L-GLUTAMIC ACID AND SUCCINIC ACID FERMENTATION BY *BREVIBACTERIUM* FLAVUM NO. 1996*

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INTRODUCTION

As regards the "L-glutamic acid fermentation", particularly the production of a great deal of L-glutamic acid in the broth from glucose with aerobic bacteria, such as, *Micrococcus* (1, 2), *Brevibacterium* (3, 4, 5), *Corynebacterium* (5), *Bacillus* (2, 6), . . . ; there have been many reports on the subject.

These microbes metabolize glucose and produce L-glutamic acid in the presence of excessive NH_4 -ion, and α -ketoglutaric acid (α KG) in the presence of a small amount of NH_4 -ion both through the pathway of TCA-cycle (11, 12, 13, 14). According to these studies the existence of the members in the TCA-cycle and of their related substances in the broth has naturally been recognized, for instance, alanine, aspartic acid, and valine etc. as amino acids, and pyruvic acid, lactic acid, α KG and succinic acid, etc. as organic acids.

However, the production of a great deal of succinic acid and the existence of L-glutamine in the broth of the L-glutamic acid fermentation has so far been never described.

Meanwhile there have been many reports on the succinic acid in the fermentation broth. The fermentative production of succinic acid may be classified into three parts according to substrates, i.e. (1) amino acids, (2) organic acids and (3) carbohydrates. Its production, the amount of which is minor, from amino acids and organic acids has been studied by workers (31, 32), and above all K. SAKAGUCHI and S. TADA (7) isolated bacteria from dairy products, one of which, *Bacterium succinicum*, produces a great deal of succinic acid from organic acids such as citric acid, tartaric acid, etc. under an anaerobic condition (2.98 g of succinic acid from 5.4 g of citric acid . . .). And yet its production from glucose has only once been reported by L. B. LOCKWOOD et al. (8) with imperfect fungi, especially with *Fusarium* species, in submerged culture (15 g glucose $\longrightarrow$ 1.25 g succinic acid). While in the L-glutamic acid fermentation, according to a report by M. TANAKA (23), K. YAMADA (3) and K. TANAKA (10) the succinic acid was accumulated in the

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broth, but its amount was very small. On the other hand, we found that succinic acid results in large quantity from glucose (9.4 g glucose $\rightarrow$ 2.8 g succinic acid) in submerged fermentation with *Brevibacterium flavum* No. 1996, one of the L-glutamic acid producing bacteria. Since it is conceivable that succinic acid is able easily to be manufactured industrially by means of this fermentation, we would like to term this as the succinic acid fermentation.

This report deals with the succinic acid fermentation by means of L-glutamic acid producing bacteria.

MATERIAL AND METHODS

i) Strains used:

Brevibacterium flavum No. 1996 was mainly used and in addition L-glutamic acid producing bacteria belonging to genera *Brevibacterium* and *Bacillus* were employed. Bouillon agar slant was used as a stock culture.

ii) Inoculum:

The constitution of the medium used for inoculum preparation is as follows: 5% of glucose, 0.8% of urea, 0.1% of KH_2PO_4 , 0.04% of $\text{MgSO}_4 \cdot 7\text{aq.}$, 0.3% of corn steep liquor (C.S.L.), 2 γ /L of biotin and pH 7.0. One loopful of this microbe grown on a stock slant medium for 24 hrs. at 30°C was transferred into 100 ml of this sterilized liquid medium in a 500 ml flask and was incubated for approximately 18 to 24 hrs. at 30°C by shaking generated by a reciprocal shaker.

iii) Fermentation:

As the constitution of fermentation medium, throughout this work, 9 to 10% of glucose, 0.8% of urea, 0.1% of KH_2PO_4 and 0.04% of $\text{MgSO}_4 \cdot 7\text{aq.}$ were employed as carbohydrate, nitrogen and mineral sources, but the amounts of C.S.L. and biotin, employed as growth factors for this microbe, were modified as indicated in several tables and figures. Also, pH of the medium was adjusted by H_2SO_4 and NaOH to about 7.0 after sterilization by autoclaving at 115°C for 10 min. The volume of the liquid medium in a flask was varied in accordance with the level of aeration. Throughout this work, the above mentioned seed was inoculated to this sterilized medium, the inoculum size being 5%. Fermentation was carried out at 30° to 31°C mainly on the reciprocal shaker (115 c.p.m., 70 mm of amplitude) and also on the rotary shaker (200 r.p.m., 75 of radia) and Monod shaker (55 r.p.m., 25° of angle) as indicated in the respective tables. During the culture pH of broth was maintained at a feeble alkali-side (pH 7.0 to 8.0) by feeding 45% aq. soln. of urea.

iv) Analysis:

Glucose was determined according to the method of FEHLING-LEHMANN-SCHOORL, and the degree of bacterial growth was measured by turbidity.

The amount of L-glutamic acid was estimated manometrically using the

L-glutamic acid decarboxylase preparation obtained from the resting cell of *E. coli crooks* (25) and bioassay using *Lact. arabinosus* 17-5 (26). The amount of succinic acid was determined manometrically using the succinic dehydrogenase in the muscle of pig's heart (27).

Alanine was quantitatively estimated according to the bioassay using *Leuc. citrovorum* 8081 (28), and then α KG and lactic acid were colorimetrically by means of FRIEDMANN HAUGEN's method (29) and BARKER and SUMMERSON's method (30), respectively. For determining the rate of oxygen supply, the sulfite oxidation method indicating Kd (9) (g-mol/cc. min. atm.) was adopted.

RESULTS OF EXPERIMENTS

- (1) Production of succinic acid by L-glutamic acid producing bacteria, and isolation and identification of succinic acid.

An increase in the volume of medium and the concentration of C.S.L. in a reciprocal flask of 500 ml resulted in activating the succinic acid production as shown in Table 1.

Table 1. Factors of succinic acid formation.

Exp. No.	CSL (g/dl)	Biotin (γ /L)	Liquid volume (ml)	Succinic acid (mg/ml)	L-Glutamic acid (mg/ml)	Residual glucose (mg/ml)	Incubation time (hr.)
1	0.3	2	20	1.95	35.7	6.4	48
2	0.3	2	300	18.34	4.0	10.8	72
3	1.0	2	20	6.16	15.7	6.8	48
4	1.0	2	300	19.42	5.4	6.6	72
5	3.0	2	20	8.26	0.2	6.8	48
6	3.0	2	300	17.18	0.1	30.1	72

Initial glucose: 9.7 g/dl, in 500 ml reciprocal shake flask.

For isolation of succinic acid from the broth, ether-extraction method was adopted.

The results of its identification are as follows:

Paper partition chromatograms....

Rf: authentic sample

0.42 0.43 ethanol-ammonia-water (80-5-10)

0.69 0.68 isoamylalcohol-formic acid-water (5-1-4)

Melting test

M.P.

(1) authentic succinic acid 185°C

(2) sample 185°C

(3) mixture of (1) and (2) 185°C

Elementary Analysis

Anal. Calcd. For $C_4H_6O_4$: C: 40.68%, H: 5.12%, O: 54.20%

Found: C: 41.07%, H: 5.31%, O: 53.62%

Succinic dehydrogenase test

enzyme source: heart-muscle of pig, O₂-absorption by Warburg manometry, recovery of sample: 100.9%

For infra-red spectra see Fig. 1.

Fig. 1. Infrared spectrum of sample and standard succinic acid.
(1): authentic succinic acid (2): sample

These data confirmed the fermentation product to be succinic acid.

(2) Effect of aeration and C.S.L. content on the succinic acid production

To determine the effect of aeration, fermentation was carried out at several levels of aeration with the changing volume of medium in the flask of 500 ml. Furthermore, C.S.L. was added for determination of its effect to each medium representing several different concentrations as noted in Table 2. In proportion to lessening aeration and increasing C.S.L. concentration,

Table 2. Production of L-Glutamic Acid and Succinic Acid.

Liquid volume	20	100	300	400	500
Kd. $\times 10^6$ CSL (g/dl)	6.5	3.2	0.84	0.65	0.41
0.3	2.1 38.4	10.5 16.3	20.6 5.9	21.3 3.2	20.2 (33.9) 0.5
0.7	6.7 36.4	17.8 17.9	24.0 4.5	20.4 (25.7) 2.5	26.6 (36.9) 0.2
1.0	9.4 25.8	19.9 12.5	24.5 3.2	28.2 (30.1) 1.6	26.8 (35.8) 0.1
2.0	13.5 16.7	22.9 11.4	25.2 0.9	27.1 (32.0) 0.3	27.6 (37.2) 0.1

In 500 ml reciprocal shake flask, at 30°, biotin: 2 γ /L, above the line: yield of succinic acid for initial glucose (%), below the line: yield of L-glutamic acid for initial glucose (%), in parentheses: yield of succinic acid for consumed glucose (%).

an increase was observed in the amount of succinic acid production.

For the highest yield of succinic acid, the optimal aeration rate was computed at K_d of 0.4 to 0.7×10^{-6} and the optimal concentration of C.S.L. was estimated to be 1.0 to 2.0% . Under this condition, its yield amounted to 38.3% of initial glucose. In contrast, the condition of K_d of 6.5×10^{-6} with 0.3% of C.S.L. was found to be optimal for L-glutamic acid production, the maximum yield of which reached 38.4% of initial glucose. While at K_d of 3.2×10^{-6} with 0.7% of C.S.L., the amounts of succinic acid and of L-

Fig. 2. L-glutamic and succinic acid fermentation in shake culture.

[I]: L-glutamic acid fermentation, 0.3% CSL, 6.5×10^{-6} Kd.

[II]: succinic acid fermentation, 1.5% CSL, 0.4×10^{-6} Kd.

—○—: residual glucose, —●—: succinic acid, —×—: L-glutamic acid, ---●---: lactic acid, ---×---: α KG, ---○---: turbidity ($-\log T$).

glutamic acid production were 17.8 and 17.9%, respectively, of initial glucose. As is manifested, possibility to convert the fermentations was observed.

Also under the "less aerobic" condition (defined as the succinic acid producing condition where aeration is limited) of producing succinic acid, growth of bacteria and utilization of glucose were much slower than those under a "more aerobic" condition (defined as the L-glutamic acid producing condition where aeration is not limited), but the amount of yielding succinic acid for consumed glucose was very large, for example, at Kd of 0.41×10^{-6} , it attained to 37%.

On the other hand, the decomposition of succinic acid was observed at later stage of fermentation under the "more aerobic" condition, but under the opposit condition, no decomposition was observed (see Fig. 2).

Under the anaerobic condition, which presumably brings about better results in the succinic acid production, more lactic acid (in surface culture) than succinic acid was produced as shown in Table 3 (80% and 21% of con

Table 3. Production of Succinic Acid in Anaerobic culture (Surface Culture)

Exp. No.	Method of time	Incubation time (hr)	Turbidity ($-\log rT$)	Residual glucose (mg/ml)	L-glutamic acid (mg/ml)	Succinic acid (mg/ml)	Lactic acid (mg/ml)
1	400 ml surface	72	0.47	19.3	0.4	—	31.2
		96	0.48	10.8	0.6	31.8	33.0
		120	0.49	6.5	0.8	31.6	26.4
2	20 ml surface	120	0.24	66.3	0.2	8.6	30.3
		216	0.23	51.7	0.2	13.1	38.3
3	400 ml surface	120	0.18	72.9	0	6.1	19.3
		193	0.15	60.7	—	—	26.4
		216	0.15	47.4	0.1	11.4	41.9

9.9 g/dl initial glucose, 1.5 g/dl CSL, 2 γ /L biotin, in 500 ml reciprocal shake flask, at 30°.

sumed glucose, respectively). From these a moderate aeration (a "less aerobic" condition) was required for a good yield of succinic acid.

In contrast to the preceding experiments in which reciprocal shake cultures were used, the effect of several methods in shake culture, i.e. rotary shake culture (rotary-motion) and Monod shake culture (angle-motion), was studied as shown in Table 4.

Two phases in fermentation, namely, (1) growth of bacteria and enzyme-system formation at earlier stages and (2) the acids-producing enzyme-reactions (glucose $\rightarrow$ L-glutamic acid and succinic acid) at later stages in fermentation, were separately studied as shown in Table 5. Throughout fermentation, a high level aeration resulted in high yield of L-glutamic acid (Exp. No. 3), whereas a low level aeration produced high yield in succinic acid (Exp. No. 4). But the L-glutamic acid production by means of high aeration only during earlier stage (Exp. No. 1) was inferior to that throughout

Table 4. Effect of Method in Shake Culture on Succinic Acid Formation.

Method of shake culture	Liquid volume (ml)	Kd. $\times 10^6$	CSL (g/dl)		
			0.7	1.0	2.0
Reciprocal	200	1.8	21.2	19.6	21.7
	500	0.4	20.1	26.4	27.1
Rotary	100	0.8	19.7	20.2	19.7
	200	0.4	25.8	25.5	25.5
Monod	20	0.6	21.7	23.7	23.9

9.4 g/dl glucose, 2 γ /L biotin, at 30°, unit: mg/ml.

Table 5. Effect of Charge of Aeration on L-Glutamic and Succinic Acid Fermentation.

Exp. No.	Incubation time	10 hrs.			72 hrs.		
	Liquid volume (ml)	turb.	r. s.		turb.	suc.	glut.
1	20	0.69	78.5	increase liquid volume to 400 ml after 10 hrs. $\rightarrow$	0.75	18.9	1.7
2	400	0.32	83.6	decrease liquid volume to 20 ml after 10 hrs. $\rightarrow$	1.06	1.9	6.8
3	20	—	—		1.09	1.0	17.1
4	400	—	—		0.45	23.8	1.0

Medium: 93.0 mg/ml glucose, 1.5% CSL, biotin: 2 γ /L, in 500 ml reciprocal shake flask, unit: mg/ml, r.s.: residual sugar, suc.: succinic acid, glut.: L-glutamic acid, turb.: turbidity ($-\log T$).

Table 6. Effect of Biotin on Succinic Acid Formation.

Liquid volume (ml)	CSL (g/dl)	Biotin (γ /L)	Incubation time (hr.)	Succinic acid (mg/ml)	L-glutamic acid (mg/ml)	Lactic acid (mg/ml)
20	0.3	0	48	1.87	16.2	0.4
		10	48	3.90	3.4	0.3
	1.5	20	48	0.13	1.6	0.2
		20	48	0.82	0.9	0.3
400	0.3	20	72	19.92	1.3	22.0
		0	72	24.70	0.9	43.5
	1.5	2	72	24.50	0.7	30.2
		20	72	23.90	0.7	34.0

On reciprocal shaker, in 500 ml flask, at 30°, 95.3 mg/ml glucose.

fermentation, and similarly a low level aeration only during the same stage resulted in a low yielding succinic acid, as shown in Exp. No. 2.

(3) Effect of biotin concentration on the production of succinic acid and lactic acid

K. TANAKA et al. recently reported that L-glutamic acid production, with *Micrococcus glutamicus*, depends upon the concentration of biotin in the broth. Its effect on succinic acid production was examined as noted in Table 6. Its high concentrations were of no avail in its production regardless of the aeration, although its effect is not clearly determinable as it is contained in C.S.L.

(4) Tendency of alanine and α KG produced through conversion to fermentation

In Fig. 3 are given the results that alanine was produced at a low level aeration in greater amount than at a high level (7.2 mg of alanine from 95 mg of glucose).

Fig. 3. Alanine formation in conversion.

9.5 g/dl glucose, in 500 ml reciprocal shake flask. (I): 0.3 g/dl CSL., (II): 0.7 g/dl CSL., (III): 1.0 g/dl CSL., (IV): 2.0 g/dl CSL.

While a α KG production in both fermentations (i and ii in Fig. 2) was the same in amount, 1.0 to 2.0 mg per ml, and its amount was not changed during fermentation.

(5) L-Glutamic and succinic acids conversion by washed cell suspension

By using the washed cell suspension which had grown under the high and low level aeration, both acids from glucose and urea as carbohydrates and nitrogen sources were produced, without salts, C.S.L. and biotin (see Exp. No. 2 and No. 3 in Table 7). And 6.57 mg of L-glutamic acid and 2.13 mg of succinic acid, respectively, could be produced from 9.87 mg of

Table 7. L-Glutamic Acid and Succinic Acid Formation by Washed Cell Suspension.

Exp. No.	Condition of cell growth	Condition of enzyme-reaction	Incubation time (hr)						
			3	5			7		
			rs.	rs.	s-a.	g-a.	rs.	s-a.	g-a.
1	less aerobic	more aerobic	2.80	—	—	—	0	0.26	1.62
2		less aerobic	2.30	0	2.17	0.50	0	2.13	0.70
3	more aerobic	more aerobic	4.53	—	—	—	0	0.05	6.57
4		less aerobic	3.88	0	1.67	1.06	0	1.06	1.07

Medium: 0.987 g/dl glucose, 0.5 g/dl urea, phosphate buffer (ph: 7.3), 20 ml liquid volume, at 30°C, unit: (mg/ml).

Condition of more aerobic (20 ml in 500 reciprocal-shake flask): Kd of 6.5×10^{-6} .

Condition of less aerobic (20 ml in 40 ml monod shake-flask): Kd of 0.38×10^{-6} .

rs.: residual sugar, s-a.: succinic acid, g-a.: L-glutamic acid.

glucose. Also, with progress of reaction, the decomposition of succinic acid was observed as shown in Exp. No. 4 (1.67 g at 5 hrs. and 1.06 g at 7 hrs.).

(6) Activity of succinic dehydrogenase

Analyses, performed successively during fermentation, showed the decreasing of succinic acid under the L-glutamic acid producing condition but that the amount of it increased constantly under the opposite condition. Therefore, the measuring of succinic dehydrogenase in the cell gave the following: at any time during fermentation the cell grown under the high level aeration have a stronger activity than those at a low level (Q_{O_2} of the former was 4.5 to 6.3 and the latter 0.1 to 0.2, respectively). The cells on bouillon agar are the most active (11.9 of Q_{O_2}).

Table 8. Succinic Dehydrogenase Activity of Cell Grown Under Condition of L-Glutamic Acid and Succinic Acid Production at Incubation Time.

Incubation time (hr.) origin of cell	Q_{O_2}				Colour of cell
	24	48	72	96	
Cell of L-glutamic acid fermentation	6.31	5.84	4.68	—	true yellow
Cell of succinic acid fermentation	—	0.17	0.12	0.19	white
Cell on bouillon agar (surface culture)	11.91	—	—	—	true yellow

Q_{O_2} : μ l of O_2 -absorbed/hr mg of cell.

Medium: 0.3 ml of 0.5M succinic acid, 0.4 ml of 0.01M methylene blue, 0.4 ml of 0.1 M KCN (ph: 7.0), 1.3 ml of 0.1M phosphate buffer (ph: 7.2), 0.2 ml of KCN+KOH, 0.2 ml of enzyme soln., by Warburg Manometry.

Fig. 4. Conversion of fermentation in submerged culture (with 201 stainless steel tank).

No. 1: 0.3 g/dl CSL, 600 r.p.m., No. 2: 1.0 g/dl CSL, 250 r.p.m., No. 3: 1.0 g/dl, 100 r.p.m. at 30°C, air flow: 1/4 V/V. min., ---○---: residual glucose, ---●---: turbidity, —●—: succinic acid, —○—: L-glutamic acid, ---×---: lactic acid, —×—: α KG.

(7) Conversion fermentation for the L-glutamic and succinic acids formation in submerged culture

To ascertain this conversion, tank culture was performed by using a 20 litre stainless steel fermenter (see Fig. 4). Thus in the L-glutamic acid fermentation (Exp. No. 1), its yield was 35.5% of initial glucose (38.4% in shake culture), while the yield of succinic acid at peak showed 6.4% (5.2% in shake culture). In contrast, in the succinic acid fermentation (Exp. No. 3), 2.35 g of succinic acid was produced from 6.6 g of consumed glucose, the yield of which was 35.6% of consumed glucose (31.5% in shake culture), and L-glutamic acid amounted to less than 1%.

In tank culture, similar to shake culture (Fig. 2), the decreasing in the rate of oxygen supply resulted in less L-glutamic acid production (but a definite decline in the production of lactic acid at later stage). The amount of α KG remained constant as in case of shake culture, as shown in Exp. No. 1 to No. 3.

(8) Production of succinic acid by various L-glutamic acid producing bacteria

Given in Table 9 are some of the results regarding the production of L-glutamic and succinic acids through the use of various L-glutamic acid producing bacteria belonging to genus *Brevibacterium* and *Bacillus*, which were isolated from the natural world by S. OKUMURA et al. (5).

Many L-glutamic acid producing bacteria which belong to genus *Brevi-*

Table 9. L-Glutamic Acid and Succinic Acid Formation by L-glutamic acid producing Bacteria.

Stration No. of L-glutamic acid producing bacteria	Condition of L-glutamic acid formation		Condition of succinic acid formation	
	glu.	suc.	glu.	suc.
<i>Brevibacterium flavum</i> No. 1996	35.2	0.79	1.3	26.4
<i>Brevibacterium flavum</i> No. 1223	41.7	2.20	4.2	21.3
<i>Brevibacterium flavum</i> No. 2247	32.6	2.80	8.0	24.9
<i>Brevibacterium lactofermentum</i> No. 2256	30.7	2.60	2.2	24.8
<i>Brevibacterium lactofermentum</i> No. 2362	21.8	0.33	0.8	13.1
<i>Brevibacterium saccharolyticum</i> No. 2237	29.9	1.20	0.1	20.2
<i>Brevibacterium immariophilum</i> No. 2237	24.3	1.47	4.9	29.3
<i>Brevibacterium roseum</i> No. 7	39.7	1.37	7.6	21.0
<i>Brevibacterium nov. sp.</i> No. 3114	7.3	0.40	1.5	17.3
<i>Bacillus megatherium</i> No. 420	32.2	0.79	0	0.7
<i>Bacillus circulans</i> No. 2158	30.9	0.85	0	0.7

Medium: 96 mg/ml glucose, 2 γ /L biotin, unit: mg/ml, condition of L-glutamic acid formation (left column): 0.3% CSL, 20 ml liquid volume, 43 hrs. incubation, condition of succinic acid formation (right column): 1.5% CSL, 400 ml liquid volume, 96 hrs. incubation, glu.: L-glutamic acid, suc.: succinic acid.

bacterium produced more or less succinic acid and above all *Brev. immariophilum* No. 2237, which has a characteristic of not reducing nitrate to nitrite, produced 2.93 g of succinic acid from 9.2 g of glucose (showing the highest yield of 31.9% for initial glucose). On the other hand, those belonging to genus *Bacillus*, failed to increase its amount even under the "less aerobic" condition.

DISCUSSION

The accumulation of succinic acid was observed by using *Brevibacterium flavum* No. 1996, one of L-glutamic acid producing bacteria, during the microbe's growth in a level aeration much lower than that employed in the production of L-glutamic acid.

Although its production most likely is placed under the influence of the concentration of C.S.L. in the broth, its effect is weaker than that of aeration.

Under a "more aerobic" condition, this microbe produces a large quantity of L-glutamic acid while accumulating little succinic acid. In contrast, under the opposite condition where aeration diminished, a great deal of succinic acid and the negligible amount of L-glutamic acid yield in the broth. However, under the anaerobic condition, lactic acid is produced in somewhat greater amount than succinic acid, but the cell growth and utilization of glucose are much slower than they are under the aerobic condition. These findings indicate that the condition suitable for succinic acid production is "less aerobic".

The decreasing of succinic acid production under the "more aerobic" condition, is conceivably attributed to the fact that the activity of succinic dehydrogenase in cells is stronger than that grown under the "less aerobic" condition. It was found that its activity depends upon the condition of cell growth. For example, it will not be lost even if aeration is reduced at the later stage. In contrast, since the cells grown under the "less aerobic" condition have little activity, succinic acid produced may be not decomposed.

The biochemical reaction to produce L-glutamic acid and succinic acid from glucose requires the aerobic condition suitable for both acids production. Accordingly, it has been found that the accumulation of succinic acid is effected by both conditions of cell-growth and acids-producing enzyme-reaction. As to details on succinic dehydrogenase, a separate report will be made in the future.

On the other hand, alanine, which tends to increase under the "less aerobic" condition, probably has no relation with this conversion. As to α KG which subsequently is turned into L-glutamic acid, its amount does not vary either in L-glutamic acid or in succinic acid fermentation. Therefore it is most likely that an intermediary, to which our attention is attracted, participates in the course of this conversion.

It is conceivable from the referenced reports that the path-way of suc-

cinic acid production follows any one of the course listed below: (1) the oxidative decarboxylation of α KG (15, 16, 17), (2) the dismutation of α KG in the existence of L-glutamic dehydrogenase (18), (3) the decomposition of citric acid into succinic acid and glyoxylic acid by isocitritase (19), (4) the reduction of fumaric acid by fumaric reductase (20, 21), and (5) the condensation of acetic acids (22). (in Fig. 5). Details on these five pathways to

Fig. 5. Conceivable five pathways to succinic acid formation.

succinic acid formation will be reported in the near future.

The L-glutamic acid producing bacteria, which belong to genus *Brevibacterium*, can produce succinic acid from glucose under a "less aerobic" condition, and have an ability to convert the process of fermentation, however, in case of genus *Bacillus*, even if the aeration is gradually lowered, succinic acid neither increase nor decreases. Hence, it may be said that bacteria belong to genus *Bacillus* have no ability to perform the conversion. Therefore, it is conceivable that the mechanism of producing L-glutamic acid and succinic acid with genus *Brevibacterium* (24) differs from that with genus *Bacillus*.

SUMMARY

It has been recognized that the conversion from L-glutamic acid fermentation to succinic acid fermentation can be performed with *Brevibacterium flavum* No. 1996.

Succinic acid can be directly produced from glucose at a high yield of 28.3% for initial glucose in submerged culture (35.8% for consumed glucose).

The optimal aeration rate and the optimal concentration of C.S.L. for the succinic acid production were 0.4 to 0.7×10^{-6} of Kd (g-mol/cc. min. atm.) and 1.0 to 2.0% of C.S.L., respectively.

The activity of succinic decomposing enzyme in cell grown under the succinic acid producing condition was more feeble than that under the L-glutamic acid producing condition.

Among the L-glutamic acid producing bacteria, genus *Brevibacterium* was able to perform this conversion of fermentations, while genus *Bacillus* was not.

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