition than the wild-type. We consider that this growth inhibition could be partly due to the inhibition of homoserine dehydrogenase activity. However, the growth inhibition obtained by a high concentration of cysteine was only partially reversed by threonine plus methionine, indicating that homoserine dehydrogenase is not the sole site of the cysteine action (Berglin *et al.*, 1982; Harris, 1981; Kari *et al.*, 1971).

As described above, *S. marcescens* has two homoserine dehydrogenases. Recently, through a strategy similar to that employed in this study, we obtained evidence for the existence of three isoenzymes of aspartokinase, another rate-limiting enzyme for biosynthesis of amino acids of the aspartate family (unpublished results). These recent findings, along with the above results, should make it easy to construct *S. marcescens* strains producing extremely large amounts of threonine, isoleucine, methionine and lysine.

We thank F. Murakami and S. Shindo for technical assistance.

REFERENCES

- ADELBERG, E. A., MANDEL, M. & CHEIN CHIN CHEN, G. (1965). Optimal conditions for mutagenesis by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in *Escherichia coli* K-12. *Biochemical and Biophysical Research Communications* **18**, 788–795.
- BACHMANN, B. J. & LOW, K. B. (1980). Linkage map of *Escherichia coli* K-12, edition 6. *Microbiological Reviews* **44**, 1–56.
- BERGLIN, E. H., EDLUND, M.-B. K., NYBERG, G. K. & CARLSSON, J. (1982). Potentiation by L-cysteine of the bactericidal effect of hydrogen peroxide in *Escherichia coli*. *Journal of Bacteriology* **152**, 81–88.
- CAFFERATA, R. J. & FREUNDLICH, M. (1970). Evidence for channelling of homoserine in *Salmonella typhimurium*. *Biochimica et biophysica acta* **222**, 671–674.
- COHEN, G. N. (1969). The aspartokinases and homoserine dehydrogenases of *Escherichia coli*. *Current Topics of Cellular Regulation* **1**, 183–231.
- COHEN, G. N., STANIER, R. Y. & LE BRAS, G. (1969). Regulation of the biosynthesis of amino acids of the aspartate family in coliform bacteria and pseudomonads. *Journal of Bacteriology* **99**, 791–801.
- DAUTRY-VARSAT, A., SIBILLI-WEIL, L. & COHEN, G. N. (1977). Subunit structure of the methionine repressible aspartokinase II-homoserine dehydrogenase II from *Escherichia coli* K-12. *European Journal of Biochemistry* **76**, 1–6.
- DAVIS, B. D. & MINGIOLI, E. S. (1950). Mutants of *Escherichia coli* requiring methionine or vitamin B₁₂. *Journal of Bacteriology* **60**, 17–28.
- HARRIS, C. L. (1981). Cysteine and growth inhibition of *Escherichia coli*: threonine deaminase as the target enzyme. *Journal of Bacteriology* **145**, 1031–1035.
- KARI, C., NAGY, A., KOVACS, P. & HERNADI, F. (1971). Mechanism of the growth inhibitory effect of cysteine on *Escherichia coli*. *Journal of General Microbiology* **68**, 349–356.
- KISUMI, M., KOMATSUBARA, S., SUGIURA, M. & CHIBATA, I. (1971). Isoleucine hydroxamate, an isoleucine antagonist. *Journal of Bacteriology* **107**, 741–745.
- KISUMI, M., KOMATSUBARA, S. & CHIBATA, I. (1977). Enhancement of isoleucine hydroxamate-mediated growth inhibition and improvement of isoleucine-producing strains of *Serratia marcescens*. *Applied and Environmental Microbiology* **34**, 647–653.
- KOMATSUBARA, S., KISUMI, M., MURATA, K. & CHIBATA, I. (1978a). Threonine production by regulatory mutants of *Serratia marcescens*. *Applied and Environmental Microbiology* **35**, 834–840.
- KOMATSUBARA, S., MURATA, K., KISUMI, M. & CHIBATA, I. (1978b). Threonine degradation by *Serratia marcescens*. *Journal of Bacteriology* **135**, 318–323.
- KOMATSUBARA, S., KISUMI, M. & CHIBATA, I. (1979a). Participation of lysine-sensitive aspartokinase in threonine production by *S*-2-aminoethylcysteine-resistant mutants of *Serratia marcescens*. *Applied and Environmental Microbiology* **38**, 777–782.
- KOMATSUBARA, S., KISUMI, M. & CHIBATA, I. (1979b). Transductional construction of a threonine-producing strain of *Serratia marcescens*. *Applied and Environmental Microbiology* **35**, 1045–1051.
- KRUEGER, J. H., JOHNSON, J. R., GREENE, R. C. & DRESSER, M. (1981). Structural studies of lambda transducing bacteriophage carrying bacterial deoxyribonucleic acid from the *metBJLF* region of the *Escherichia coli* chromosome. *Journal of Bacteriology* **147**, 612–621.
- MATSUMOTO, H., TAZAKI, T. & HOSOGAYA, S. (1973). A generalized transducing phage of *Serratia marcescens*. *Japanese Journal of Microbiology* **17**, 473–479.
- MATSUMOTO, H., HOSOGAYA, S., SUZUKI, K. & TAZAKI, T. (1975). Arginine gene cluster of *Serratia marcescens*. *Japanese Journal of Microbiology* **19**, 35–44.
- MORIMOTO, T., ITOH, H. & CHIBATA, I. (1980). An automated recording incubator for tube culture of

- microorganisms. *Enzyme and Microbiological Technology* **2**, 194–200.
- PATTE, J. C., TRUFFA-BACHI, P. & COHEN, G. N. (1966). The threonine-sensitive homoserine dehydrogenase and aspartokinase activities of *Escherichia coli*. I. Evidence that the two activities are carried by a single protein. *Biochimica et biophysica acta* **128**, 426–439.
- STUTTARD, C. (1973). Genetic analysis of *thr* mutations in *Salmonella typhimurium*. *Journal of Bacteriology* **116**, 1–11.
- THÈZE, J. & SAINT-GIRONS, I. (1974). Threonine locus of *Escherichia coli* K-12: genetic structure and evidence for an operon. *Journal of Bacteriology* **118**, 990–998.
- THÈZE, J., MARGARITA, D., COHEN, G. N., BORNE, F. & PATTE, J. C. (1974). Mapping of the structural genes of the three aspartokinases and the two homoserine dehydrogenases of *Escherichia coli* K-12. *Journal of Bacteriology* **117**, 133–143.
- TOSA, T. & PIZER, L. I. (1971). Effect of serine hydroxamate on the growth of *Escherichia coli*. *Journal of Bacteriology* **106**, 966–971.
- UMBARGER, H. E. (1978). Amino acid biosynthesis and its regulation. *Annual Review of Biochemistry* **47**, 533–606.
- WEINER, R. M., VOLL, M. J. & COOK, T. M. (1974). Nalidixic acid for enrichment of auxotrophs in cultures of *Salmonella typhimurium*. *Applied Microbiology* **28**, 579–581.