Production of O-Succinyl-L-homoserine by Auxotrophic Mutants of *Aerobacter aerogenes*

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Ten of Nineteen methionine-requiring mutants isolated from *Aerobacter aerogenes* ATCC 8308 by treatment with N-methyl-N'-nitro-N-nitrosoguanidine were found to accumulate in a culture broth a large amount of O-succinyl-L-homoserine (OSH) which was an intermediate in the biosynthesis of methionine in *Escherichia coli* and *Salmonella typhimurium*. OSH was isolated from the culture broth and identified by the behavior in paper chromatography, elementary analysis, melting point, optical density and infrared spectrum. Among these mutants, *A. aerogenes* KY 7056 which responds to methionine, homocysteine or cystathionine was used to investigate culture conditions for OSH production. The amount of OSH accumulation reached a level of 8.36 mg/ml with the medium containing 10% fructose and 1% ammonium sulfate. Addition of L-homoserine (10 mg/ml) increased the amount of OSH accumulation to a level of 15.8 mg/ml. Methionine or cystathionine suppressed the accumulation of OSH. Addition of δ -hydroxylysine to the fermentation medium almost abolished the OSH accumulation.

O-Succinyl-L-homoserine (OSH) has recently been found to be an intermediate in the biosynthesis of methionine in *Escherichia coli*¹⁻³⁾ and *Salmonella typhimurium*³⁾ (Fig. 1). In the course of the studies on the production of amino acids using microbial auxotrophic mu-

nants, a large amount of OSH was found to be accumulated by methionine-requiring mutants of *Aerobacter aerogenes*. The accumulation of OSH by microbial mutant has been reported only in *E. coli* and *S. typhimurium*. Therefore, the isolation and identification of OSH

FIG. 1. Pathway of Methionine Synthesis in *Escherichia coli* and *Salmonella typhimurium*

1) R. J. Rowbury, *J. Gen. Microbiol.*, **28**, 5P (1962).

2) R. J. Rowbury and D. D. Woods, *ibid.*, **36**, 341

(1964).

3) R. J. Rowbury, *J. Gen. Microbiol.*, **37**, 171 (1964).

from the culture broth were attempted. Some cultural conditions for the production of OSH by the mutant were also investigated.

MATERIALS AND METHODS

Microorganism. Nineteen methionine-requiring mutants were isolated from *A. aerogenes* ATCC 8308 by treatment with N-methyl-N'-nitro-N-nitrosoguanidine, according to the procedure of Adelberg *et al.*⁴⁾ Ten of them accumulated OSH in a culture broth. Among these mutants, *A. aerogenes* KY 7056 was mainly employed for the investigation of fermentation condition for OSH production.

Culture method. Thirty milliliters of a seed medium in a 250 ml Erlenmyer flask was inoculated with one loopful of organism grown on a bouillon agar slant, and incubated on a rotatory shaker operated at 220 rpm. After incubation for 24 hr at 30°C, 1 ml of this seed culture was transferred into a large test tube (25 mm × 19 cm) containing 10 ml of a fermentation medium. This test tube was incubated at 30°C on a test tube shaker for 72 hr. The composition of the medium is described in the footnote of each table and figure. The composition of the seed medium was as follows; 2% glucose, 1% peptone, 1% yeast extract and 0.25% sodium chloride (pH 7.4).

Growth experiment. A large test tube containing 10 ml medium was inoculated with one drop of cell suspension from bouillon agar slant (finally, *ca.* 10⁵ cells/ml), and incubated at 30°C on a test tube shaker. After 24 hr, the optical density of the culture broth at a wave length of 660 mμ was read using a Tokyo Kodon colorimeter.

Analysis. OSH, and the other amino acids were estimated by colorimetry using ninhydrin after paper chromatographical separation. The paper chromatography was performed on Toyo Roshi No. 50 paper using the following solvent system; *n*-butanol-acetic acid-water (4:1:2). For the estimation of growth in a fermentation medium containing calcium carbonate, 1 ml of 6N HCl was added to 1 ml aliquot of the broth to dissolve the calcium carbonate, and the optical density was measured after dilution 1:10.

RESULTS

1. Isolation and identification

When some of the methionine-requiring mutants of *A. aerogenes* were incubated in the medium with limited quantities of methionine, an unknown compound was noticed to be accumulated, which showed a ninhydrin positive spot at *R_f* 0.35 when chromatographed on paper in the solvent mixture described in the section, Materials and Methods. The compound gave a yellow brown color spot (which later turned purple) on heating with ninhydrin. The compound was very unstable in mild alkaline solution, but more stable in mild acid solution. After heating the broth adjusted to 4N in hydrochloric acid concentration at 100°C for 2 hr, the spot corresponding to the compound was no more detected on paper chromatogram, while a new spot corresponding to homoserine appeared. These properties agreed closely with those of OSH described in the paper by Rowbury.^{2,3)} Then, the attempt was made to isolate OSH from the culture broth. The isolation procedure follow-

FIG. 2. Isolation of OSH from Broth Supernatant.

ed that by Rowbury³⁾ (Fig. 2). The broth was centrifuged, and the supernatant was adjusted to pH 7.4 with conc. ammonia, and chromatographed on a column of strongly basic anion exchange resin, Diaion PA 416, in the acetate form. Elution was performed

4) E. A. Adelberg, M. Mandel and G. C. C. Chen, *Biochem. Biophys. Res. Commun.*, **18**, 788 (1965).

FIG. 3. Infrared Absorption Spectrum of OSH.

FIG. 4. Growth Response of *A. aerogenes* KY 7056 to Homocysteine, Cystathionine and Methionine.

○—○ DL-Homocysteine, △—△ DL-Cystathionine
●—● L-Methionine

Medium; glucose 10 g, $\text{NH}_4\text{H}_2\text{PO}_4$ 1 g, KCl 0.2 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g, trace element solution* 1 ml, distilled water 1 liter (pH 7.2).

* $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ 88 mg, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{27} \cdot 4\text{H}_2\text{O}$ 37 mg, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 8.8 mg, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 270 mg, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 7.2 mg, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 970 mg, distilled water 1 liter.

** optical density

with water, then with 0.2N acetic acid. OSH was eluted with 0.2N acetic acid and separated from the other amino acids, homoserine and valine, which were accumulated in small amounts in the culture broth. The OSH rich fractions were combined and evaporated to dryness under reduced pressure. The residue was dissolved in water. The solution was treated with active carbon, and concentrated *in vacuo*, to crystallize OSH. The product was recrystallized from ethanol. The data of the

elementary analysis was essentially identical with theoretical value; Found: H, 6.13; C, 44.05; N, 6.22. Calcd. for $\text{C}_8\text{H}_{13}\text{O}_6\text{N}$: H, 5.94; C, 43.83; N, 6.39%. Melting point (193°C), optical rotation, $[\alpha]_D^{20} +7.8$ ($c=1.0$, H_2O), and infrared spectrum (Fig. 3) are in good agreement with those in the paper by M. Flavin *et al.*⁵⁾

5) M. Flavin and C. Slaughter, *Biochemistry*, **4**, 1370 (1965).

2. Growth response to methionine, homocysteine and cystathionine

As shown in Fig. 4, *A. aerogenes* KY 7056 responded to methionine, homocysteine or cystathionine. No growth was obtained when methionine was replaced by either cystine or cysteine. The genetic block must, therefore, occur between OSH and cystathionine.

3. Effect of methionine concentration on OSH accumulation

As shown in Fig. 5, the lower were the methionine levels in fermentation media, the

FIG. 5. Effect of Methionine on OSH Accumulation.

○—○ OSH, ●—● Growth.

Basal medium; glucose 10%, $(\text{NH}_4)_2\text{SO}_4$ 2%, KH_2PO_4 0.05%, K_2HPO_4 0.05%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.025%, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.001%, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 0.001%, CaCO_3 2% (pH 7.4).

better were OSH yields. The highest yield was obtained without added methionine. It is assumed that suitable amount of methionine to produce OSH was transferred from a seed culture to a fermentation medium. Excess of methionine suppressed the accumulation of OSH.

4. Effect of carbon source on OSH production

Seven sugars and soluble starch were investigated at 5 and 10% levels to determine the most favorable carbon source for OSH production. As shown in Table I, fructose, sorbitol, mannitol, lactose and sucrose gave high yields. Soluble starch seemed to be hardly utilized by this organism. With glucose or xylose, accumulation was moderately good.

TABLE I. EFFECT OF CARBON SOURCE ON OSH ACCUMULATION

	%	pH	O.D.	OSH mg/ml
Glucose	5	7.6	0.615	3.29
	10	7.5	0.855	4.73
Fructose	5	7.0	0.720	7.00
	10	6.7	0.970	6.40
Xylose	5	7.0	0.700	5.47
	10	5.8	1.075	4.97
Lactose	5	5.6	0.770	4.41
	10	4.9	0.995	6.02
Mannitol	5	7.5	0.748	4.71
	10	5.7	1.050	6.18
Sorbitol	5	6.7	0.795	7.55
	10	6.9	1.050	5.40
Sucrose	5	7.4	0.698	4.78
	10	7.3	0.965	6.19
Soluble starch	5	7.4	0.259	1.69
	10	5.8	0.400	1.41

Basal medium; $(\text{NH}_4)_2\text{SO}_4$ 2%, KH_2PO_4 0.05%, K_2HPO_4 0.05%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.025%, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.001%, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 0.001%, CaCO_3 2%, (pH 7.4).

5. Effect of inorganic nitrogen source on OSH production

TABLE II. EFFECT OF NITROGEN SOURCE ON OSH ACCUMULATION

	%	pH	O.D.	OSH mg/ml
$(\text{NH}_4)_2\text{SO}_4$	0.5	6.1	0.835	5.86
	1.0	6.7	0.915	5.03
	2.0	7.6	0.855	4.55
NH_4Cl	0.4	5.8	0.815	7.21
	0.8	6.1	0.945	6.55
	1.6	6.9	0.940	5.00
$(\text{NH}_4)_2\text{HPO}_4$	0.5	5.3	0.488	4.72
	1.0	6.6	0.668	4.23
	2.0	5.8	0.355	1.68
NH_4NO_3	0.3	7.3	0.850	4.75
	0.6	7.0	0.928	3.91
	1.2	7.2	0.800	4.93
KNO_3	0.4	6.5	0.335	0.43
	0.8	5.4	0.228	—
	1.5	5.4	0.223	—
$\text{CH}_3\text{COONH}_4$	0.6	7.1	0.795	2.41
	1.2	6.3	0.705	1.22
	2.3	6.4	0.458	0.08
Urea	0.2	5.6	0.570	4.72
	0.5	7.8	0.800	3.52
	1.0	7.5	0.800	0.82

Basal medium; glucose 10%, KH_2PO_4 0.05%, K_2HPO_4 0.05%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.025%, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.001%, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 0.001%, CaCO_3 2% (pH 7.4).

TABLE III. EFFECT OF CARBON SOURCE AND AMMONIUM SULFATE ON OSH ACCUMULATION

	% (NH ₄) ₂ SO ₄ %	pH	O.D.	OSH mg/ml
Fructose	10	0.5	0.665	5.86
		1.0	0.880	8.36
		2.0	0.800	4.14
	5	0.5	0.605	4.55
		1.0	0.740	5.81
		2.0	0.660	7.53
Sorbitol	10	0.5	0.750	4.56
		1.0	0.753	5.44
		2.0	0.880	4.42
	5	0.5	0.643	5.30
		1.0	0.618	4.82
		2.0	0.708	5.53

Basal medium; KH₂PO₄ 0.05%, K₂HPO₄ 0.05%, MgSO₄·7H₂O 0.025%, FeSO₄·7H₂O 0.001%, MnSO₄·4H₂O 0.001%, CaCO₃ 2% (pH 7.4).

TABLE IV. EFFECT OF INORGANIC SALTS ON OSH ACCUMULATION

KH ₂ PO ₄ ⁺ K ₂ HPO ₄ ^{a)} %	MgSO ₄ · 7H ₂ O %	pH	O.D.	OSH mg/ml
0.0063	0.0125	5.1	0.760	3.63
	0.025	5.2	0.723	3.30
	0.05	5.2	0.758	3.26
	0.1	5.1	0.750	3.45
	0.2	5.1	0.745	3.21
0.0125	0.0125	5.0	0.850	5.75
	0.025	4.9	0.880	5.36
	0.05	4.9	0.850	5.63
	0.1	5.0	0.860	6.29
	0.2	5.2	0.885	6.77
0.025	0.0125	6.0	0.885	6.53
	0.025	5.8	0.955	7.41
	0.05	5.1	1.015	7.40
	0.1	5.0	0.990	7.44
	0.2	5.3	1.015	7.92
0.05	0.0125	6.2	0.850	6.20
	0.025	6.1	0.950	7.35
	0.05	5.1	0.975	5.30
	0.1	5.8	1.033	6.63
	0.2	5.6	1.030	6.97

^{a)} KH₂PO₄ and K₂HPO₄ were used in 1:1 ratio on weight basis. The concentration in the table represents the level of each phosphate.

Basal medium; fructose 10%, (NH₄)₂SO₄ 1%, FeSO₄·7H₂O 0.001%, MnSO₄·4H₂O 0.001%, CaCO₃ 2%, (pH 7.4).

Various nitrogen sources were tested for their effects on OSH production, at the concentrations equal to 0.5, 1.0 and 2.0% ammonium sulfate on a nitrogen basis. Ammonium salts, and urea gave high yields, and potassium nitrate was found to be unutilized by this organism (Table II). These results were obtained with the medium containing 10% glucose. Then, experiments were carried out to determine the favorable combination of carbon source and ammonium sulfate and maximum yield (8.36 mg/ml) was obtained with the medium containing 10% fructose and 1% ammonium sulfate (Table III).

6. Effect of inorganic salts on OSH production

The effect of the concentration of phosphate and magnesium ion on OSH accumulation was studied. The proper ranges of the concentration of dipotassium hydrogen phosphate and potassium dihydrogen phosphate were each 0.0125~0.05% and those of magnesium sulfate were 0.025~0.2% (Table IV).

7. Effect of amino acids, vitamins, and precursors on OSH accumulation

The effect of 24 natural amino acids on OSH production was investigated. A part of the results is presented in Table V. Addition of homoserine was found to increase OSH accumulation markedly. Ten milligrams per milliliter of homoserine led to the OSH accumulation of 15.8 mg/ml. Addition of L-lysine or α , ϵ -diaminopimelic acid at low levels enhanced OSH accumulation, while the addition at high levels led to the accumulation of cadaverine, a catabolite of lysine. By addition of L-arginine, glycine, L-glutamic acid, L-glutamine, or L-asparagine, OSH accumulation was stimulated. High concentration of L-cystine, L-cysteine, L-aspartic acid, L-proline, L-serine, or L-leucine inhibited OSH production. Addition of α -aminobutyric acid at high levels led to the decrease in OSH production with concurrent accumulation of homoserine and isoleucine.

Besides these natural amino acids, some

TABLE V. EFFECT OF AMINO ACIDS ON OSH ACCUMULATION

	mg/ml	pH	O.D.	OSH mg/ml		mg/ml	pH	O.D.	OSH mg/ml
L-Homoserine	0.2	7.0	0.645	6.44	L-Cystine	0.2	7.2	0.710	5.11
	1	7.0	0.655	7.53		1	6.0	0.820	4.14
	5	6.4	0.748	8.69		5	5.8	0.580	0.47
	10	6.5	0.890	15.80		0.2	7.0	0.703	5.21
L-Lysine	0.2	5.8	0.730	7.45	L-Cysteine	1	5.5	0.845	4.15
	1	6.4	0.715	7.51		5	5.8	0.720	1.11
	5	5.1	0.945	5.21		0.2	7.1	0.708	5.59
DAP ^{a)}	0.2	6.4	0.730	6.73	L-Serine	1	7.1	0.730	5.68
	1	5.1	0.890	4.11		5	5.1	0.905	3.82
L-Arginine	0.2	6.4	0.780	6.65		10	5.1	0.975	1.57
	1	5.7	0.895	6.47	L-Leucine	0.2	7.0	0.675	3.87
Glycine	0.2	7.0	0.660	6.76		1	7.2	0.740	3.37
	1	5.8	0.885	6.67		5	7.0	0.795	2.19
L-Glutamic acid	0.2	7.2	0.695	6.86		10	6.2	0.805	1.73
	1	7.1	0.775	6.60	L-Aspartic acid	0.2	7.0	0.705	4.58
L-Proline	0.2	5.8	0.905	6.64		1	6.7	0.740	4.75
	1	5.6	0.950	2.96		5	7.0	0.780	1.63
L-Glutamine	0.2	6.3	0.745	6.64	DL- α -Aminobutyric acid	0.2	7.1	0.700	5.52
	1	5.8	0.780	6.66		1	6.2	0.785	4.34
L-Asparagine	0.2	6.1	0.683	7.01		5	6.6	0.815	2.66
	1	6.0	0.770	4.66	Control	10	5.8	0.313	—
						—	6.3	0.630	4.30

^{a)} α, ϵ -Diaminopimelic acid.Basal medium; glucose 10%, $(\text{NH}_4)_2\text{SO}_4$ 1%, KH_2PO_4 0.05%, K_2HPO_4 0.05%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.025%, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.001%, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 0.001%, CaCO_3 2% (pH 7.4).

TABLE VI. EFFECT OF SOME AMINO ACIDS ON OSH ACCUMULATION

	mg/ml	pH	O.D.	OSH mg/ml	Other amino acids mg/ml
Cystathionine	0.2	6.0	0.915	1.28	val $\pm \sim +$
	1	6.9	1.010	tr.	val $++$
	5	6.0	1.065	tr.	Met 0.63
	10	6.0	0.950	tr.	Met 1.78
δ -Hydroxy- lysine	0.2	5.4	0.910	5.38	
	1	6.9	0.925	4.85	
	5	6.8	0.850	0.51	
	10	5.8	0.710	0.30	
Thialysine	0.2	5.8	0.875	5.16	
	1	5.2	0.860	5.21	
	5	5.2	0.840	4.61	
	10	5.9	0.715	4.76	
Lanthionine	0.2	6.5	0.940	6.14	
	1	6.5	0.950	5.61	
	5	6.3	1.010	6.09	
	10	6.3	1.060	7.76	
Control	—	6.2	0.930	5.41	

Basal medium; fructose 10%, $(\text{NH}_4)_2\text{SO}_4$ 1%, KH_2PO_4 0.05%, K_2HPO_4 0.05%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.025%, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.001%, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 0.001%, CaCO_3 2% (pH 7.4).

TABLE VII. EFFECT OF SOME AMINO ACIDS ON OSH ACCUMULATION

	mg/ml	pH	O.D.	OSH mg/ml
D-Lysine	0.2	5.8	1.045	6.46
	1	5.8	0.955	7.12
	5	6.4	0.910	4.05
	10	5.6	0.855	3.31
α -Ketobutyric acid	0.2	5.8	0.975	3.44
	1	6.0	0.940	5.51
	5	5.9	0.361	—
	10	5.8	0.236	—
nor-Leucine	0.2	5.4	0.990	—
	1	5.8	0.398	—
	5	5.8	0.293	—
	10	5.8	0.270	—
nor-Valine	0.2	5.3	0.920	4.93
	1	5.8	0.615	0.94
	5	5.9	0.658	0.30
	10	5.8	0.292	tr.
Control	—	6.0	0.927	3.81

Basal medium; fructose 10%, $(\text{NH}_4)_2\text{SO}_4$ 1%, KH_2PO_4 0.05%, K_2HPO_4 0.05%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.025%, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.001%, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 0.001%, CaCO_3 2% (pH 7.4).

other amino acids including lysine analogues, δ -hydroxylysine, thialysine (S- β -aminoethylcysteine), and diaminopimelic acid analogue, lanthionine, were investigated on their effects on OSH production. The results are presented in Tables VI and VII. δ -Hydroxylysine suppressed OSH accumulation at high levels, while the influence of thialysine was slight. Although the effect of D-lysine was similar to that of L-lysine, cadaverine accumulation was not noted even at high D-lysine levels. Furthermore, the addition of high levels (1~10 mg/ml) of D-lysine led to the accumulation of small amount of threonine. Lanthionine was found to promote OSH accumulation to some extent. A methionine precursor, cystathionine, suppressed the OSH production markedly. Besides, low concentration of cystathionine stimulated the production of valine, and high concentration of it caused the accumulation of methionine. α -Ketobutyric acid, nor-valine, and nor-leucine retarded both growth and OSH production.

Nine vitamins, including thiamine, riboflavin, *p*-aminobenzoic acid, pyridoxine, nicotinic acid, biotin, folic acid, cyanocobalamin and inositol (0.2 μ g/liter and 2 μ g/liter), fumaric acid and succinic acid (5 mg/ml and 10 mg/ml) showed slight effect at best.

FIG. 6. Time Course of OSH Fermentation.

○—○ OSH, ●—● Growth, ■—■ pH.
Medium; fructose 10%, $(\text{NH}_4)_2\text{SO}_4$ 1%, KH_2PO_4 0.05%, K_2HPO_4 0.05%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.025%, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.001%, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 0.001%, CaCO_3 2% (pH 7.4).

8. Changes occurring during fermentation

An example of the chemical changes occurring during the fermentation under optimal conditions is shown in Fig. 6. Production of OSH paralleled growth, reached a maximum in about 48 hr, after which OSH concentration steadily declined with a concurrent rise of pH-value, and rapidly declined after 120 hr.

DISCUSSION

OSH has been found to be accumulated by the mutants of *A. aerogenes* which are blocked between homoserine and cystathionine. This finding indicates that OSH is an intermediate in methionine biosynthesis in *A. aerogenes*, as has been reported in *E. coli* and *S. typhimurium*. L-Methionine inhibited the formation of OSH (Fig. 5). This fact suggests that the regulation mechanism in methionine synthesis reported in *E. coli*¹⁻³ and *S. typhimurium*³ functions also in *A. aerogenes*. Addition of cystathionine suppressed the OSH formation, when methionine accumulated. It may be considered that cystathionine inhibited the formation of OSH after conversion into methionine.

O-Succinylhomoserine transsuccinylase may be speculated to be the step which receives feedback type inhibition by methionine although the evidence supporting this speculation awaits to be obtained. Addition of homoserine increased OSH accumulation markedly (Table V). This fact indicates that homoserine is a precursor of OSH. In contrast, succinic acid did not stimulate the OSH formation. This organism may be able to supply the amount of succinic acid required endogenously while the formation of homoserine is limited.

Addition of α -aminobutyric acid at high levels (5~10 mg/ml) led to the decrease in OSH production with the concurrent accumulation of homoserine and isoleucine. The production of isoleucine from α -aminobutyric acid has been well documented.^{6,7} However, it is not clear whether homoserine accumula-

tion in addition of α -aminobutyric acid is due to the decomposition of OSH or to the inhibition by α -aminobutyric acid of the reaction between homoserine and OSH.

δ -Hydroxylysine suppressed OSH accumulation at high levels (Table VI). The inhibition of aspartokinase by δ -hydroxylysine in

Streptococcus faecalis has been reported.⁸⁾ Whether similar type of inhibition led to the suppression of OSH accumulation requires further investigation.

During the fermentation, OSH concentration was rapidly declined after 120 hr. Although the factor involved is not clear, the possibility that the induction of an anabolic pathway other than the synthetic pathway of methionine may be considered.

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