L-Glutamic Acid Fermentation

Part II. The Production of L-Glutamic Acid by an Oleic Acid-Requiring Mutant*

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The effect of biotin, oleic acid and other unsaturated fatty acids on the production of L-glutamic acid was investigated by using an oleic acid-requiring mutant of *Brevibacterium thiogenitalis* No. 653. The results described in the present paper showed that the oleic acid-requiring mutant D-248 produced a large amount of L-glutamic acid in the excess biotin-containing media, and that oleic acid seemed to be completely replaced by other unsaturated fatty acids such as palmitoleic acid and linoleic acid.

INTRODUCTION

In the previous paper,¹⁾ the authors reported that the oleic acid-requiring mutant of *Brevibacterium thiogenitalis* No. 653 showed no growth response to biotin.

It has been well known that L-glutamic acid-producing bacteria generally require biotin for their growth and, in a biotin-rich medium, they grow vigorously and do not accumulate L-glutamic acid.

The present investigation is undertaken to examine the effect of biotin, oleic acid and other unsaturated fatty acids on the production of L-glutamic acid.

MATERIALS AND METHODS

Organisms. *Brevibacterium thiogenitalis* No. 653 and its oleic acid-requiring mutant, D-248, were used.

Culture. The culture conditions for shaker and 200-l fermentor are shown in Tables I and II, respectively.

Estimation of bacterial growth. Bacterial growth was conveniently indicated by optical density. The culture broth was diluted twentyfold and its optical density was measured at 590 m μ .

Determination of L-glutamic acid. L-Glutamic acid accumulated was determined by manometric method with the decarboxylase of *Escherichia coli*.

TABLE I. CULTURE CONDITIONS FOR SHAKER

Kind of medium	Seed (%)	Main (%)
Articles	(%)	(%)
Glucose	2.0	10.0
Urea	0.5	0.3
CSL*	0.5	0.3
KH ₂ PO ₄	0.1	0.1
MgSO ₄ ·7 aq	0.04	0.04
Na-oleate	100 μ g/ml	50~2000 μ g/ml
Biotin	—	0~300 μ g/l
Phenol red	—	10 μ g/l
Temperature	28°C	32°C
Volume/200-ml flask	20 ml	15 ml
r.p.m.	200	200
Inoculum size	1 loopful	5%
Culture time	16 hr.	48 hr.
pH	7.0 (start)	7~8**

* This work was presented at the 236th Meeting of Kansai Division of Agricultural Chemical Society of Japan, held on February 25, 1967, at Kyoto.

1) T. Kanzaki, K. Isobe, H. Okazaki, K. Motizuki and H. Fukuda, This Journal, **31**, 1307 (1967).

* CSL: corn steep liquor.

** Controlled with urea during the fermentation.

TABLE II. CULTURE CONDITIONS FOR 200-L FERMENTOR

Kind of medium	Seed (%)	Main (%)
Articles		
Cane molasses	—	13.0 (as total sugar)
Glucose	2.0	—
Urea	0.5	0.5
CSL	1.0	0.5
KH ₂ PO ₄	0.1	—
MgSO ₄ ·7 aq	0.04	—
Na-oleate	100 µg/ml	150 µg/ml
Temperature	28°C	32°C
Agitation	200 r.p.m.	250 r.p.m.
Aeration	15 l/min.	30 l/min.
Culture time	18 hr.	48 hr.
Volume of medium	30 l	100 l
Fermentor capacity	50 l	200 l
pH	7.0 (at start)	7.2~7.4*

* Controlled with ammonium hydroxide during the fermentation.

Chemicals. Oleic acid, palmitoleic acid and linoleic acid were obtained commercially and their purities analyzed by gas chromatography were 90%, 99% and 95%, respectively.

RESULTS

Effect of Oleic Acid Concentration on the Growth and the Production of L-Glutamic Acid

The data presented in Fig. 1 show that the growth-stimulating effect of oleic acid on D-248 was exhibited gradually up to 0.7 mg/ml, and yet its effect was not changed by the concentration of biotin. On the other hand, the growth of the parent strain, No. 653, was stimulated by a small amount of biotin contained in corn steep liquor, which was added to the medium for the purpose of exclusion of the growth-inhibitory effect of oleic acid and by the addition of which the effect of oleic acid on the growth was reduced considerably.

In relation to the production of L-glutamic acid, both No. 653 and D-248 accumulated a large amount of L-glutamic acid when a suitable amount of biotin or oleic acid was added to the medium. In the medium which contained excess biotin, however, the productivity of L-glutamic acid of D-248 was not remark-

FIG. 1. Effect of Oleic Acid Concentration on Bacterial Growth.

●—● 30 µg/l of biotin added.
○---○ No biotin added.

FIG. 2. Effect of Oleic Acid Concentration on the Production of L-Glutamic Acid.

●—● 30 µg/l of biotin added.
○---○ No biotin added.

ably changed but that of No. 653 was reduced to nearly zero as shown in Fig. 2.

Effect of Biotin Concentration on the Growth and the Production of L-Glutamic Acid

In view of the fact that the effect of biotin on the growth and the production of L-glutamic acid was different between the two strains as mentioned above, the effect of biotin was studied in detail. Figs. 3 and 4 show the growth response and the productivity of L-glutamic acid in these strains.

In D-248, the growth and the productivity of L-glutamic acid if in the presence of oleic acid, were not affected by biotin concentration. The growth of No. 653 was dependent on biotin and the growth-stimulating effect of oleic acid in this case was apparently reduced in the biotin-containing medium.

The production of L-glutamic acid by No. 653 was decreased in proportion to increasing

FIG. 3. Effect of Biotin Concentration on Bacterial Growth.

●—● 300 µg/ml of oleic acid added.
○---○ No oleic acid added.

FIG. 4. Effect of Biotin Concentration on the Production of L-Glutamic Acid.

●—● 300 µg/ml oleic acid added.
○---○ No oleic acid added.

biotin concentration and so almost no L-glutamic acid was produced when more than 5 to 10 µg/l of biotin was added to the medium.

L-Glutamic Acid Fermentation with Cane Molasses

As mentioned above, it was evident that the production of L-glutamic acid was not affected by the biotin concentration in the media when D-248 was used.

It is well known that cane molasses contains a large amount of biotin-vitamer and, without any treatment such as the addition of penicillin²⁾ or a surface-active agent,^{3~5)} the

FIG. 5. L-Glutamic Acid Fermentation with Cane Molasses.

●—● D-248. ○---○ No. 653.

normal L-glutamic acid fermentation is not expected.

When the culture conditions shown in Table II were employed, a time course of the fermentation is presented in Fig. 5.

As might have been expected, there was no difference between the two strains in the consumption rate of sugar, but, in respect of L-glutamic acid accumulation, a marked difference was observed.

Only 5 mg/ml of L-glutamic acid was accumulated when No. 653 was used, but, in D-248, 63 mg/ml of L-glutamic acid was accumulated and the yield of over 50%, which was calculated from the consumed sugar basis, was obtained.

Effect of Palmitoleic Acid and Linoleic Acid on the Production of L-Glutamic Acid

As reported previously,¹⁾ D-248 was able to grow in the presence of an unsaturated fatty acid in place of oleic acid. Then palmitoleic acid and linoleic acid were tested in this experiment.

The productivity of L-glutamic acid was

2) N. L. Somerson and T. Phillips, Belg. Patent, No. 593,807 (1961).

3) K. Udagawa, S. Abe and S. Kinoshita, *J. Ferment. Tech.*, **40**, 614 (1962).

4) K. Yamada, J. Takahashi and J. Nakamura, *J. Ferment. Assoc. Japan*, **20**, 348 (1962).

5) H. Okada, K. Takinami and T. Tsunoda, *This Journal*, **28**, 114 (1964).

TABLE III. EFFECT OF PALMITOLEIC ACID AND LINOLEIC ACID ON THE PRODUCTION OF L-GLUTAMIC ACID

C_{16}^{**} $\mu\text{g/ml}$	50		100		200		300	
	L-GA*	O.D.	L-GA	O.D.	L-GA	O.D.	L-GA	O.D.
Biotin 0 $\mu\text{g/l}$	6.0	0.15	15.0	0.23	36.5	0.43	42.0	0.49
30	7.0	0.17	10.0	0.19	35.5	0.42	42.5	0.47

C_{18}^{**} $\mu\text{g/ml}$	200		300		500		700	
	L-GA*	O.D.	L-GA	O.D.	L-GA	O.D.	L-GA	O.D.
Biotin 0 $\mu\text{g/l}$	15.5	0.15	27.5	0.27	37.0	0.38	39.0	0.41
30	22.0	0.15	35.5	0.28	41.5	0.39	40.0	0.48

* L-Glutamic acid produced mg/ml.

** C_{16}^{**} Palmitoleic acid, C_{18}^{**} linoleic acid.

not changed when one of these unsaturated fatty acids was used in place of oleic acid as shown in Table III.

Therefore, it seems reasonable to assume that these unsaturated fatty acids can replace for oleic acid in the L-glutamic acid fermentation.

DISCUSSION

The methods of L-glutamic acid fermentation with the excess biotin-containing media have been studied by several groups of investigators in recent years²⁻⁷⁾ and it seems that the most useful methods are the addition of penicillin or a surface-active agent to the culture medium.

On the other hand, K. Tanaka, K. Oshima, K. Kimura and S. Kinoshita⁸⁾ reported that adenine-requiring mutants of *Micrococcus glutamicus* accumulated L-glutamic acid in biotin-rich media but its yield was low as compared with that in the biotin-deficient media.

R. Kodaira, S. Ono, U. Akimoto and O. Kanemitsu⁹⁾ also reported interesting pheno-

mena using histidine-or/and purine-requiring mutants of *Microbacterium ammoniaphilum*.

The reason why the oleic acid-requiring mutant can produce a large amount of L-glutamic acid in the biotin-rich media can not yet be explained.

However, the present data suggest that oleic acid was more closely related to L-glutamic acid fermentation than biotin, and the function of oleic acid was completely substituted by palmitoleic acid or linoleic acid.

Recent works^{10,11)} have shown that oleic acid is one of the constitutive cellular fatty acids of the L-glutamic acid-producing bacteria and, according to current hypothesis,^{10,11)} the cellular acids of bacteria are closely related to the productivity of L-glutamic acid.

Then, it will be worthwhile to consider the role of oleic acid in the L-glutamic acid-producing bacteria, the relation between oleic acid and biotin, and the relation between oleic acid and other unsaturated fatty acids.

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8) K. Tanaka, K. Oshima, K. Kimura and S. Kinoshita, *Amino Acid and Nucleic Acid*, **11**, 106 (1965).

9) R. Kodaira, S. Ono, U. Akimoto and O. Kanemitsu, *The Symposium of Amino Acid and Nucleic Acid*, November 11, 1965, at Nagoya.

10) T. Otani, S. Ôtsuka, R. Tsugo and S. Okumura, *Amino Acid and Nucleic Acid*, **11**, 89 (1965).

11) M. Shibukawa, K. Komatsu, H. Takahashi and T. Ohsawa, *J. Agr. Chem. Soc. Japan*, **39**, 150 (1965).