GROWTH OF A GLUTAMIC ACID PRODUCING BACTERIUM AND RELATED BACTERIA

I. EFFECT OF IRON SALTS, FERRICHROME, AMINO ACIDS AND SOME OTHER COMPOUNDS

KIYOSHI NAKAYAMA, ZENROKU SATO
and SHUKUO KINOSHITA

*Tokyo Research Laboratory, Kyowa Hakko Kogyo Co., Ltd.
Machida, Tokyo.*

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Many bacteria have been reported to produce L-glutamic acid directly from sugar in high yield since the discovery of *Micrococcus glutamicus* by KINOSHITA *et al.* (1). Although they have been classified into various genera, they seem to constitute a group of bacteria consisting of intimately related members belonging to so-called coryne-form bacteria (2).

All were reported to require biotin for their growth, and the accumulation of glutamic acid occurs at a sub-optimum concentrations of biotin for growth (3). Some biotin-related substances or its precursors have been found to replace biotin (4, 5). A kind of surface-active agent (6) and a few plant hormones (7) have been reported to have biotin-like activity. Some strains require thiamine besides biotin (2). TERAMOTO *et al.* (8) have reported that ferrous sulfate in addition to potassium phosphate and magnesium sulfate promote the growth of the bacterium. They found that cystine and tyrosine promote growth and glutamic acid production. MIYAI *et al.* (9) also found cystine promotes the growth of one strain, though in another strain the effect was not clear. OKUMURA *et al.* (10) observed that some amino acids promote and others reduce the growth, but the effects were different in various strains. They found cysteine reduced the amount of diaminopelargonic acid required to replace biotin in glutamic acid fermentation. According to them, the effects of sulfur-containing amino acids were not noticed in the biotin-containing medium. Recently, OSANO *et al.* (11) have reported that a glutamic acid producing bacterium became to require some amino acids when incubated at high temperatures.

Formerly, the authors have reported the competition among some amino acids in supporting the growth of some amino acid-auxotrophic mutants of *M. glutamicus* (12). In the experimental media used, growth response conforming to the amount of amino acid added was noticeable within 24 hr with mutants. Recently it was found that the growth of the bacterium remains at a level less than 2 mg per ml even when the incubation was continued for several days. Accordingly, the present study was undertaken to investigate factors necessary to attain good growth in a

synthetic medium. This paper presents the results of these studies, especially the effects of iron salts, ferrichrome, amino acids and organic acids.

MATERIALS AND METHODS

Microorganism. *Micrococcus glutamicus* No. 534-348, an adenine-auxotrophic mutant derived from *M. glutamicus* No. 534 by UV-irradiation was used in this study. *Arthrobacter terregens* (13) No. 857 and *Arth. flavescens* No. 860 were supplied from Dr. A.G. Lochhead (Canadian Department of Agriculture, Central Experimental Farm, Ottawa).

Growth studies. The 24 hr growth on a nutrient agar slant was washed into 10 ml distilled water. After washing two times, one to three drops of the final suspension (10 ml) was used as an inoculum for 30 ml medium in a 250 ml Erlenmeyer flask. The final cell number was from 5×10^6 to 1.7×10^7 cells per ml. The flasks were incubated at 28° on a rotary shaker operating at 220 rpm. The media were autoclaved for 10 min at 1 kg per cm². Glucose in growth media was autoclaved separately from other components and added to the flasks before inoculation. An aliquot of the culture was centrifuged, the precipitate containing the cells was suspended in water, and diluted after solubilization of the calcium carbonate and iron salts, if necessary. Optical density of the diluted suspension was measured with a Beckman DU spectrophotometer. The amount of growth was represented as dry weight of the cells per ml after calculation by using a calibration curve (optical density *vs.* dry weight of the cells).

Materials. Ferrichrome (14) and ferrichrome A (15) were generously provided by Dr. J. B. NEILANDS (University of California). Ferroverdin (16) was obtained from Dr. E. B. CHAIN (International Research Center for Chemical Microbiology, Rome). Vitamin-free casamino acids and other reagents were available commercially. DL-Amino acids were used at 2-fold concentrations of L-amino acids.

RESULTS AND DISCUSSION

Effect of iron compounds, ferrichrome and ascorbic acid

The growth of *M. glutamicus* No. 534-348 remained at levels of about 1 mg per ml, even when casamino acids, L-tryptophan, L-cysteine and vitamin B₁₂ were added to the growth medium in addition to biotin and adenine. Consequently, the basal medium (Table 1) containing these substances was used in testing the activity of some other substances.

The following substances were ineffective:

(1) Compounds known as a growth factors in some microorganisms —oleic acid, linoleic acid, linolenic acid, lecithin, glutathione, vitamin A acetate, betaine hydrochloride, DL- α -lipoic acid, α -tocopherol acetate, menadione, spermine, N-acetylglucosamine, and sodium thioglycolate.

(2) Organic acids—sodium succinate, sodium citrate, sodium fumarate, sodium malate, sodium gluconate.

(3) Oils and surface-active agent—soybean oil, white mineral oil, and Tween 80.

(4) Combinations—vitamins plus bases, vitamins plus bases plus sodium thioglycolate (vitamin mixture contained: thiamine, riboflavin, *p*-aminobenzoic acid, pyridoxal, pantothenic acid, nicotinic acid, folic acid, choline and inositol; base mixture contained: uracil, guanine, xanthine and adenine).

Table 1. Composition of basal medium.

Constituents	Concentration
Glucose	5% (sterilized separately)
NH ₄ Cl	0.4%
K ₂ HPO ₄	0.2%
KH ₂ PO ₄	0.1%
MgSO ₄ ·7H ₂ O	0.05%
Biotin	30 µg/liter
Adenine	25 µg/ml
Casamino acids ^a	1.0%
Trace element solution	1 ml/liter
Trace element solution (per liter):	
Borax (Na ₂ B ₄ O ₇ ·10H ₂ O)	88 mg (B 10 µg)
Ammonium molybdate (NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	37 mg (Mo 20 µg)
Manganous chloride MnCl ₂ ·4H ₂ O	72 mg (Mn 20 µg)
Ferric chloride FeCl ₃ ·6H ₂ O	970 mg (Fe 200 µg)
Zinc sulfate ZnSO ₄ ·7H ₂ O	8.8 mg (Zn 2 µg)
Cupric sulfate CuSO ₄ ·5H ₂ O	20 mg (Cu 5.1 µg)

^a Free of vitamin. pH of the medium was adjusted to 7.3~7.4 with NaOH (before sterilization).

During the investigation of various substances, flasks supplemented with ferrous sulfide (100 mg powder per 30 ml) gave a growth of about 10 mg per ml in 48 hr. Since ferrous sulfide powder has been reported to be effective in continuously supplying ferrous ion required by a bacterium, it was tested in these experiments. On the other hand, ascorbic acid (5 mg per ml) gave a growth of about 6 mg per ml. Ascorbic acid was tested on the basis of the personal communication that this acid promoted the growth of *M. glutamicus* in culture media. Ascorbic acid and ferrous sulfide were effective also in the medium not supplemented with vitamins and bases. *M. glutamicus* is related to *Arthrobacter* in some taxonomical respects (2). Terregens factor is known as a growth factor in *Arthrobacter terregens* (13). Ferrichrome and aspergillilic acid can

replace the terregens factor (17). Hemin is also effective in *Arth. terregens* though at much higher concentrations (17). Ferrichrome, hydroxyaspergillic acid, a hydroxy derivative of aspergillic acid, and hemin were tested

Table 2. Effect of some compounds on growth of *M. glutamicus* 534-348.

Addenda to basal medium ^a	Concentration	Growth	
		24 hr	48 hr
Ferrichrome	5 μ g/ml	6.60	
	1 μ g/ml	4.31	
Ferrichrome A	5 μ g/ml	6.36	
	1 μ g/ml	5.65	
Hydroxyaspergillic acid	10 μ g/ml		2.52
L-Ascorbic acid	5 mg/ml	2.70	
Araboascorbic acid	5 mg/ml	2.58	
Hemin	1000 μ g/ml		ca.7.2
	100 μ g/ml		ca.2.3
Cytochrome C	100 μ g/ml		0.28
Ferrous sulfide	100 mg/30 ml		5.40
	10 mg/30 ml		0.78
FeSO ₄ ·7H ₂ O	100 μ g/ml	4.10	
FeSO ₄ ·7H ₂ O + L-Ascorbic acid	(5 mg/ml)		
	100 μ g/ml	4.60	
None		—	—

^a Basal Medium: following substances added to the medium shown in Table 1: L-tryptophan, 50 μ g/ml; L-cysteine, 50 μ g/ml; and vitamin B₁₂, 0.1 g/ml.

^b Milligram cells per ml (on dry weight basis).

with *M. glutamicus* No. 534-348. The results of the tests of some compounds including the above mentioned compounds are shown in Table 2. Ferrichrome, ferrichrome A and hydroxyaspergillic acid as well as araboascorbic acid and ferrous sulfate were effective. Hemin and cytochrome C had a slight effect at high concentrations. Ferroverdin, an iron-containing pigment from streptomyces, was ineffective.

As shown in Table 3, ferric chloride was also effective. Casamino acids were necessary to attain sufficient growth although tryptophan, cysteine and vitamin B₁₂ could be eliminated from the basal medium. The growth response of the bacterium to ferrichrome in the basal medium (Table 1) is shown in Fig. 1.

Relation between iron salt and ferrichrome

According to NEILANDS (18), ferrichrome seems to be concerned with

Table 3. Effect of Fe^{++} , Fe^{+++} , ferrichrome and L-ascorbic acid on growth of *M. glutamicus* 534-348 in various basal media.

Addenda to basal medium		Growth ^a in basal medium		
		A ^b	B ^b	C ^b
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	5 mg/ml	1.28	8.54	11.1
	2.5 mg/ml	1.12	4.70	8.8
	1.0 mg/ml	0	4.70	7.27
	500 $\mu\text{g}/\text{ml}$	0	4.10	7.00
	250 $\mu\text{g}/\text{ml}$	0	4.70	7.15
	100 $\mu\text{g}/\text{ml}$	0	0.75	5.42
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	5 mg/ml	trace	11.0	10.2
	2.5 mg/ml	0	6.94	6.81
	100 $\mu\text{g}/\text{ml}$	0	0.10	trace
Ferrichrome	10 $\mu\text{g}/\text{ml}$	1.41	6.71	7.15
	5 $\mu\text{g}/\text{ml}$	1.03	6.18	7.81
	1.0 g/ml	1.72	5.63	5.17
	0.1 g/ml	0	0.64	0.90
L-Ascorbic acid	10 mg/ml	1.41	5.17	6.10
	5 mg/ml	1.62	4.51	6.06
	1 mg/ml	1.85	3.40	5.36
L-Ascorbic acid (5mg/ml) + FeCl_3	5 mg/ml		7.93	11.40
	2.5 mg/ml		8.60	9.24
	100 $\mu\text{g}/\text{ml}$		3.52	2.70

^a Milligram cells per ml (on dry weight basis). Incubation period: 24 hr.^b Composition of basal media:

A: Same as in Table 1, except for that casamino acids were excluded.

B: Same as in Table 1.

C: B + L-tryptophan 50 $\mu\text{g}/\text{ml}$ + L-cysteine 50 $\mu\text{g}/\text{ml}$ + vitamin B₁₂ 0.1 $\mu\text{g}/\text{ml}$. Fig. 1. Growth response of *M. glutamicus* 534-348 to ferrichrome.

the utilization of iron in microorganisms. If this concept is correct, the effect of the ferrous sulfate may be synergistically increased by the presence of ferrichrome. But, as shown in Table 4, synergistic effect

Table 4. Additive effect of ferrichrome and ferrous sulfate at low concentrations^a.

Ferrichrome ($\mu\text{g/ml}$)	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ($\mu\text{g/ml}$)			
	0	125	500	1000
0	0	2.31	4.33	4.82
0.26	1.71	4.16	4.73	5.13
0.53	2.48	5.11	5.28	5.72
16.6	4.52	4.89	5.50	4.85

^a Values represent the growth of the bacterium after 24 hr (mg/ml).

between ferrous sulfate and ferrichrome could not be found and an additional effect was noted. Consequently, it is suggested that the bacterium does not require a large amount of iron salt, but rather the effect of a large amount of iron salt may be caused by the mutual action with another component in the medium. This speculation is also suggested by the fact that the iron salt added in medium remains as precipitate (possibly ferric hydroxide) until the end of the incubation. Consideration of the common characteristics between ferrichrome and ascorbic acid suggested the interaction with chelating agents, and the activity of some chelating compounds was demonstrated. These results will be described in the following paper, and here some observation on the effect of organic acids will be described.

The growth promoting effect of some organic acids in complex medium was found. Consequently, the effect of the combination of organic acids and iron salt was tested. As shown in Table 5, sodium citrate promoted

Table 5. Combination effect of ferrous sulfate and sodium citrate or sodium gluconate^a.

		$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ($\mu\text{g/ml}$)		
		125	500	2000
Sodium citrate (mg/ml)	0	2.31	4.33	4.82
	0.1	3.86	4.68	5.60
	1.0	5.40	6.05	7.31
	5.0	4.60	4.96	4.37
Sodium gluconate (mg/ml)	0.1	1.99	4.53	4.56
	1.0	3.36	3.68	4.62
	5.0	3.87	4.83	4.35

^a Values represent the growth of the bacterium after 24 hr (mg/ml).

the growth at a concentration below 1 mg per ml. Sodium gluconate promoted the growth at low concentrations ($125\text{ }\mu\text{g}$ per ml) of ferrous sulfate, but it had no promoting effect at its high concentrations. The growth response of the bacterium to ferric citrate and ferrous lactate is shown in Fig. 2.

Fig. 2. Response of *M. glutamicus* 534-348 to ferric-citrate and ferrous-lactate.
(—— ferric-citrate, ----- ferrous-lactate)

Comparison with *Arthrobacter terregens*

M. glutamicus responded to ferrichrome as well as to a high concentration of iron salt. Consequently, *Arthrobacter terregens* No. 857, a bacterium requiring ferrichrome, was tested to see if it also responds to high concentrations of iron salt. *Arth. flavescens* No. 860, another bacterium requiring ferrichrome, was also tested. In this investigation, the medium of LOCHHEAD and BURTON (13) was modified as follows: glucose, 10 g; NH_4Cl , 1 g; KNO_3 , 0.5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.01 g; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.01 g; yeast extract, 2 g; vitamin B_{12} , $4\text{ }\mu\text{g}$; water to 1 liter. *Arth. terregens* No. 857 and *Arth. flavescens* No. 860 did not respond to ferrous sulfate (4 levels: 2 mg, 1 mg, $500\text{ }\mu\text{g}$, $250\text{ }\mu\text{g}$ each per ml) added in the basal medium described above. Both organisms did not respond to yeast extract while *M. glutamicus* responded to it. Therefore, the behavior of *M. glutamicus* is different from that of the two arthrobacters, although all three bacteria responded to ferrichrome.

Effect of amino acids

As described before, casamino acids were necessary even in the presence of a large amount of iron salt or ferrichrome. Fig. 3 shows the growth response of the bacterium to casamino acids. A mixture of 21

Fig. 3. Response of *M. glutamicus* 534-348 to casamino acids. (Growth medium: basal medium (Table 1) + $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (2 mg/ml) + casamino acids indicated). Incubation: 24 hr.

amino acids substantially replaced the casamino acids, though the mixture (total 1.05 mg per ml) gave somewhat lower growth level than casamino acids (10 mg per ml). Therefore, we next attempted to determine the amino acids required by the bacterium. The result of a single omission test is shown in Table 6. Though the response to the complete mixture was lower than that of the mixture deficient in a single amino acid, the following amino acids were suggested to be important in the growth of the bacterium: glutamic acid, alanine, histidine, glutamine, cysteine, leucine, cystine, tyrosine, proline and aspartic acid. The results of single addition tests are shown in Table 7. The result of two experiments (Experiments I and II) were somewhat different and the important amino acids suggested were: Experiment I—lysine (only at 48 hr), cystine, leucine (only at 24 hr), phenylalanine, tyrosine, and histidine; Experiment II—methionine, cystine, leucine, phenylalanine, tyrosine, tryptophan, histidine, and cysteine.

From the results of single omission and single addition tests, the following amino acids were picked out as the important amino acids: cystine, leucine, tyrosine, histidine, glutamic acid, aspartic acid, alanine, cysteine and glutamic acid. The mixture of the above selected amino acids

Table 6. Effect of single amino acid omission from amino acid mixture.

Omitted amino acid	Growth ^a	
	24 hr	48 hr
L-Lysine	3.82	5.94
L-Arginine	4.25	5.88
L-Methionine	4.45	7.60
L-Cystine	3.94	3.72
L-Leucine	2.86	2.66
DL-Isoleucine	3.77	6.50
DL-Valine	3.56	7.20
L-Phenylalanine	4.04	7.40
DL-Tyrosine	3.94	3.26
L-Tryptophan	3.94	7.65
DL-Histidine	2.18	2.96
DL-Threonine	3.11	9.00
L-Glutamic acid	1.68	3.50
L-Proline	2.76	3.32
L-Aspartic acid	2.87	4.40
DL-Alanine	2.53	2.23
Glycine	2.43	5.78
L-Serine	3.52	6.05
DL- α -Aminobutyric acid	2.90	6.54
L-Cysteine	2.24	3.15
L-Glutamine	1.74	3.75
None (Amino acid mixture)	1.16	2.84
Amino acids were not added	0.37	2.11

^a Milligram cells per ml (on dry weight basis). Basal medium: Same as in Fig. 6.

and the mixture deficient in one of the amino acids were tested. The result of the experiment is shown in Table 8. Distinct reduction in growth was noted when any one of the following three amino acids was deficient: leucine, tyrosine and histidine.

Based on the above described experiments, the following amino acids were suggested to be important and the single addition at various concentration levels was tried: cystine, leucine, phenylalanine, tyrosine, tryptophan, histidine and cysteine. The result of the experiment is shown in Table 9. Though the single addition of these amino acids gave considerable growth in 48 hr, the growth in 24 hr was distinctly lower than the growth on the mixture of these amino acids (shown in Table 10).

Table 7. Effect of single amino acid addition to the growth of *M. glutamicus* 534-348.

Amino acid added	Exp. I		Exp. II	
	24 hr	48 hr	24 hr	48 hr
	mg/ml	mg/ml	mg/ml	mg/ml
L-Lysine	0.47	7.46	0.12	0.38
L-Arginine	2.10	3.26	0.04	0.31
L-Methionine	1.33	2.35	0.35	0.35
L-Cystine	0.43	4.52	0.27	1.01
L-Leucine	1.21	2.43	0.19	1.47
DL-Isoleucine	0.34	2.07	0.13	0.36
DL-Valine	0.71	3.25	0.12	0.42
L-Phenylalanine	0.95	6.80	0.54	2.45
DL-Tyrosine	1.30	5.76	0.49	4.22
L-Tryptophan	0.35	2.67	0.40	1.62
DL-Histidine	0.32	5.68	0.52	6.88
DL-Threonine	0.30	0.51	0.07	0.49
L-Glutamic acid	0.30	2.17	0.18	0.24
L-Proline	0.30	2.01	0.12	0.22
L-Aspartic acid	0.57	1.33	0.19	0.78
DL-Alanine	0.33	0.66	0.12	0.30
Glycine	0.33	0.55	0.16	0.27
L-Serine	0.285	2.04	0.19	0.23
DL- α -Aminobutyric acid	0.18	3.19	—	—
L-Cysteine	0.53	2.70	0.41	1.48
L-Glutamine	0.61	2.33	0.12	0.20
Not added	0.46	2.55	0.09	0.25

Exp. I. Each amino acid: 167 μ g/ml (DL-amino acid was added in 2-fold).

Exp. II. Each amino acid: 50 μ g/ml (DL-amino acid was added in 2-fold).

Basal medium: Same as in of Fig. 6.

Table 8. Effect of single amino acid omission from the mixture of important amino acids.

Amino acid omitted	Growth	
	24 hr	48 hr
None (mixture of amino acids ^a)	3.62 mg/ml	10.10 mg/ml
L-Cystine	3.42	8.93
L-Leucine	2.41	9.68
L-Tyrosine	2.18	5.90
L-Histidine·HCl·H ₂ O	2.20	8.61
L-Glutamic acid	3.80	10.50
L-Proline	3.74	10.19
L-Aspartic acid	3.72	9.98
DL-Alanine	3.97	11.55
L-Cysteine	3.97	10.21
L-Glutamine	3.97	10.92

^a Mixture of L-cystine, L-leucine, L-tyrosine, L-histidine, L-glutamic acid, L-proline, L-aspartic acid, DL-alanine, L-cysteine, and L-glutamine.
 Concentration of each amino acid was 33 μ g/ml (final).
 Basal medium: Same as in Fig. 6.

Table 9. Effect of some amino acid on growth of *M. glutamicus* 534-348.

Amino acid added		Growth (mg/ml)		Amino acid added		Growth (mg/ml)	
		24 hr	48 hr			24 hr	48 hr
L-Cystine	μ g/ml 200	0.86	5.48	DL-Tryptophan	μ g/ml 200	0.72	3.05
	100	1.41	5.52		100	1.61	2.00
	50	1.51	2.46		50	1.63	1.94
	10	1.81	2.31		10	1.75	1.45
L-Leucine	200	1.27	1.49	DL-Histidine	200	0.70	5.92
	100	1.06	1.72		100	1.08	11.13
	50	2.06	6.26		50	1.26	8.61
	10	1.00	1.74		10	0.65	2.21
L-Phenylalanine	200	1.53	6.57	L-Cysteine	200	1.69	3.40
	100	1.59	2.84		100	1.13	5.52
	50	1.40	2.55		50	1.37	7.35
	10	1.75	3.31		10	0.87	4.33
DL-Tyrosine	200	1.34	4.83	None		0.65	1.77
	100	2.01	2.52				
	50	1.08	4.83				
	10	0.86	2.63				

Basal medium: Same as in Fig. 6.

Table 10. Effect of some amino acid combinations on growth of *M. glutamicus* 534-348.

Amino acid added	Growth (mg/ml)	
	24 hr	48 hr
C+L+P+Tr+Tp+H+Cy	4.91	10.90
L+Tr+H	4.43	8.42
L+Tr+H+C	4.96	9.22
L+Tr+H+Cy	5.61	11.30
L+Tr+H+C+P	5.98	8.42
L+Tr+H+C+Tp	4.07	7.77
C+L+H+Tp	3.65	8.65
C+L+H+P	4.85	9.14
None	0.65	1.77

C: L-cystine
 L: L-leucine
 P: L-phenylalanine
 Tr: DL-tyrosine
 Tp: DL-tryptophan
 H: DL-histidine
 Cy: L-cysteine (each 50 μ g/ml)

Fig. 4. Response of *M. glutamicus* 534-348 to casamino acids and mixture of amino acids (L-cysteine, L-leucine, DL-histidine and DL-tyrosine). Basal medium: Same with that of Fig. 6.

×—× Amino acids, ●---● Casamino acids.

Leucine, histidine and cysteine gave a rather low growth at higher concentrations than the optimum concentration of each amino acid. Close biosynthetic relationships exist between cystine and cysteine, and among each aromatic amino acid. Accordingly, the mutual replacement effect of the each amino acid in the two groups of amino acids was tested. The result of the experiment is shown in Table 10. Cystine and cysteine were replacable by each other, but cysteine was slightly more effective than cystine. Aromatic amino acids were also replaced by each other, though tryptophan was less effective than others.

There are competitive relationships between some amino acids. Since the relationships among each amino acid in the mixture is very complex, it seems difficult to obtain clearcut results. But, the most important amino acids may be limited to the following ones: cysteine or cystine, leucine, histidine, and phenylalanine or tyrosine or tryptophan. Comparison of the effects of casamino acids and the mixture of the four amino acids described above, is shown in Fig. 4. This mixture could replace casamino acids to the extent of about one-twentieth in effective concentration. In this experiment, considerable growth was obtained after 48 hr even in the absence of casamino acids. As indicated by this result, the necessity of amino acids is apt to fluctuate with the inoculum used, and considerable growth was sometimes obtainable with long incubation periods. The growth levels of 20 mg per ml at 48 hr suggests the occurrence of the reversion of the adenine-auxotrophy to prototype.

The bacterium used in the present study was an adenine-auxotrophic mutant of *M. glutamicus*. But the result found in this study may be applicable to the parent bacterium except the requirement of adenine. The validity of this consideration will be confirmed by the experiment described in a later paper.

SUMMARY

The growth of a strain of the glutamic acid producing bacterium *Micrococcus glutamicus* remained at very low levels even when the incubation was continued for several days. The addition of both casamino acids and ferrichrome to the synthetic medium was necessary to increase the growth level of the bacterium.

Ferrichrome could be replaced by the following compounds: ferrichrome A, hydroxyaspergillilic acid, large amounts of ascorbic acid or araboascorbic acid, and large quantities of iron salts.

Cysteine or cystine, histidine, leucine and any one of the aromatic amino acids were concluded to be most important amino acids. The mixture of these four amino acids could replace casamino acids.

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