JOURNAL OF BACTERIOLOGY, Aug. 1978, p. 318-323
0021-9193/78/0135-0318$02.00/0
Copyright © 1978 American Society for Microbiology
Vol. 135, No. 2
Printed in U.S.A.

Threonine Degradation by *Serratia marcescens*

SABURO KOMATSUBARA,* KOUSAKU MURATA, MASAHIKO KISUMI, AND ICHIRO CHIBATA

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

Received for publication 30 December 1977

The wild strain of *Serratia marcescens* rapidly degraded threonine and formed aminoacetone in a medium containing glucose and urea. Extracts of this strain showed high threonine dehydrogenase and "biosynthetic" threonine deaminase activities, but no threonine aldolase activity. Threonine dehydrogenase-deficient strain Mu-910 was selected among mutants unable to grow on threonine as the carbon source. This strain did not form aminoacetone from threonine, but it slowly degraded threonine. Strain D-60, deficient in both threonine dehydrogenase and threonine deaminase, was derived from strain Mu-910 and barely degraded threonine. A glycine-requiring strain derived from the wild strain grew in minimal medium containing threonine as the glycine source, whereas a glycine-requiring strain derived from strain Mu-910 did not grow. This indicates that threonine dehydrogenase participates in glycine formation from threonine (via α-amino-β-ketobutyrate) as well as in threonine degradation to aminoacetone.

The following four enzymes are involved in threonine degradation by microorganisms. Threonine dehydrogenase (EC 1.1.1.103) degrades threonine to α-amino-β-ketobutyrate in *Escherichia coli* (29), an *Arthrobacter* species (21), and others (3, 6, 19). Threonine aldolase (EC 2.1.2.1) cleaves threonine to acetaldehyde and glycine in some microorganisms (10, 17, 24, 33). Threonine deaminase (EC 4.2.1.16) converts threonine to α-ketobutyrate and ammonium ion. In *E. coli* (9, 30) and *Salmonella typhimurium* (20), "catabolic" threonine deaminase degrades threonine under anaerobic culture conditions. "Biosynthetic" threonine deaminase is possibly concerned with threonine degradation as well as with isoleucine biosynthesis (4).

During a study on isoleucine production from threonine, we observed that a wild strain of *Serratia marcescens* rapidly degraded threonine (11). Moreover, we obtained regulatory mutants producing large amounts of isoleucine in a medium containing glucose and urea (13, 15). This finding indicated that *S. marcescens* possesses a high potential activity for threonine synthesis. Therefore, we expected that *S. marcescens* would produce large amounts of threonine if threonine degradation was prevented and threonine biosynthesis was released from feedback controls.

To establish microbial production of threonine, we examined threonine degradation by *S. marcescens*, isolated a mutant deficient in the degrading enzymes, and, furthermore, derived regulatory mutants from it. This paper deals with the features of threonine degradation by *S. marcescens*.

MATERIALS AND METHODS

Bacterial strains. The wild strain and derivatives of *S. marcescens* Sr41 (23) listed in Table 1 were used in our experiments.

Media. Threonine degradation by growing cells was examined with a medium containing 10% glucose, 1% urea, 0.1% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.0002% $FeSO_4 \cdot 7H_2O$, 3% $CaCO_3$, and 1 or 2% L-threonine. Glucose was autoclaved separately and mixed with the other components.

To determine the growth of strains and the enzyme activities of cells and to isolate mutants, the media in Table 2 were used. When threonine was used as the carbon source, the nitrogen source, or both, the medium contained yeast extract at a concentration of 0.01%, where it accelerates growth but minimally serves as the carbon or nitrogen source.

Isolation of mutants. Cells were mutated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine by a modification of the method of Adelberg et al. (2). For the selection of threonine dehydrogenase-deficient strains, mutated cells were spread on Thr-C agar plates. After 5 days of incubation at 30°C, small colonies found were picked and placed on nutrient agar slants and tested for aminoacetone formation from L-threonine with cell suspensions. Mutants lacking this activity were purified by single-colony isolation. For the selection of amino acid auxotrophs, mutated cells were further treated with nalidixic acid to enrich auxotrophs by the method of Weiner et al. (32).

Threonine degradation by growing cells. The medium given above was used for threonine degradation by growing cells. A loopful of cells grown on nutrient agar slants overnight was inoculated into 15

TABLE 1. *Strains of S. marcescens Sr41 used*

Strain	Parent	Auxotrophy	Threonine dehydrogenase	Threonine deaminase
8000 (wild)		None	+	+
Mu-910	8000	None	−	+
D-60	Mu-910	Isoleucine	−	−
I-102	8000	Glycine or serine	+	+
D-62	Mu-910	Glycine or serine	−	+

TABLE 2. *Media used for growth study, enzyme assay, and isolation of mutants*

Medium[a]	Additive[b] (%)			
	L-Threonine	Glucose	$(NH_4)_2SO_4$	Yeast extract
Minimal	0	0.5	0.1	0
Thr-C	0.5	0	0.1	0.01
Thr-N	0.5	0.5	0	0.01
Thr-CN	0.5	0	0	0.01

[a] Media Thr-C, Thr-N, and Thr-CN are those containing threonine as the carbon source, the nitrogen source, and both, respectively. For minimal medium, glucose was used as the carbon source, unless otherwise noted.

[b] The compounds were added to a salt solution containing 0.7% K_2HPO_4, 0.3% KH_2PO_4, and 0.01% $MgSO_4 \cdot 7H_2O$.

ml of the medium in 500-ml Sakaguchi shaking flasks. The other culture conditions and the estimation of the growth were described previously (12). Growth is expressed as dry cell weight. For assays of L-threonine, aminoacetone, and glycine in the medium, the culture broth was centrifuged to obtain the supernatant. L-Threonine was measured by bioassay, using *Leuconostoc mesenteroides* P-60. Aminoacetone was determined by the method of Urata and Granick (31). Glycine was detected by paper chromatography.

Growth study. Cells were grown in test tubes (15 by 150 mm) containing 3 ml of medium with a Hitachi automated recording incubator system (5). Incubation was at 30°C with shaking (140 rpm, 4-cm stroke). Growth was turbidimetrically measured at 660 nm at 1- or 6-h intervals by the automatic method. An optical density of 0.10 corresponded to 3×10^8 cells per ml.

Enzyme assays. Cells were grown in 500-ml shaking flasks containing 150 ml of medium at 30°C with shaking. Cells were harvested from exponentially growing culture by centrifugation and washed twice with 50 mM tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.5). Cells were suspended in the same buffer, and cell-free extracts were prepared as described previously (14). The extracts prepared thus were used for the assay of threonine dehydrogenase and threonine aldolase. Cell-free extracts for threonine deaminase were prepared by the method stated previously (14).

Threonine dehydrogenase was assayed as follows. The reaction mixture contained 20 μmol of L-threonine, 0.5 μmol of nicotinamide adenine dinucleotide, 100 μmol of tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.5), and extracts (0.2 to 1.0 mg of protein) in a total volume of 1.0 ml. Incubation was carried out at 30°C for 30 min. The reaction was stopped by the addition of 1.0 ml of 0.3 M trichloroacetic acid and deproteinized by centrifugation. Aminoacetone in the supernatant was determined by the method of Urata and Granick (31). The activities in the extracts were stable at 0 to 10°C for several days.

Threonine aldolase was assayed as follows. The reaction mixture contained 20 μmol of L-threonine, 0.2 μmol of pyridoxal phosphate, 100 μmol of tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.5), 1 μmol of L-isoleucine, and extracts (0.2 to 1.0 mg of protein) in a total volume of 1.0 ml. Isoleucine was added to inhibit α-ketobutyrate formation by threonine deaminase. Incubation was performed at 30°C for 30 min. The reaction was stopped by the addition of 1.0 ml of 0.3 M trichloroacetic acid, and the mixture was deproteinized by centrifugation. The carbonyl compound in the supernatant was determined by the methods of Paz et al. (26) and Greenberg (8).

Threonine deaminase was assayed as described previously (14), except that the reaction mixture was incubated at 30°C.

Specific activities are expressed as micromoles of products per milligram of protein per minute.

Chemicals. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine was obtained from Aldrich Chemical Co., Milwaukee, Wis. All amino acids were the L-isomers. All reagents were of the highest quality commercially available.

RESULTS

Threonine degradation by growing cells of the wild strain. Threonine degradation by the wild strain was studied using the medium containing glucose and urea as the carbon and nitrogen sources (Fig. 1). Threonine in the medium decreased as the culture proceeded. Large amounts of aminoacetone were found in the supernatant of the medium during the exponential growth phase. Glycine was not formed during the period tested.

Threonine-degrading enzymes in the wild strain. To determine the pathways of threonine degradation in *S. marcescens*, threonine dehydrogenase, threonine aldolase, and threonine deaminase were investigated with extracts prepared from cells grown in various media (Table 3). Threonine dehydrogenase activities in the cells grown on threonine as the carbon

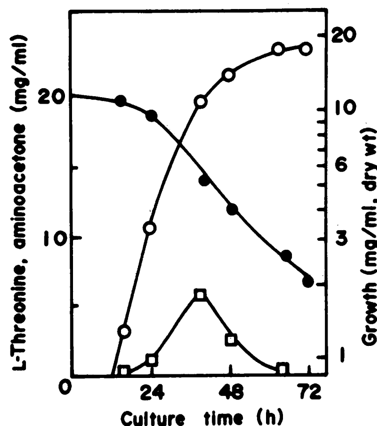

FIG. 1. *Threonine degradation and aminoacetone formation by growing cells of the wild strain. The medium for threonine degradation was used. Symbols: ○, growth; ●, L-threonine found in the supernatant of the medium; □, aminoacetone found in the supernatant of the medium.*

TABLE 3. *Activities of threonine dehydrogenase, threonine aldolase, and threonine deaminase in the wild strain*

Medium[a]	Sp act[b]			
	Threonine dehydrogenase	Threonine aldolase	Threonine deaminase	
			pH 9.0	pH 7.4[c]
Minimal	0.0029	ND[d]	0.088	0.065
Thr-C	0.020	ND[d]	0.062	0.042
Thr-N	0.0095	ND[d]	0.030	0.021
Thr-CN	0.018	ND[d]	0.033	0.021

[a] The components were described in Table 2.
[b] Micromoles of product per milligram of protein per minute.
[c] For catabolic threonine deaminase (28), the activities were determined at pH 7.4 in the presence of 1 mM AMP.
[d] ND, Not detected.

source, the nitrogen source, or both (medium Thr-C, Thr-N, or Thr-CN) were higher than the minimal medium. No threonine aldolase activity was observed with the cells grown in any medium.

Threonine deaminase activities were examined at pH 9.0 and 7.4 in the presence and absence of isoleucine to determine the existence of catabolic threonine deaminase (28). The activity at pH 7.4 in cells grown on threonine was lower than that in cells grown in the minimal medium and was completely inhibited by isoleucine, as in the case of the activity at pH 9.0. These data indicate that *S. marcescens* has no threonine deaminase other than the biosynthetic deaminase under the aerobic conditions. However, this does not exclude the possibility that biosynthetic threonine deaminase is involved in threonine degradation.

Isolation of a threonine dehydrogenase-deficient mutant. The growth of the wild strain on threonine was examined to determine the conditions for selecting threonine dehydrogenase-deficient mutants. The wild strain grew on threonine added as the carbon or nitrogen source, although slowly. The growth was accelerated by a small amount of yeast extract (Fig. 2; media Thr-C and Thr-N).

Strain Mu-910 was selected among small colonies on plates containing threonine plus limiting amounts of yeast extract as the carbon sources. This strain completely lacked threonine dehydrogenase activity and did not grow in medium Thr-C. Nevertheless, this mutant grew in medium Thr-N, although more slowly than the wild strain. Therefore, we supposed that enzymes other than threonine dehydrogenase also degrade threonine to the nitrogen source.

Threonine degradation by growing cells of strains Mu-910 and D-60. Threonine degradation by strain Mu-910 in the medium containing glucose and urea was significantly slower than that by the wild strain (Table 4). Threonine deaminase-deficient strain D-60 was isolated from strain Mu-910 as an isoleucine auxotroph,

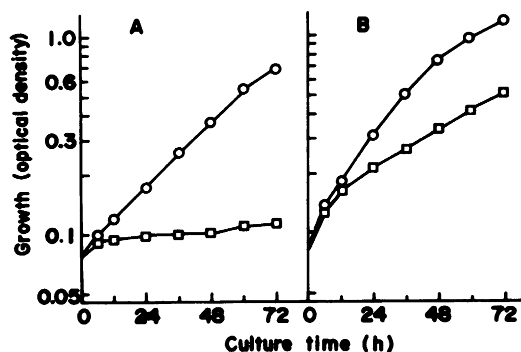

FIG. 2. *Growth of the wild strain and strain Mu-910 on threonine. Media: A, Thr-C; B, Thr-N. Strains: ○, wild strain; □, Mu-910.*

TABLE 4. *Threonine degradation by strains Mu-910 and D-60*[a]

Strain	L-Threonine found (mg/ml) at:			
	24 h	48 h	72 h	96 h
Wild	9.6	6.0	3.1	1.4
Mu-910	9.9	8.8	7.9	6.1
D-60	10.2	9.5	8.8	8.6

[a] The medium for threonine degradation (1% L-threonine) was used. For strain D-60, 10 mM L-isoleucine was added. The three strains showed similar growth curves.

since threonine deaminase is possibly involved in threonine degradation. This strain barely degraded threonine under the same conditions.

Controls of threonine dehydrogenase formation. In *E. coli,* threonine dehydrogenase was recently reported to be induced by leucine, but not by threonine (25). Therefore, the effect of both amino acids on the formation of threonine dehydrogenase in *S. marcescens* was studied (Table 5). The activities of threonine dehydrogenase in the medium containing succinate as the carbon source were higher than those in the medium containing glucose. Threonine had no effect on formation of the enzyme. The activities were markedly elevated by leucine in the presence of either glucose or succinate. These data suggest that threonine dehydrogenase is induced by leucine and possibly controlled by catabolic repression.

Some properties of threonine dehydrogenase. Properties of threonine dehydrogenase were studied with crude extracts. Optimal pH was near 10 in glycine-KOH buffer. The activity was stable, since a decrease of the activity was not observed when the extracts were treated at 40°C for 5 min. The apparent K_m value for threonine was calculated to be 0.55 mM. The enzyme required nicotinamide adenine dinucleotide as a coenzyme. The activity was completely inhibited by low concentrations of *p*-chloromercuribenzoate and $HgCl_2$, indicating that the enzyme has SH groups for the active center. K^+ and NH_4^+ ions did not enhance the activity, in contrast to the enzyme of *Staphylococcus aureus* (6). Substrate specificity was also investigated. DL-Hydroxynorvaline and DL-allothreonine were converted to aminoketones by the crude extracts. Since threonine dehydrogenase-deficient strain Mu-910 did not produce aminoketones from these compounds, threonine dehydrogenase is considered to catalyze the formation of aminoketones from these threonine analogs. Aminoketone formation was not observed when D-threonine, L-homoserine, and DL-β-hydroxyleucine were used as substrates.

Growth of glycine-requiring strains on threonine as the glycine source. There was the possibility that threonine would be converted to glycine via α-amino-β-ketobutyrate. To clarify the role of threonine dehydrogenase in glycine formation, glycine-requiring strains I-102 and D-62 were derived from the wild strain and threonine dehydrogenase-deficient strain Mu-910, respectively. Strain I-102 slowly grew on threonine alone in the minimal medium, and the growth on threonine was accelerated by leucine (Table 6). This strain exhibited higher growth rates on threonine and leucine when succinate was used as the carbon source than when glucose was used. We suppose that this is due to the higher activities of threonine dehydrogenase in the medium containing succinate. On the other hand, the growth of strain D-62 was observed neither on threonine nor on threonine plus leucine.

TABLE 5. *Leucine-mediated induction of threonine dehydrogenase in the wild strain*[a]

Carbon source	Addition to minimal medium (mM)	Sp act[b] of threonine dehydrogenase
Glucose	None	0.0026
	L-Threonine (10)	0.0030
	L-Leucine (1)	0.0062
	L-Threonine (10) + L-leucine (1)	0.0080
Succinate	None	0.0081
	L-Threonine (10)	0.0094
	L-Leucine (1)	0.027
	L-Threonine (10) + L-leucine (1)	0.016

[a] Cells were grown in minimal medium containing 0.5% glucose or 1.4% sodium succinate hexahydrate as the carbon source.

[b] Micromoles of product per milligram of protein per minute.

DISCUSSION

Threonine was rapidly degraded by the wild strain of *S. marcescens* in the medium containing glucose and urea as the carbon and nitrogen

TABLE 6. *Effect of amino acids on the growth rates of strains I-102 and D-62*

Strain	Addition to minimal medium[a] (mM)	Specific growth rate (k)[b]	
		Glucose	Succinate
I-102	None	<0.02	<0.02
	Glycine (3)	0.28	0.37
	L-Serine (3)	0.69	0.46
	L-Leucine (1)	0.03	0.02
	L-Threonine (10)	0.06	0.10
	L-Threonine (10) + L-leucine (1)	0.11	0.19
D-62	None	<0.02	<0.02
	Glycine (3)	0.28	0.41
	L-Serine (3)	0.48	0.46
	L-Leucine (1)	<0.02	<0.02
	L-Threonine (10)	<0.02	<0.02
	L-Threonine (10) + L-leucine (1)	<0.02	<0.02

[a] Cells were grown in minimal medium containing 0.5% glucose or 1.4% sodium succinate hexahydrate as the carbon source.

[b] The specific growth rate constant, k, is defined as: k (h^{-1}) = ln 2/mass-doubling time (h).

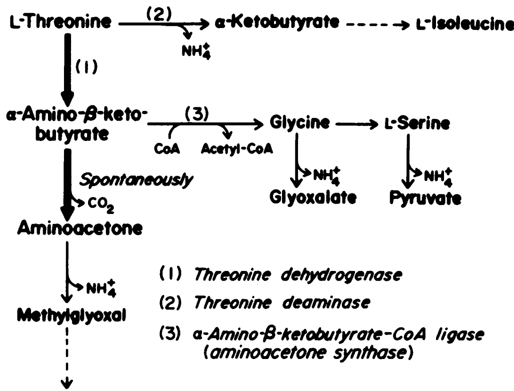

FIG. 3. *Proposed pathways for threonine degradation in S. marcescens.*

sources. This is disadvantageous for the construction of threonine-producing strains of *S. marcescens*. For these reasons, we examined the threonine-degrading enzymes and isolated a mutant deficient in both enzymes. Furthermore, we derived threonine-producing strains from this deficient mutant (16).

Threonine dehydrogenase degrades threonine to α-amino-β-ketobutyrate. Two pathways for the degradation of α-amino-β-ketobutyrate are possible: the aminoacetone route and the glycine route (Fig. 3). *S. marcescens* accumulated large amounts of the direct or indirect product of the reaction catalyzed by threonine dehydrogenase in the medium containing threonine. The product, which is likely to be aminoacetone because α-amino-β-ketobutyrate is decarboxylated nonenzymatically and rapidly (18), disappeared in the early stationary phase. Aminoacetone is deaminated to methylglyoxal in an *Arthrobacter* species (7). *S. marcescens* did not grow on aminoacetone as the nitrogen or carbon source (data not shown). This may be due to a toxic action caused by a high concentration of methylglyoxal accumulated in the cells (1). Therefore, the further pathway for aminoacetone degradation is not known in *S. marcescens.*

Another pathway for α-amino-β-ketobutyrate degradation is the glycine route. α-Amino-β-ketobutyrate-coenzyme A ligase cleaves the substrate to glycine and acetyl coenzyme A in the presence of coenzyme A (22). This ligase was first studied as an enzyme forming aminoacetone from acetyl coenzyme A and glycine (31). Thereafter, the role of this enzyme in glycine formation from α-amino-β-ketobutyrate was suggested in an *Arthrobacter* species (21). Newman et al. revealed the participation of threonine dehydrogenase in glycine formation from threonine in vivo in *E. coli* (25). Recently, Potter et al. demonstrated that the glycine thus formed was converted to serine, which acted as the actual nitrogen donor (27).

In the minimal medium, *S. marcescens* produces glycine primarily from serine, as other bacteria do. But in the minimal medium containing threonine, threonine dehydrogenase is involved in glycine formation from threonine. This was confirmed by the growth of glycine-requiring mutants with or without threonine dehydrogenase activity. Therefore, α-amino-β-ketobutyrate-coenzyme A ligase probably degrades α-amino-β-ketobutyrate to glycine and acetyl coenzyme A, although we have not yet examined this enzyme in *S. marcescens*. Nevertheless, this route seems to be a secondary one, since the other product, aminoacetone, was found in large amounts when *S. marcescens* was grown on threonine.

Threonine dehydrogenase of *S. marcescens* is controlled by catabolic repression and by leucine-mediated induction, as observed in *E. coli* (25). This is consistent with the observation that a glycine-requiring strain grew most rapidly in the medium containing threonine plus leucine as the glycine source and succinate as the carbon source. Nevertheless, the physiological significance of the leucine-mediated induction of threonine dehydrogenase is not clear at present.

ACKNOWLEDGMENTS

We thank F. Murakami and S. Sindo for their technical assistance.

LITERATURE CITED

1. **Ackerman, R. S., N. R. Cozzarelli, and W. Epstein.** 1974. Accumulation of toxic concentrations of methylglyoxal by wild-type *Escherichia coli* K-12. J. Bacteriol. **119**:357–362.
2. **Adelberg, E. A., M. Mandel, and G. C. C. Chen.** 1965. Optimal conditions for mutagenesis by N-methyl-N′-nitro-N-nitrosoguanidine in *Escherichia coli* K-12. Biochem. Biophys. Res. Commun. **18**:788–795.
3. **Bell, S. C., and J. M. Turner.** 1976. Bacterial catabolism of threonine. Threonine degradation initiated by L-threonine-NAD$^+$ oxidoreductase. Biochem. J. **156**:449–458.
4. **Bell, S. C., and J. M. Turner.** 1977. Bacterial catabolism of threonine. Threonine degradation initiated by L-threonine hydro-lyase (deaminating) in a species of *Corynebacterium*. Biochem. J. **164**:579–587.
5. **Chibata, I., H. Itoh, and T. Morimoto.** 1977. Automated recording incubator for tube culture of microorganism. Chem. Econ. Eng. Rev. **9**:11–15.
6. **Green, M. L., and W. H. Elliot.** 1964. The enzymic formation of aminoacetone from threonine and its further metabolism. Biochem. J. **92**:537–549.
7. **Green, M. L., and J. B. Lewis.** 1968. The oxidation of aminoacetone by a species of *Arthrobacter*. Biochem. J. **106**:267–270.
8. **Greenberg, D. M.** 1962. Threonine and allothreonine aldolase. Methods Enzymol. **5**:931–942.
9. **Hirata, M., M. Tokushige, A. Inagaki, and O. Hayaishi.** 1965. Nucleotide activation of threonine deaminase from *Escherichia coli*. J. Biol. Chem. **240**:1711–1717.
10. **Jungermann, K. A., W. Schmidt, F. H. Kirchniawy,**

E. H. Rupprecht, and R. K. Thauer. 1970. Glycine formation *via* threonine and serine aldolase. Its interrelation with the pyruvate formate lyase pathway of one-carbon unit synthesis in *Clostridium kluyveri*. Eur. J. Biochem. **16**:424–429.

11. **Kisumi, M.** 1962. Studies on the isoleucine fermentation. I. Screening of organisms and investigation of culture conditions. J. Biochem. (Tokyo) **52**:390–399.
12. **Kisumi, M., S. Komatsubara, and I. Chibata.** 1971. Valine accumulation by α-aminobutyric acid-resistant mutants of *Serratia marcescens*. J. Bacteriol. **106**:493–499.
13. **Kisumi, M., S. Komatsubara, and I. Chibata.** 1977. Enhancement of isoleucine hydroxamate-mediated growth inhibition and improvement of isoleucine-producing strains of *Serratia marcescens*. Appl. Environ. Microbiol. **34**:647–653.
14. **Kisumi, M., S. Komatsubara, M. Sugiura, and I. Chibata.** 1971. Isoleucine hydroxamate, an isoleucine antagonist. J. Bacteriol. **107**:741–745.
15. **Kisumi, M., S. Komatsubara, M. Sugiura, and I. Chibata.** 1972. Isoleucine accumulation by regulatory mutants of *Serratia marcescens:* lack of both feedback inhibition and repression. J. Bacteriol. **110**:761–763.
16. **Komatsubara, S., M. Kisumi, K. Murata, and I. Chibata.** 1978. Threonine production by regulatory mutants of *Serratia marcescens*. Appl. Environ. Microbiol. **35**:834–840.
17. **Kumagai, H., T. Nagate, H. Yoshida, and H. Yamada.** 1972. Threonine aldolase from *Candida humicola*. II. Purification, crystallization and properties. Biochim. Biophys. Acta **258**:779–790.
18. **Laver, W. G., A. Neuberger, and J. J. Scott.** 1959. α-Amino-β-keto acids. Part II. Rates of decarboxylation of the free acids and the behaviour of derivatives on titration. J. Chem. Soc., p. 1483–1491.
19. **Lessie, T. G., and H. R. Whiteley.** 1969. Properties of threonine deaminase from a bacterium able to use threonine as sole source of carbon. J. Bacteriol. **100**:878–889.
20. **Luginbuhl, G. H., J. G. Hofler, C. J. Decedue, and R. O. Burns.** 1974. Biodegradative L-threonine deaminase of *Salmonella typhimurium*. J. Bacteriol. **120**:559–561.
21. **McGilvray, D., and J. G. Morris.** 1969. Utilization of L-threonine by a species of *Arthrobacter*. A novel catabolic role for 'aminoacetone synthase'. Biochem. J. **112**:657–671.
22. **McGilvray, D., and J. G. Morris.** 1970. Aminoacetone synthase (*Arthrobacter*). Methods Enzymol. **17B**: 585–589.
23. **Matsumoto, H., S. Hosogaya, K. Suzuki, and T. Tazaki.** 1975. Arginine gene cluster of *Serratia marcescens*. Jpn. J. Microbiol. **19**:35–44.
24. **Morris, J. G.** 1969. Utilization of L-threonine by a pseudomonad: a catabolic role for L-threonine aldolase. Biochem. J. **115**:603–605.
25. **Newman, E. B., V. Kapoor, and R. Potter.** 1976. Role of L-threonine dehydrogenase in the catabolism of threonine and synthesis of glycine by *Escherichia coli*. J. Bacteriol. **126**:1245–1249.
26. **Paz, M. A., O. O. Blunefeld, M. Rojkind, E. Henso, C. Furfine, and P. M. Gallop.** 1965. Determination of carbonyl compounds with *N*-methyl-benzothiazole hydrazone. Arch. Biochem. Biophys. **109**:548–559.
27. **Potter, R., V. Kapoor, and E. B. Newman.** 1977. Role of threonine dehydrogenase in *Escherichia coli* threonine degradation. J. Bacteriol. **132**:385–391.
28. **Shizuta, Y., and O. Hayaishi.** 1970. Regulation of biodegradative threonine deaminase synthesis in *Escherichia coli* by cyclic adenosine 3',5'-monophosphate. J. Biol. Chem. **245**:5416–5423.
29. **Turner, J. M.** 1967. Microbial metabolism of amino ketone. L-1-Aminopropan-2-ol dehydrogenase and L-threonine dehydrogenase in *Escherichia coli*. Biochem. J. **104**:112–121.
30. **Umbarger, H. E., and B. Brown.** 1957. Threonine deamination in *Escherichia coli*. II. Evidence for two L-threonine deaminases. J. Bacteriol. **73**:105–112.
31. **Urata, G., and S. Granick.** 1963. Biosynthesis of α-aminoketones and the metabolism of aminoacetone. J. Biol. Chem. **238**:811–820.
32. **Weiner, R. M., M. J. Voll, and T. M. Cook.** 1974. Nalidixic acid for enrichment of auxotrophs in cultures of *Salmonella typhimurium*. Appl. Microbiol. **28**:579–581.
33. **Willetts, A. J., and J. M. Turner.** 1971. L-Threonine acetaldehyde-lyase in a strain of *Bacillus subtilis*. Biochim. Biophys. Acta **252**:105–110.