Note

L-Serine Production Improved by Analogue-resistant Mutants of a Methanol-utilizing Bacterium[†]

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In previous papers,^{1,2)} we reported that a facultative methylotroph, *Pseudomonas* MS 31, produced L-serine when glycine was added to methanol-containing medium after adequate growth and that the L-serine accumulation was improved by the use of temperature-sensitive mutants which were defective in L-serine degradation activity. A temperature-sensitive mutant strain, ts 162, derived from *Pseudomonas* MS 31 produced 6.8 mg/ml of L-serine from 12 mg/ml of glycine. However, the yield of L-serine and the conversion rate of glycine to L-serine were still unsatisfactory for industrial purposes. The fact that a considerable amount of glycine remained in the medium after cultivation indicated the importance of the improvement of the L-serine synthesizing activity for higher accumulation of L-serine.

Serine transhydroxymethylase (STHM) (EC 2.1.2.1) which catalyzes the conversion of glycine to L-serine is known to be regulated by feedback inhibition and repression. In *Salmonella typhimurium*, the synthesis of STHM has been reported to be repressed by L-serine or glycine.³⁾ In a facultative methylotroph *Pseudomonas* AM1, the

isozymes of STHM were found. One was a constitutive enzyme catalyzing the formation of glycine from L-serine. The other was an inducible enzyme catalyzing the formation of L-serine from glycine in assimilation of C₁ compounds.⁴⁾ O'Connor and Hanson⁵⁾ also found a similar isozyme system in a facultative methylotroph, *Methylobacterium organophilum*, and reported that these isozymes were strongly inhibited by glycine. The above mentioned regulatory features of this enzyme imply that the derivation of mutants resistant to analogues of L-serine or glycine is a useful way to improve the STHM activity.

The growth of temperature-sensitive mutant ts 162 was inhibited by several L-serine analogues such as D-serine, O-methyl-DL-serine, DL-serine hydroxamate, β -chloro-DL-alanine and glycine. Since ts 162 was highly sensitive to L-serine as described previously,²⁾ the growth inhibition by these analogues was not relieved by addition of L-serine to the medium.

Derivation of mutants resistant to these analogues was conducted. After mutagenic treatment with N-methyl-N'-nitro-N-nitrosoguanidine (250 μ g/ml),²⁾ the cells of ts 162 were spread over BM agar plates containing appropriate concentrations (1 to 4 mg/ml) of the analogues. Colonies, which appeared after cultivation at 30°C for 9 to 15 days, were picked up as analogue-resistant mutants, and then inoculated into test tubes containing 3 ml of L-serine production medium,²⁾ followed by cultivation at 34°C for 96 hr. Table I shows the distribution of L-serine productivity in analogue-resistant mutants. Mutants exhibiting improved productivity were obtained from the plates containing D-serine, O-methyl-DL-serine or glycine. After single colony isolations, the productivities of the potent L-serine-producing mutants were re-examined. Typical results of L-serine production by these mutants are shown in Table II. O-Methyl-DL-serine-resistant mutant strain S 395 which accumulated 12.3 mg/ml of L-serine in a test tube culture was selected as the most potent strain. S 395 was resistant to 0.2 mg/ml of O-methyl-DL-serine when cul-

TABLE I. DISTRIBUTION OF L-SERINE PRODUCTIVITY IN ANALOGUE-RESISTANT MUTANTS

The mutants were cultured in 3 ml of production medium²⁾ in test tubes. Glycine (16 mg/ml) and methanol (4% v/v) were added at 48-hr cultivation. The parental strain, ts 162 accumulated 3.0 mg/ml of L-serine under these conditions.

Isolation plate			Tested mutants	L-Serine productivity (mg/ml)				
				0~2	2~4	4~6	6~8	8~10
D-Serine	1 mg/ml	200	189	3	7	1	0	
	2	34	25	2	6	1	0	
O-Methyl-DL-serine	2	120	97	12	7	2	2	
Glycine	1	60	43	11	4	1	1	

[†] Production of L-Serine from Glycine by Methanol-utilizing Bacteria. Part III. Presented at the Annual Meeting of the Soc. of Ferment. Technol., Osaka, Japan, 1981.

TABLE II. L-SERINE PRODUCTION
BY TYPICAL MUTANTS

Cultivation was carried out in large test tubes (total volume, 50 ml) containing 10 ml of the production medium²⁾ for 96 hr at 34°C with reciprocal shaking. The addition of methanol and glycine was performed as described in the legend to Table I.

Strain No.	Genetic marker	L-Serine (mg/ml)	Conversion rate (%) ^a
ts 162	ts ^b (parent)	5.8	26
S 355	ts, D-Ser ^r	11.8	53
S 395	ts, O-CH ₃ -Ser ^r	12.3	55
S 276	ts, Gly ^r	11.6	52

^a Conversion rate of added glycine to L-serine (mol/mol).

^b Temperature-sensitive.

tured in a liquid BM medium, whereas the growth of ts 162 was inhibited approximately 50% under this condition. On the other hand, S 395 was still sensitive to higher concentrations of the analogue than 1 mg/ml.

S 395 accumulated 10 to 12 mg/ml of L-serine after 5 days cultivation at 34°C when 4% (v/v) methanol and 15 mg/ml glycine were added after 40 to 48 hr cultivation in shake flasks. The molar conversion rate of glycine to L-serine was 57% at the maximum. Only a small amount (less than 1 mg/ml) of glycine remained in the medium after cultivation. Though the accumulated amount of L-serine by this mutant was somewhat lower than those of

the known heterotrophic L-serine producers such as *Corynebacterium glycinophilum*,⁶⁾ *Nocardia butanica*⁷⁾ and *Sarcina albida*,⁸⁾ the conversion rate of glycine to L-serine of this mutant was considerably higher than those of the latter. Thus, this methylotrophic L-serine production process is promising from an economical viewpoint.

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