TAXONOMIC STUDIES ON CORYNEFORM BACTERIA

IV. MORPHOLOGICAL, CULTURAL, BIOCHEMICAL, AND PHYSIOLOGICAL CHARACTERISTICS

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Morphological, cultural, biochemical, and physiological characteristics of 112 strains of coryneform bacteria were examined. Coryneform bacteria were taxonomically divided into seven groups on the basis of the combination of the characteristics of gram-stain, motility, presence of metachromatic granules, pleomorphism, effect of citrate on cell form, acid formation from carbohydrates, assimilation of organic acids, hydrolysis of gelatin, extracellular DNase, urease, and cell wall composition.

In the course of taxonomic studies on the so-called coryneform bacteria, the present authors (1, 2) reported that coryneform bacteria were distinguished from the true bacteria on the basis of the mode of cell division, and that they were divided into three groups by the principal amino acids, DL- and LL-diaminopimelic acid (DAP), and lysine in the cell wall. Further, the guanine-cytosine content (GC content) in DNA of these organisms was distributed from 46 to 78%, and the specific range of GC content which characterizes the genus was not found except in *Cellulomonas* (3). The present paper deals with the morphological, cultural, biochemical, and physiological characteristics of coryneform bacteria including *Corynebacterium*, *Microbacterium*, *Cellulomonas*, *Arthrobacter*, and *Brevibacterium*, and with the results of re-examination of cell wall composition because an incomplete identification of principal amino acids was supposed. The taxonomic significance of these characteristics is discussed.

MATERIALS AND METHODS

Microorganisms. Bacteria tested comprised 112 strains which were ob-

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Table 1. Bacteria tested.

Genus	Number of species	Number of strains
<i>Arthrobacter</i>	12	19
<i>Brevibacterium</i>	23	45
<i>Cellulomonas</i>	5	7
<i>Corynebacterium</i>	17	32
<i>Microbacterium</i>	2	2
<i>Micrococcus</i>	1	1
Unidentified coryneform bacteria		6
Total	60	112

tained from the culture collections or freshly isolated by the present authors. They were confirmed to be pure. They belonged to 60 species of 6 genera. *Micrococcus glutamicus* was also used in this work because this bacterium had been considered to belong to the category of coryneform bacteria (1, 2, 4). Number of bacteria tested is shown in Table 1.

Determination Methods. 1) Morphological characteristics: Cell form, cell size, pleomorphism, and gram-stain were observed on the cells grown on the three media shown in Table 2, after 6 hr, 24 hr, 4 days, 10 days, and 20 days. HUCKER's modification (5) was employed for gram-stain. Motility was investigated by hanging drop and flagellation was confirmed by the staining method of TODA (6). Metachromatic granules were observed on the cells grown on R agar medium for 24 hr by the staining methods of ALBERT's (6) and of LAYBOURN's modification (5). The cells grown on R agar medium for 14 to 48 hr were negatively stained by 2% phosphotungstic acid and the septation of cells was observed by electron microscopy. Further, cell form and septation were observed on the cells grown on R agar medium containing 2% sodium citrate by the same manner, because a considerable number of strains had shown abnormal cell forms by the presence of citrate as described in the previous paper (1).

2) Cultural characteristics: Growth, surface structure, pigmentation, etc., on the plate culture of nutrient and R agar media were observed after 1 to 10 days' incubation. Turbidity and formation of pellicle and pigment, etc., were observed in the same liquid cultures. For milk culture, bromocresol purple (B.C.P.) was used as a pH indicator.

3) Biochemical characteristics:

a) Cleavage of carbohydrates. For the test of cleavage of carbohydrates, the medium composed of 3.0 g, peptone; 2.5 g, NaCl; 5.0 g, carbohydrate; and 1,000 ml distilled water, adjusted to pH 7.2 and colored with B.C.P., was employed. Production of acid was investigated for a month. Twenty-eight compounds as listed in Table 4 were used. Further, the type of cleavage of

Table 2. Composition of the media employed.

R (Rich) medium		P (Poor) medium		G (Glucose-glutamate) medium	
Bacto-Peptone (Difco)	10.0 g	Yeast extract (Difco)	0.7 g	Glucose	10.0 g
Yeast extract (Difco)	5.0 g	Proteose Peptone (Difco)	1.0 g	Proteose Peptone (Difco)	5.0 g
Casamino acids (Difco)	5.0 g	Glucose	1.0 g	Sodium glutamate	5.0 g
Beef extract (Difco)	2.0 g	(NH ₄) ₂ SO ₄	0.25g	K ₂ HPO ₄	1.0 g
Malt extract (Difco)	5.0 g	K ₂ HPO ₄	1.0 g	MgSO ₄ ·7H ₂ O	0.2 g
Glycerol	2.0 g	MgSO ₄ ·7H ₂ O	0.25g	KCl	0.1 g
MgSO ₄ ·7H ₂ O	1.0 g	Distilled water	1,000 ml	Distilled water	1,000 ml
Tween 80	0.05g				
Distilled water	1,000 ml				

pH is adjusted to 7.2, and 20 g of Bacto-Agar (Difco) is added in the case of solid media.

Table 3. Basal medium employed for assimilation of organic acids.

Organic acid ^a	5.0 g
Glucose	0.2 g
Yeast extract (Difco)	0.1 g
Trypticase (B.B.L.)	0.1 g
K ₂ HPO ₄	1.0 g
NaCl	5.0 g
Phenol Red	0.012 g
Bacto-Agar (Difco)	20.0 g
Distilled water	1,000 ml
	(pH 7.0)

^a Organic acids are added as sodium salt.

glucose was determined by the method of HUGH and LEIFSON (7).

b) Assimilation of organic acids. Assimilation of 22 organic acids was tested by using CHRISTENSEN's medium (8) modified by the present authors. The original CHRISTENSEN's medium was not adequate for this purpose because a considerable number of strains changed the medium to alkaline reaction in the absence of citrate. Therefore, concentration of yeast extract was reduced to 0.01% and peptone was added to the concentration of 0.01%, as shown in Table 3. Organic acids were added at the concentration of 0.5% and the basal medium without organic acid was used as a

control. Sodium D- and L- lactate, and sodium oxaloacetate were sterilized through Seitz filter. Assimilation of the compounds was detected by the change in the color of the indicator after 10 days' incubation.

c) Hydrolysis of gelatin. Two methods were employed. One was carried out by the stab culture of nutrient gelatin containing 0.2% Brain-Heart Infusion (Difco) and hydrolysis was detected after 20 days' incubation at 25° and 30°. The other was tested by the method of SMITH *et al.* (9) after 1, 3, 5, and 7 days' incubation at 30° using R agar medium containing 0.4% gelatin.

d) Extracellular DNase. DNase Test Medium (Difco) was used and the activity was detected after 1, 3, 5, and 7 days' incubation. For the poorly growing strains, 0.2% Brain-Heart Infusion (Difco) was supplemented to the medium.

e) Urease. Urease was detected by using Urease Medium (Nissui Seiyaku, Tokyo) after an overnight incubation.

f) Cellulolytic activity. Cellulolytic activity was detected by the method described in "Laboratory Methods in Microbiology" (10) after one-month incubation.

g) Other biochemical tests. Reduction of nitrate, Methyl-Red and Voges-Proskauer reactions, production of indole, hydrolysis of starch, and catalase were tested by the methods described in "Manual of Microbiological Methods" (5). Production of hydrogen sulfide was detected by lead acetate paper. The commercial media (Nissui Seiyaku, Tokyo) were employed for the tests of lysine decarboxylase, utilization of malonate, and production of phenylpyruvic acid from phenylalanine. Cytochrome oxidase was detected by the test paper (Nissui Seiyaku, Tokyo). Tyrosinase was tested by browning around the streak after 10 days' incubation using R agar plate containing 0.5% L-tyrosine. The following characteristics were observed by the methods described in the literatures: Nitrate respiration (11), hydrolysis of tributyrin (10), production of dihydroxyacetone from glycerol (12), and of 2-ketogluconate from gluconate (13), reduction of trimethylamine oxide (14), and of 2, 3, 5-triphenyltetrazolium chloride (10), egg yolk reaction (15), static action of bile acid (10), and of tellulite (10), and phosphatase reaction (16).

All the biochemical tests were carried out at 30° unless otherwise mentioned, and cells grown on R agar medium at 30° for 48 hr were used as inoculum.

4) Physiological characteristics:

a) Relation to temperature. Growth with shaking at 25°, 30°, 37°, and 42° was compared using R medium broth.

b) Relation to NaCl. Growth with shaking at concentrations of 6.0, 7.0, 8.0, and 10.0% NaCl at 30° was compared using Brain-Heart Infusion Broth (Difco) as a basal medium.

5) Detection of principal amino acids in the cell wall: After preparation and hydrolysis of cell wall in the same manner as reported by the present authors

(2), the hydrolysate was subjected to paper chromatography and paper electrophoresis. The three solvent systems used in the previous work were employed for paper chromatography. The amino acids were also examined by paper electrophoresis using a buffer solution of 0.025M sodium carbonate, pH 11.5, at 9 V/cm for 4 hr (17).

RESULTS

Morphological, cultural, biochemical, and physiological characteristics significant for the classification of coryneform bacteria are summarized in Table 4.

Morphological characteristics

Fundamental cell form of the strains tested was rod-shaped. The large cells, in general, were found after 6 hr of incubation or nearly so, and later, they became smaller on all the media used. About one-third of the strains showed a little change in cell form, but the remainder changed from rod-shaped to coccoid. Sometimes large spherical bodies, cystites, were found as shown in Figs. 3 and 6. An extreme morphological change during the cultivation was termed as pleomorphism. Pleomorphism was found more remarkably on the cells grown on nutritionally poor media than on rich media. The degree of pleomorphism was arbitrarily scored as ++, +, and -.

Forty-nine strains was strongly positive in gram-stain regardless of the composition of media and culture periods. The remainder generally lost positivity in gram-stain with cultural age, and some strains of this group became almost gram-negative in old culture. The former is expressed as "strongly gram-positive" and the latter, "weakly gram-positive".

An interesting relation between gram-stain and pleomorphism was found. Almost all the strongly gram-positive strains were not pleomorphic but, in contrast, weakly gram-positive strains exhibited more distinctive pleomorphism. *Corynebacterium diphtheriae* ATCC 11913, *Corynebacterium xerosis* ATCC 7711, *Brevibacterium ammoniagenes* ATCC 6872, *Brevibacterium flavum* ATCC 14067, etc., were strongly gram-positive, and *Arthrobacter globiformis* ATCC 8010, *Arthrobacter tumescens* ATCC 6947, *Arthrobacter nicotianae* ATCC 15236, *Corynebacterium poinsettiae* CCM 1587, *Corynebacterium michiganense* ATCC 492, *Corynebacterium flaccumfaciens* ATCC 6887, *Corynebacterium aquaticum* ATCC 14665, *Brevibacterium citreum* IAM 1514, *Cellulomonas biazotea* ATCC 486, *Cellulomonas fimi* ATCC 484, etc., were weakly gram-positive. Typical changes in morphology of some strains are shown in Figs. 1-7.

Twenty-eight strains showed motility and their flagellation was lateral with variation of single, two, or more flagella. It is interesting that all the motile strains were weakly gram-positive.

Metachromatic granules were observed in the cells of 37 strains which

were non-pleomorphic and strongly gram-positive. None of weakly gram-positive strains exhibited metachromatic granules. Two or more septated cells were frequently found in strongly gram-positive strains such as *C. diphtheriae* ATCC 11913, *B. ammoniagenes* ATCC 6872, etc., while multicellularity was hardly found in weakly gram-positive strains. The effect of sodium citrate on cell morphology differed from strain to strain. Generally, strongly gram-positive strains such as *Brevibacterium lactofermentum* ATCC 13869, *M. glutamicus* ATCC 13032, etc., showed a remarkable elongation and branching as previously reported (1).

Cultural characteristics

Most strains demonstrated moderate growth on nutrient agar, and some strains such as *C. diphtheriae* ATCC 11913, *C. michiganense* ATCC 492, *Corynebacterium sepedonicum* ATCC 9850, etc., showed scanty growth. All the strains grew well on R agar medium but *Cellulomonas* species, except *C. fimi* ATCC 8183, grew slowly on this medium. Scanty or no growth of many strains was recognized on synthetic media such as glutamate agar (18).

Colonies of coryneform bacteria were circular, raised to convex, entire, and butyrous. Most bacteria showed smooth surface, but a few rough. White, gray, yellow, orange, rose, or red chromogenesis was observed. All the strains of strongly gram-positive group formed opaque colonies and generally those of weakly gram-positive group did opalescent colonies.

Biochemical characteristics

a) *Cleavage of carbohydrates.* Acidification of the test media within 5 days' incubation was judged as positive, +. Certain bacteria changed the indicator slowly after 7 to 20 days. In such a case, the results were scored as weakly positive, (+). Cleavage of sugars showed a close correlation with other characteristics, while those of alcohols and glucosides did not seem to be significant for the classification. Bacteria of non-pleomorphic and strongly gram-positive group were not subdivided into any distinctive type from the pattern of acid production. About half the members of this group produced acid from one compound or more, though the sugars were restricted to mono- and disaccharides of hexose such as glucose, fructose, mannose, sucrose, or maltose. The remainder did not produce acid from any sugars. As an exception, *Corynebacterium hoagii* AJ 1374 produced acid from rhamnose, lactose, and dextrin, in addition to the above-mentioned hexoses. On the other hand, weakly gram-positive strains were divided into the following three types on the basis of acid production from sugars:

i) Bacteria producing acid from sugars. *Cellulomonas* species, except *C. fimi* ATCC 8183, rapidly produced acid from all the sugars tested except sorbose and inulin. *Brevibacterium saperdae* CCEB 366, *C. poinsettiae* CCM 1587, etc., produced acid relatively slowly from a wide variety of sugars.

Fig. 1. Morphological changes in cells of *Brevibacterium ammoniagenes* ATCC 6872 grown on G agar medium at 30°.

A: 6 hr. B: 24 hr. C: 4 days. D: 10 days. E: 20 days.

Fig. 2. Morphological changes in cells of *Brevibacterium linens* ATCC 9172 grown on P agar medium at 30°.

A: 6 hr. B: 24 hr. C: 4 days. D: 10 days. E: 20 days.

Fig. 3. Morphological changes in cells of *Arthrobacter nicotianae* ATCC 15236 grown on G agar medium at 30°.

A: 6 hr. B: 24 hr. C: 4 days. D: 10 days. E: 20 days.

Fig. 4. Morphological changes in cells of *Cellulomonas biazotea* ATCC 486 grown on P agar medium at 30°.

A: 6 hr. B: 24 hr. C: 4 days. D: 10 days. E: 20 days.

Fig. 5. Morphological changes in cells of *Brevibacterium citreum* IAM 1514 grown on P agar medium at 30°.

A: 6 hr. B: 24 hr. C: 4 days. D: 10 days. E: 20 days.

Fig. 6. Morphological changes in cells of *Arthrobacter tumescens* ATCC 6947 grown on G agar medium at 30°.

A: 6 hr. B: 24 hr. C: 4 days. D: 10 days. E: 20 days.

Fig. 7. Morphological changes in cells of *Corynebacterium michiganense* ATCC 492 grown on P agar medium at 30°.

A: 6 hr. B: 24 hr. C: 4 days. D: 10 days. E: 20 days.

- ii) Bacteria producing acid weakly and slowly. *Brevibacterium albidum* IAM 1631, *Brevibacterium citreum* IAM 1614, *Brevibacterium pusillum* IAM 1479, *B. testaceum* IAM 1537, etc., were included in this type.
- iii) Bacteria producing no acid from any sugars. *A. globiformis* ATCC 8010, *A. ureafaciens* ATCC 7562, etc., belonged to this type.

It is interesting that the type of glucose cleavage of all the acid producers of coryneform bacteria is "fermentative" according to the terminology of HUGH and LEIFSON (7).

b) *Assimilation of organic acids.* Most coryneform bacteria assimilated many kinds of organic acids. Especially, the members of weakly gram-positive group exhibited a wide assimilation pattern. *Cellulomonas* species assimilated only low-molecular organic acids such as acetic acid, pyruvic acid, and L-lactic acid.

c) *Hydrolysis of gelatin.* About 80 strains hydrolyzed gelatin. Almost the same results were obtained in the ordinary stab culture and SMITH's method. *C. poinsettiae* CCM 1587, *B. citreum* IAM 1514 and IAM 1614, *B. pusillum* IAM 1479, and *B. saperdae* CCEB 366 hydrolyzed gelatin within 7 days by the SMITH's method, but the liquefaction was not detected after 30 days by stab culture. The results of these bacteria were scored as positive. None of the strains of nonpleomorphic and strongly gram-positive group hydrolyzed gelatin except 5 strains of *Brevibacterium linens*. On the contrary, most strains of weakly gram-positive group hydrolyzed gelatin. In this group, *Corynebacterium aquaticum* ATCC 14665 and *Corynebacterium insidiosum* ATCC 10253 did not hydrolyze gelatin.

d) *Extracellular DNase.* Bacteria belonging to non-pleomorphic and strongly gram-positive group did not exhibit extracellular DNase activity except 5 strains of *B. linens*, while a large number of weakly gram-positive strains showed its activity. *A. globiformis* ATCC 8010 and *Arthrobacter pascens* CCM 1653 hydrolyzed DNA very slowly.

e) *Urease.* Thirty-two strains showed urease activity and 28 were strongly gram-positive. In the weakly gram-positive group, only three strains of *Brevibacterium sulfureum* and one strain of *Arthrobacter citreus* showed urease activity.

f) *Cellulolytic activity.* All the tested bacteria including *Cellulomonas* species did not exhibit cellulolytic activity under the present experimental condition.

g) *Other biochemical characteristics.* The following characteristics were variable from strain to strain: Cytochrome oxidase, hydrolysis of starch, reduction of nitrate, production of hydrogen sulfide, tyrosinase, static action of tellurite and of bile acid, Methyl-Red test, decomposition of tributyrin, lysine decarboxylase, formation of 2-ketogluconate from gluconate, and utilization of malonate. All the tested strains gave negative results for the following properties: Nitrate respiration, Voges-Proskauer reaction, indole test, coagulase, and egg yolk test.

Table 4. Morphological, biochemical and physiological characteristics of coryneform bacteria

[illegible]

Table 4. (Continued 1)

[illegible]

Table 4. (Continued 2)

Physiological characteristics

a) *Relation to temperature.* All the tested bacteria grew well at 25° and 30°. Optimum temperatures for growth of most strains were 30° and 37°, while the bacteria such as four strains of *C. michiganense*, *Corynebacterium renale* ATCC 10848, and *C. sepeidonicum* ATCC 9850 could not grow at 37°. None of the strains developed at 42°.

b) *Relation to NaCl.* All the strains of strongly gram-positive group grew in the medium containing 5% NaCl, and about half of the strains of this group did even in 10%. On the other hand, in weakly gram-positive group, 51 strains developed in 5% NaCl and the remaining 17 strains did not, and only 5 strains grew in 10% NaCl. Generally, salt tolerance of strongly gram-positive group seemed to be higher than that of weakly gram-positive group.

Principal amino acid in the cell wall

The results of re-examination of principal amino acid in the cell wall are shown in Table 4. Of 73 strains whose principal amino acid was previously reported to be lysine or LL-diaminopimelic acid (DAP), 18 strains contained ornithine, 11 possessed α , γ -diaminobutyric acid (DAB), and 5 had DL-DAP. The bacteria containing DL-DAP showed the same results as reported in the previous paper. Composition of other amino acids and amino sugars was the same as previous analyses.

DISCUSSION

From the results obtained, it is apparent that the strains labeled in a certain genus did not necessarily show common morphological, cultural, biochemical, and physiological characteristics. This fact may indicate that bacterial taxonomists have given different evaluations for the criteria presently employed in the classification of the coryneform bacteria. However, when the names labeled in each genus are disregarded, the coryneform bacteria tested are divided into seven groups on the basis of the combination of the following characteristics: Gram-stain, motility, presence of metachromatic granules, pleomorphism, effect of citrate on cell form, acid production from sugars, assimilation of organic acids, hydrolysis of gelatin, extracellular DNase, urease, and cell wall composition.

Group 1. Bacteria of this group are strongly gram-positive and non-motile. Metachromatic granules and septa are found in the cells grown on ordinary media. Pleomorphism is not distinctive but elongated and branching cells appear in the media containing 2% sodium citrate. Some strains produce acid fermentatively from glucose, fructose, mannose, maltose, sucrose, and trehalose. Many strains utilize organic acids related to TCA cycle but some of these do not utilize citrate. None of the strains hydrolyzes

gelatin. Extracellular DNase is not found. Many strains exhibited urease activity. All the strains grow in the medium containing 5% NaCl and about half of these do even in 10%. DL-DAP is found in the cell wall as a principal amino acid. In this group, 37 strains consisting of *C. diphtheriae* ATCC 11913, *C. xerosis* ATCC 373, *Corynebacterium equi* ATCC 6939, *B. ammoniagenes* ATCC 6872, *Microbacterium flavum* ATCC 10340, and all the glutamic acid-producing bacteria tested such as *B. flavum* ATCC 14067, *Brevibacterium divaricatum* NRRL B-2312, etc., were included.

Group 2. Bacteria of this group are strongly gram-positive and non-motile. Metachromatic granules are not found in the cells grown on ordinary media. Pleomorphism is not recognized. The bacteria grow on the media containing 2% sodium citrate but the marked effect of this substance to cell form is not distinctive. None of the strains produces acid from any kind of sugars. They assimilate almost all the organic acids tested. Gelatin is hydrolyzed. Extracellular DNase is found. Urease is negative. All the strains grow in the medium containing 10% NaCl. They contain DL-DAP in the cell wall as a principal amino acid. This group consisted of only six strains of *B. linens*.

Group 3. Bacteria of this group are weakly gram-positive. Most strains are non-motile. Metachromatic granules are not found in the cells grown on ordinary media. Remarkable pleomorphism is observed. Many short or coccoid cells are found and frequently large spherical bodies, cystites, appear in old cultures. The cystites are strongly gram-positive. The bacteria grow on the media containing 2% sodium citrate but marked effect of this substance to cell form is not recognized. None of the strains produces acid from any kind of sugars. Almost all of organic acids tested are assimilated. Gelatin is hydrolyzed. Extracellular DNase is found. Urease is generally negative but a small number of strains show its activity. Most strains do not grow in the medium containing 10% NaCl but some strains did. Lysine is contained in the cell wall. In this group, 27 strains consisting of *A. globiformis* ATCC 8010, *A. ureafaciens* ATCC 7562, *A. citreus* ATCC 11624, *C. fimi* ATCC 8183, etc., were included. Of these strains, 15 had been identified as *Arthrobacter* and 10 as *Brevibacterium*.

Group 4. Bacteria of this group are weakly gram-positive. Most of the cells become gram-negative in old cultures. Motile and non-motile strains are found. Metachromatic granules are not found in the cells grown on ordinary media. Pleomorphism is fairly distinctive. The members of this group grow slowly on ordinary media. Growth is inhibited by the presence of 2% sodium citrate in the medium. All the strains produce acid fermentatively from all the sugars tested except sorbose and inulin. Only low-molecular organic acids such as sodium acetate and sodium pyruvate are assimilated. Sodium L-lactate is utilized by some strains. The bacteria of this group slowly hydrolyze gelatin. They produce extracellular DNase but not urease, and do not grow in the medium containing 5% NaCl. Ornithine is

found in the cell wall as a principal amino acid. This group consisted only of *Cellulomonas* species (6 strains) but they did not show cellulolytic activity.

Group 5. Bacteria of this group are weakly gram-positive. All the strains are motile except *B. albidum* IAM 1631. Metachromatic granules are not found in the cells grown on ordinary media. Pleomorphism is recognized but the degree is lower than that of *Group 3*. Faint growth is found in the medium containing 2% sodium citrate, and effect of this substance to cell morphology is negligible. Members of this group produce acid slowly and weakly from various kinds of sugar. They assimilate a considerable number of organic acids. Gelatin is hydrolyzed. All the strains produce extracellular DNase but not urease. All the strains grow in the medium containing 5% NaCl but not in 10%. Ornithine is contained in the cell wall. In this group, 16 strains consisting of *B. citreum* IAM 1514, *B. testaceum* IAM 1537, *B. pusillum* IAM 1479, etc., were included. Many strains of this group are isolated from plant materials and correspond to "motile brevibacteria" reported by KOMAGATA *et al.* (19).

Group 6. Bacteria of this group are weakly gram-positive. Some strains are motile. Metachromatic granules are not found in the cells grown on ordinary media. The bacteria exhibited considerably large, elongated, or complicated branching cell form. Pleomorphism is distinctive and large spherical bodies which are strongly gram-positive are frequently found. Change of cell form by the presence of 2% sodium citrate is not distinctive. No acid is produced from any carbohydrates. Many kinds of organic acids tested are assimilated. Gelatin is hydrolyzed. All the strains produce extracellular DNase but not urease. They grow in the medium containing 10% NaCl. LL-DAP is found in the cell wall. In this group 6 strains consisting of *Brevibacterium lipolyticum* IAM 1398, *Arthrobacter variabilis* CCM 1565, *Arthrobacter tumescens* ATCC 6947, etc., were included.

Group 7. Bacteria of this group are weakly gram-positive. Motile and non-motile strains are found. Metachromatic granules are not found in the cells grown on ordinary media. Comparatively short rod cells are found. Pleomorphism is recognized. Change of cell form by the presence of 2% sodium citrate is not distinctive. No acid is produced from any carbohydrates. Some organic acids related to TCA cycle are assimilated. Most strains hydrolyze gelatin but a few do not. All the strains exhibit extracellular DNase but not urease. Some strains grow in the medium containing 5% NaCl but no strain does in the medium containing 10% NaCl. Diaminobutyric acid is contained in the cell wall as a principal amino acid. In this group 6 strains consisting of *C. aquaticum* ATCC 14655, *Corynebacterium insidiosum* ATCC 10253 and 5 strains of *Corynebacterium michiganense* were included.

Of the strains employed in this work, the following strains could not be included in any group mentioned above: *C. bovis* ATCC 7715 and AJ 1407, *C. hydrocarboclastus* IAM 1399 and IAM 1494, *C. sepeidonicum* ATCC 9850,

B. minutiferula IFM AU-27, *B. imperiale* ATCC 8365, and *M. lacticum* ATCC 8180.

Details of taxonomic relation of above-mentioned characteristics, mode of cell division, and GC content in DNA will be discussed in the following paper.

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