O-Acetylhomoserine as an Intermediate in Methionine Biosynthesis in *Arthrobacter paraffineus*, *Corynebacterium glutamicum* and *Bacillus* Species

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Certain methionine auxotrophs of *Arthrobacter paraffineus* and *Bacillus* species produce large amounts of O-acetylhomoserine (OAH). The methionine requirement of these auxotrophs could be satisfied by either cystathionine or homocysteine but not by homoserine. The cell-free extracts from the auxotrophs were found to be deficient in cystathionine γ -synthase activity. OAH and O-succinylhomoserine (OSH) could replace methionine in the auxotrophs which are deficient in homoserine-O-transacetylase. A methionine auxotroph of *Corynebacterium glutamicum* also produced OAH, and the blocked step in the auxotroph appeared to be between cystathionine and homocysteine.

Cell-free extracts of *A. paraffineus*, *C. glutamicum* and *Bacillus* species catalyzed the formation of OAH from acetyl-CoA and homoserine, while a corresponding reaction with succinyl-CoA was not detected. Cystathionine γ -synthases in extracts of *C. glutamicum* and *Bacillus* species were specific for OAH, while the enzyme in extract of *A. paraffineus* was rather specific for OSH though it reacted with OAH to a certain extent.

These results indicate that the biosynthesis of L-methionine in these bacteria involves OAH.

It has been demonstrated in *Escherichia coli*^{1,2)} and *Salmonella typhimurium*³⁾ that the first step of methionine biosynthesis is transsuccinylation from succinyl-CoA to homoserine, leading to the formation of O-succinylhomoserine (OSH). The observation that a methionine auxotroph of *Aerobacter aerogenes* produce a large amount of OSH suggested that OSH is an intermediate in methionine biosynthesis in the bacterium.⁴⁾ On the other hand, acetylated derivative of homoserine has been reported to be involved in methionine biosynthesis in *Neurospora crassa*⁵⁾ and *Saccharomyces cerevisiae*.⁶⁾ Accumulation of O-acetylhomoserine (OAH) was reported by the authors⁷⁾ in certain methionine auxotrophs of *Arthrobacter paraffineus* and *Bacillus* species. The results suggested that the acetylated intermediate was not restricted in fungi and yeast. More recently, the acylation of homoserine in *B. subtilis*,⁸⁾ *B. polymixa*⁸⁾ and *Brevibacterium flavum*⁹⁾ was reported to involve acetyl-CoA.

In a previous paper,¹⁰⁾ it was reported that

some regulatory mutants of *Corynebacterium glutamicum* produced a large amount of OAH and that OAH is an intermediate in methionine biosynthesis in the bacterium. We here report in further detail our observations on *A. paraffineus*, *C. glutamicum* and *Bacillus* species together with further enzymic studies using cell-free extracts of these bacteria. The results indicate that OAH is an intermediate in methionine pathway in these bacteria.

MATERIALS AND METHODS

Bacteria. The bacterial strains used in this study are described in Table I. *A. paraffineus* KY 7104 (Ile⁻) was derived from KY 4302.¹¹⁾ *A. paraffineus* KY 7137 (Ile⁻, Met⁻/Homocyst⁻/Cystath⁻)¹²⁾ and KY 8743 and KY 8744 (Ile⁻, Met⁻/Homocyst⁻/Cystath⁻/OAH⁻) were derived from KY 7104. *Bacillus* species KY 8042 (Met⁻), KY 8043 (Met⁻/Homocyst⁻), KY 8041 (Met⁻/Homocyst⁻/Cystath⁻) and KY 8044 (Met⁻/Homocyst⁻/Cystath⁻/OAH⁻) were derived from *Bacillus* species KK (wild type). *C. glutamicum* TLD-32 (Thr⁻, Met⁻/Homocyst⁻) and TLD-25 (Thr⁻, Met⁻) were derived from KY 9276 (Thr⁻).¹³⁾ Mutations were induced by treatment of N-methyl-N'-nitro-

TABLE I. BACTERIAL STRAINS USED

<i>Arthrobacter paraffineus</i>	
KY 4302	wild type
KY 7104	Ile ⁻
KY 7137	Ile ⁻ , Met ⁻ /Homocyst ⁻ /Cystath ⁻
KY 8743	Ile ⁻ , Met ⁻ /Homocyst ⁻ /Cystath ⁻ / OAH ⁻
KY 8744	Ile ⁻ , Met ⁻ /Homocyst ⁻ /Cystath ⁻ / OAH ⁻
<i>Bacillus</i> species	
KK	wild type
KY 8042	Met ⁻
KY 8043	Met ⁻ /Homocyst ⁻
KY 8041	Met ⁻ /Homocyst ⁻ /Cystath ⁻
KY 8044	Met ⁻ /Homocyst ⁻ /Cystath ⁻ /OAH ⁻
<i>Corynebacterium glutamicum</i>	
KY 9276	Thr ⁻
TLD-25	Thr ⁻ , Met ⁻ /B ₁₂ ⁻
TLD-32	Thr ⁻ , Met ⁻ /Homocyst ⁻

Abbreviations: Homocyst, homocysteine; Cystath, cystathionine; OAH, O-acetylhomoserine.

N-nitrosoguanidine¹⁴⁾ or ethyl methanesulfonate¹⁰⁾ or by UV irradiation.¹⁴⁾

Media. The composition of glucose-minimal medium was as follows: glucose, 10 g; NH₄H₂PO₄, 1 g; KCl, 0.2 g; MgSO₄·7H₂O, 0.2 g; biotin, 30 µg; trace element solution, 1 ml and deionized water, 1 liter (pH 7.2). The trace element solution contained 88 mg Na₂B₄O₇·10H₂O, 37 mg (NH₄)₆Mo₇O₂₇·4H₂O, 8.8 mg ZnSO₄·7H₂O, 270 mg CuSO₄·5H₂O, 7.2 mg MnCl₂·4H₂O and 970 mg FeCl₃·6H₂O in a liter of deionized water. For the preparation of sorbitol-minimal medium, glucose in the glucose-minimal medium was replaced by an equal amount of sorbitol and 1 mg/liter of thiamine hydrochloride was added to the medium. The compositions of the seed medium and the fermentation medium for OAH production are shown in Table II.

Culture. Thirty milliliters of a seed medium in a 250 ml Erlenmeyer flask were inoculated with a loopful of organisms grown on a bouillon-agar slant overnight and incubated on a rotary shaker. After incubation for 24 hr at 30°C, an aliquot of this seed culture was transferred into a large test tube (2.5 × 19 cm) or 250 ml flask containing 5~10 ml of a fermentation medium and incubated for 72~168 hr at 30°C. For harvesting the cells to get enzyme preparation, 30 ml of the seed culture was transferred into a 2 liter flask containing 300 ml of a fermentation medium, which was incubated for 40~48 hr.

Growth experiments. The method used for growth assay was as follows. Ten milliliters of a medium in a

large test tube were inoculated with one drop of the cell-suspension prepared from cells grown on a bouillon agar slant overnight to the final concentration of 10⁶~10⁷ cells/ml and incubated on a test tube shaker for 24~48 hr at 30°C. Growth was measured with "Tokyo Kodon" spectrophotometer at 660 nm.

Preparation of extracts. The culture broth was first centrifuged at low speed to eliminate calcium carbonate from the broth. The broth was again centrifuged for 10 min at 3,000 rev/min in the cold. The supernatant fluid was discarded and cells were washed twice with 0.05 M potassium phosphate buffer (pH 7.5) containing 10 mM ethylenediaminetetraacetic acid, 0.1 mM β-mercaptothiopropanoic acid and 0.05 mM pyridoxal phosphate. The washed cells were resuspended in the buffer (50 mg dry weight of cells/ml), and disrupted for 30 min in a "Kubota" 10 KC sonic oscillator cooled with ice-cold water. The disrupted-cell suspension was centrifuged at 15,000 × g for 20 min at 2°C and the supernatant extract was recovered. Five milliliters aliquot of the extract was then dialyzed at 4°C for a total 2.5 hr against two changes of 2 liters of the same buffer.

Enzyme assays. Assay for homoserine-O-trans-acetylase measures the homoserine dependent release of free CoA from acetyl CoA. The reaction mixture contained 50 µmoles of Tris-hydrochloride buffer (pH 7.5), 1.25 µmoles of L-homoserine, 0.14 µmole of acetyl-CoA and dialyzed extract in a total volume of 0.5 ml. L-Homoserine was omitted in the control tube. The reaction was carried out at 30°C for 10 min and terminated by the addition of 1 ml of absolute alcohol. The sulfhydryl group of CoA was measured spectrophotometrically by the use of 5,5'-dithiobis-(2-nitrobenzoic acid) as a reagent for the determination of the sulfhydryl group ($E_{M412nm} = 13,600$).¹⁵⁾

Cystathionine γ-synthase was assayed by the modified method of Kaplan and Flavin ("assay C"),¹⁶⁾ measuring the rate of cysteine disappearance caused by γ-replacement reaction catalyzed by the enzyme (OAH + L-cysteine → L-cystathionine + acetic acid). The reaction was carried out anaerobically at 25°C in test tubes containing, in 2.0 ml final volume, 100 µmole of potassium pyrophosphate (pH 7.5), 0.2 µmole of pyridoxal phosphate, 10 µmoles of OAH, 1 µmole of L-cysteine and dialysed extract. Control mixture contained no OAH. First water and buffer are added to the tubes and nitrogen was bubbled through the solution for 30 sec and over its surface for 10 min. Then enzyme, L-cysteine, and OAH were added in this order. While the flow of gas over the solution surface is maintained, 0.2 ml aliquots were withdrawn at 2 min intervals for 10 min and added into 2.8 ml solution containing 300 µmoles of potassium phosphate (pH 7.4), 0.6 µmole of 5,5'-dithiobis-(2-nitrobenzoic acid) and 1 ml of ab-

TABLE II. COMPOSITION OF MEDIA

	S-a ^{b)}	S-b ^{c)}	F-I	F-II	F-III (%)
<i>n</i> -Paraffin (C ₁₁ ~C ₁₄)			10		
Glucose		2		10	10
Sorbitol	5				
(NH ₄) ₂ SO ₄			2	2	2
KH ₂ PO ₄			0.2	0.05	0.05
K ₂ HPO ₄			0.2	0.05	0.05
MgSO ₄ ·7H ₂ O			0.05	0.025	0.1
FeSO ₄ ·7H ₂ O				0.001	0.001
MnSO ₄ ·4H ₂ O				0.001	0.001
Trace element solution ^{a)}			1ml/liter		
Thiamine			2mg/liter		
Biotin					100μg/liter
L-Isoleucine			0.002		
Meat extract	1				
Peptone	1	1			
Yeast extract	0.5	1			
NaCl	0.3	0.25			
CaCO ₃			2	2	2
pH	7.4	7.4	7.4	7.4	7.4

^{a)} The composition of the trace element solution was described in the text.

^{b)} The seed medium for *A. paraffineus*.

^{c)} The seed medium for *C. glutamicum* and *Bacillus* species.

solute alcohol. After 10 min, the optical density at 412 nm (molar absorbance=13,600) was measured. In the absence of OAH, there was no decline in sulfhydryl titer of cysteine.

Determination of protein. Protein concentrations were determined by the method of Lowry *et al.*¹⁷⁾

Chemicals. The purest sources commercially available were employed. OAH was synthesized by the method of Sakami and Toennis.¹⁸⁾ OSH was prepared from a culture broth of *A. aerogenes* KY 7025 (Met⁻).⁴⁾

RESULTS

1. OAH production by methionine auxotrophs of various bacteria

i) *A. paraffineus*. When a methionine-requiring mutant of *A. paraffineus*, KY 7137, was cultured in medium F-I supplemented with 2 mg/ml of DL-methionine, an unknown compound was produced. The compound gave a yellow-brown color spot (which later turned purple) on heating with ninhydrin at *Rf* 0.38 in paperchromatography using the solvent system, *n*-butanol-acetic acid-water (4: 1: 2). The compound was very unstable in mild alkaline solution but more stable in acid

solution. Heating an aliquot of the broth adjusted to 4 N in hydrogen chloride concentration at 100°C for 2 hr resulted in the disappearance of the spot corresponding to the compound and the appearance of a new spot corresponding to homoserine in paperchromatography. In 5 min after the broth was adjusted to pH 11, the compound decomposed. These properties of the compound agreed closely with those of OSH or OAH, an intermediate in methionine biosynthesis. Paperchromatographic mobilities of the compounds in several solvent systems were compared with those of authentic OSH and OAH. As shown in Table III, in all solvent systems the compound migrated identically to OAH but quite differently from OSH. All of these studies indicate that the compound produced by the strain KY 7137 is OAH. The amount of OAH produced by KY 7137 was 2.5 mg/ml. OAH was also found to be produced when KY 7137 was grown on sorbitol as a sole source of carbon.

Other methionine auxotrophs and the parental strain were examined with respect to

TABLE III. PAPERCHROMATOGRAPHIC MOBILITIES OF HOMOSERINE DERIVATIVES

Toyo Roshi No. 50 was used for paperchromatography. Spots were revealed by ninhydrin. Solvent A, *n*-butanol-acetic acid-water (4:1:2); solvent B, water-saturated phenol; solvent C, *n*-butanol-pyridine-water (1:1:1).

Substances	R _f -value		
	Solvent A	Solvent B	Solvent C
O-Succinylhomoserine	0.35	0.45	0.25
O-Acetylhomoserine	0.38	0.80	0.80
Compound produced by <i>A. paraffineus</i> KY 7137	0.38	0.80	0.80

TABLE IV. O-ACETYLHOMOSERINE PRODUCTION BY *A. paraffineus*

Each strain was cultured in 10 ml of medium F-I supplemented with 2 mg/ml of DL-methionine in 250 ml Erlenmeyer flask for 7 days at 30°C.

Strain No.	O-Acetylhomoserine produced mg/ml
KY 4302 (wild)	0
KY 7104 (Ile ⁻)	0
KY 7137 (Ile ⁻ , Met ⁻)	2.5
KY 8743 (Ile ⁻ , Met ⁻)	0
KY 8744 (Ile ⁻ , Met ⁻)	0

OAH production in F-I medium supplemented with 2 mg/ml of DL-methionine (Table IV). KY 4302 (wild type strain), KY 7104 (Ile⁻) and two methionine requiring mutants derived from KY 7104, KY 8743 and KY 8744 did not produce OAH. Growth responses of these auxotrophs to some intermediates of methionine biosynthesis were investigated (Table V). KY 7137 responded to methionine, homocysteine, or cystathionine but slightly to OAH, OSH or homoserine. On the other hand, KY 8743 and KY 8744 responded to methionine, homocysteine, cystathionine, OAH and OSH, but slightly to homoserine. OAH could fully be replaced by OSH in these strains. These results suggested that the blocked step in KY 7137 was between cystathionine and acylhomoserine and that in KY 8743 and KY 8744 the blocked step was between acylhomoserine and homoserine.

TABLE V. NUTRITIONAL RESPONSES OF VARIOUS METHIONINE AUXOTROPHS OF *A. paraffineus* TO METHIONINE AND THE INTERMEDIATES IN METHIONINE BIOSYNTHESIS

Growth responses were measured after 48 hr of incubation at 30°C.

Supplement to SMM ^{a)}	Amount μ g/ml	Growth ^{b)}		
		KY 7137	KY 8743	KY 8744
None	—	0.05	0.13	0.05
L-Homoserine	100	0.14	0.13	0.13
O-Acetyl-L-homoserine	100	0.18	0.57	0.35
O-Succinyl-L-homoserine	100	0.14	0.61	0.34
DL-Cystathionine	200	0.57	0.62	0.60
DL-Homocysteine	200	0.57	0.56	0.60
L-Methionine	100	0.53	0.53	0.53

^{a)} Sorbitol-minimal medium supplemented with 20 μ g/ml of L-isoleucine.

^{b)} Expressed as optical density at 660 nm.

ii) *Bacillus species*. A methionine auxotroph derived from *Bacillus* species KK, KY 8041, was also found to produce OAH. The amount of OAH produced by KY 8041 was 1.1 mg/ml. Other methionine auxotrophs derived from *Bacillus* species KK did not produce OAH (Table VI). As shown in Table VII,

TABLE VI. O-ACETYLHOMOSERINE PRODUCTION BY *Bacillus* SPECIES

Each strain was cultured in 10 ml of medium F-II supplemented with 100 μ g/ml of L-methionine in a large test tube for 3 days at 30°C.

Strain No.	O-Acetylhomoserine produced mg/ml
KK (wild)	0
KY 8042 (Met ⁻)	0
KY 8043 (Met ⁻)	0
KY 8041 (Met ⁻)	1.1
KY 8044 (Met ⁻)	0

KY 8041 responded to methionine, homocysteine and cystathionine but not to OAH, OSH or homoserine. Therefore, the blocked step in KY 8041 was suggested to be between acylhomoserine and cystathionine. OAH non-producing mutants showed patterns of growth response different from KY 8041 and the

TABLE VII. NUTRITIONAL RESPONSES OF VARIOUS METHIONINE AUXOTROPHS IN *Bacillus* SPECIES TO METHIONINE AND THE INTERMEDIATES IN METHIONINE BIOSYNTHESIS

Supplement to GMM ^{a)}	Amount $\mu\text{g/ml}$	Growth ^{b)}			
		KY 8042	KY 8043	KY 8041	KY 8044
None	—	—	—	—	—
L-Homoserine	20	—	—	—	—
O-Acetyl-L-homoserine	20	—	—	—	++
O-Succinyl-L-homoserine	20	—	—	—	±
DL-Cystathionine	40	—	—	+	+
DL-Homocysteine	40	—	++	++	++
L-Methionine	20	++	++	++	++

^{a)} Glucose-minimal medium

^{b)} —, no growth; ±, poor growth; +, moderate growth; ++, abundant growth.

blocked steps in these mutants seemed to be different from that in KY 8041. KY 8044 grew well on OAH but hardly grew on OSH in contrast with the results with *A. paraffineus* KY 8743 and KY 8744.

iii) *C. glutamicum*. Production of OAH by regulatory mutants of *C. glutamicum* has already been reported by the authors.¹⁰⁾ In this study, we report OAH production by a methionine auxotroph of the bacterium, TLD-32. OAH production by TLD-32 was affected by methionine added to the medium (Fig. 1). Optimal level of L-methionine for OAH production was about 100 $\mu\text{g/ml}$ when OAH was produced at a level of 1.5 mg/ml. Addition of higher level (200 $\mu\text{g/ml}$) of L-methionine repressed OAH production. Methionine requirement of TLD-32 could be satisfied by homocysteine but not by cystathionine, OAH or homoserine.

2. Homoserine-O-transacetylase

Presence of homoserine-O-transacetylase activity was confirmed in crude extracts of *A. paraffineus* KY 7104, *Bacillus* species KY 8043 and *C. glutamicum* TLD-25. When the extracts of these strains were incubated with succinyl-CoA, little acylation of homoserine could be detected while in the presence of

FIG. 1. Effect of Methionine on O-Acetylhomoserine Production in *C. glutamicum* TLD-32.

TLD-32 was cultured in 5 ml of medium F-III supplemented with increasing concentrations of L-methionine in a large test tube for 4 days at 30°C.

acetyl-CoA rapid formation of OAH was observed (Table VIII). *A. paraffineus* KY 8743 and KY 8744 and *Bacillus* species KY 8044 had no detectable enzyme activity.

TABLE VIII. ACYLATION OF HOMOSERINE CATALYZED BY EXTRACTS OF *A. paraffineus*, *Bacillus* SPECIES AND *C. glutamicum*

Source of extract ^{a)}	Acyl donor	Specific rate of reaction, nmole/min/mg protein
<i>A. paraffineus</i> (KY 7104)	Acetyl-CoA	11.9
	Succinyl-CoA	0
<i>Bacillus</i> species (KY 8043)	Acetyl-CoA	4.25
	Succinyl-CoA	0.9
<i>C. glutamicum</i> (TLD-25)	Acetyl-CoA	37.7
	Succinyl-CoA	0

^{a)} Extracts of *A. paraffineus* KY 7104, *Bacillus* species KY 8043 and *C. glutamicum* TLD-25 were prepared from cells grown on medium F-I, medium F-II supplemented with 20 $\mu\text{g/ml}$ of L-methionine and medium F-III supplemented with 10 $\mu\text{g/ml}$ of L-methionine, respectively.

3. Cystathionine γ -synthase

Cystathionine γ -synthase activities were proved to be present in the crude extracts of *A. paraffineus* KY 7104, *Bacillus* species KY 8043 and *C. glutamicum* TLD-25. The substrate specificities of the enzymes from these strains were examined (Table IX). The enzymes in the extracts of *Bacillus* species and *C. glutamicum* were specific for OAH and

TABLE IX. CYSTATHIONINE SYNTHESIS FROM
ACYLHOMOSERINE CATALYZED BY EXTRACTS
OF *A. paraffineus*, *Bacillus* SPECIES AND
C. glutamicum

Source of extract ^{a)}	Substrate	Specific rate of reaction nmole/min/mg protein
<i>A. paraffineus</i> (KY 7104)	O-Acetyl-L-homoserine	1.6
	O-Succinyl-L-homoserine	4.43
<i>Bacillus</i> species (KY 8043)	O-Acetyl-L-homoserine	193
	O-Succinyl-L-homoserine	33
<i>C. glutamicum</i> (TLD-25)	O-Acetyl-L-homoserine	12.6
	O-Succinyl-L-homoserine	0.25

^{a)} Extracts were prepared as described in the legend of Table VIII.

catalyzed at a much slower rate of reaction with OAH. On the contrary, the extract of *A. paraffineus* could utilize OSH better than OAH for cystathionine synthesis. The previous observation that *A. paraffineus* KY 8743 and KY 8744 could grow well on OSH as well as on OAH may be explained by the low specificity of cystathionine γ -synthase.

A. paraffineus KY 7137 and *Bacillus* species KY 8041, both of which produced OAH and grew on methionine, homocysteine or cystathionine but not on OAH or homoserine, had no detectable activities of cystathionine γ -synthase. *C. glutamicum* TLD-32, an OAH producer, had enzyme activity at the same level with that of TLD-25. On the basis of nutritional data, it may be concluded that TLD-32 is deficient in β -cystathionase.

DISCUSSION

Results presented here indicate that OAH is an intermediate in methionine biosynthesis in *A. paraffineus*, *C. glutamicum* and *Bacillus* species. Evidence was present from the following observations: (a) Methionine auxotrophs of these bacteria produced a large amount of OAH. Except for *C. glutamicum*,

the auxotrophs were deficient in cystathionine γ -synthase activity. (b) Cell-free extracts of these bacteria catalyzed the acetylation but not the succinylation of homoserine. (c) The methionine-requiring mutants of *Bacillus* species and *A. paraffineus* which could utilize homocysteine, cystathionine, OAH but not homoserine in place of methionine were deficient in homoserine-O-transacetylase activity. (d) Homoserine-O-transacetylase in *C. glutamicum* was subject to repression control by S-adenosylmethionine.¹⁹⁾ It is interesting that diversity was found in methionine biosynthesis in bacteria. An analogous diversity has been found to exist in the lysine pathway of bacteria, *i. e.*, N-succinyldiaminopimelic acid is an intermediate in lysine biosynthesis in *E. coli*,²⁰⁾ while the corresponding intermediate in *Bacillus* is N-acetyldiaminopimelic acid.^{21, 22)} The observations that diaminopimelic acid-requiring mutants of *A. aerogenes* and *S. marcescens* produced a large amount of N-succinyldiaminopimelic acid²³⁾ suggested that succinyl derivative of diaminopimelic acid is an intermediate in lysine biosynthesis in these bacteria belonging to *Enterobacteriaceae*. These observations suggest that acyl donor specificity in the aspartate pathway represents an evolutionary divergence between *Enterobacteriaceae* and Gram-positive rod bacteria such as *Bacillus*, *A. paraffineus*, *Brev. flavum* and *C. glutamicum*. At least in *Bacillaceae*, the parallel change in the specificity of acyl donor in both pathways would occur in evolutionary process; nevertheless, the chemical roles of acyl groups in each biosynthesis are quite different and the two acylation reactions are carried out by different enzymes. Brush and Paulus⁸⁾ have discussed that differences in the regulation of intermediary metabolism might bring about the change in the acyl donor specificity; the more efficient acyl donor was in one case acetyl CoA and in the other succinyl CoA. Weinberger and Gilvarg²²⁾ have demonstrated that the utilization of acetyl blocking group in diaminopimelate-lysine pathway was limited to the genus *Bacillus* and other bacteria including *A. globiformis*, *Micrococcus lysodeikticus*

utilized succinyl blocking group. From a taxonomic point of view, there may be the possibility that *A. paraffineus* or *C. glutamicum* also utilizes the succinyl blocking group in lysine pathway. In those bacteria, the parallel change in specificity of acyl donor in lysine and methionine pathway might not occur during evolutionary process.

In contrast with the specific utilization of acetyl-CoA for acylhomoserine synthesis in *A. paraffineus*, cystathionine γ -synthase of the bacterium was rather specific for OSH. The facts may be compatible with the result of the nutritional responses of methionine auxotrophs, KY 8743 and KY 8744; the mutants could grow well on OSH as well as on OAH. It has been reported²⁴⁾ that *S. typhimurium* cannot synthesize OAH but can utilize it in place of OSH for cystathionine synthesis. *Salmonella* cystathionine γ -synthase can form cystathionine about one-tenth as fast from OAH as from OSH. As mentioned above, the enzymes in *Bacillus* and *C. glutamicum* were specific for OAH and catalyzed much slow reactions with OSH. Thus, *A. paraffineus* might be an intermediary type between *Enterobacteriaceae* and *Bacillus* or *Corynebacterium* with respect to evolutionary change in the specificity of acyl groups in methionine biosynthesis.

REFERENCES

- 1) R. J. Rowbury, *Biochem. J.*, **81**, 42 p. (1961).
- 2) R. J. Rowbury and D. D. Woods, *J. Gen. Microbiol.*, **36**, 341 (1964).
- 3) M. Flavin, C. Delavier-Klutcho and C. Slaughter, *Science*, **143**, 50 (1964).
- 4) H. Kase, K. Nakayama and S. Kinoshita, *Agr. Biol. Chem.*, **34**, 274 (1970).
- 5) S. Nagai and M. Flavin, *J. Biol. Chem.*, **242**, 3884 (1967).
- 6) H. de Robichon-Szulmajster and H. Cherest, *Biochem. Biophys. Res. Commun.*, **28**, 256 (1967).
- 7) K. Nakayama, H. Kase and S. Kinoshita, *Agr. Biol. Chem.*, **33**, 1664 (1969).
- 8) A. Brush and H. Paulus, *Biochem. Biophys. Res. Commun.*, **45**, 735 (1971).
- 9) R. Miyajima and I. Shioo, *J. Biochem.*, **73**, 1061 (1973).
- 10) H. Kase and K. Nakayama, *Agr. Biol. Chem.*, **38**, 2021 (1974).
- 11) K. Takayama, S. Abe and S. Kinoshita, *Amino Acid and Nucleic Acid*, No. **19**, 121 (1969).
- 12) H. Kase and K. Nakayama, *Agr. Biol. Chem.*, **37**, 1643 (1973).
- 13) H. Kase and K. Nakayama, *ibid.*, **38**, 2235 (1974).
- 14) H. Kase, H. Tanaka and K. Nakayama, *ibid.*, **35**, 2089 (1971).
- 15) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).
- 16) M. M. Kaplan and M. Flavin, *J. Biol. Chem.*, **241**, 4463 (1966).
- 17) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *ibid.*, **193**, 265 (1951).
- 18) W. Sakami and G. Toennis, *ibid.*, **144**, 203 (1942).
- 19) H. Kase and K. Nakayama, *Agr. Biol. Chem.*, **39**, 161 (1975).
- 20) S. H. Kindler and C. Gilverg, *J. Biol. Chem.*, **235**, 3532 (1960).
- 21) S. Sundharadas and C. Gilverg, *ibid.*, **242**, 3983 (1967).
- 22) S. Weinberger and C. Gilverg, *J. Bacteriol.*, **101**, 323 (1970).
- 23) K. Nakayama, H. Kase and S. Kinoshita, *Agr. Biol. Chem.*, **34**, 282 (1970).
- 24) M. Flavin and C. Slaughter, *Biochim. Biophys. Acta*, **132**, 400 (1967).