TAXONOMIC STUDIES ON CORYNEFORM BACTERIA

V. CLASSIFICATION OF CORYNEFORM BACTERIA

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Classification and concepts of the genera of coryneform bacteria were described on the basis of the results obtained previously. Coryneform bacteria were divided into seven groups at the generic level by the combination of mode of cell division, principal amino acid in the cell wall, the guanine-cytosine content (GC content) in DNA, and biochemical and physiological characteristics. The concepts of several genera were made clearly and a new genus Curtobacterium was proposed. The scheme for differentiation of the genera of these bacteria was presented, and problems for classification of coryneform bacteria were discussed.

Aerobic, gram-positive, not acid-fast, non-spore forming, rod-shaped bacteria are called "coryneform bacteria" as a whole, and are taxonomically classified into the genera of Corynebacterium, Microbacterium, Cellulomonas, Arthrobacter, and Brevibacterium in the system of Bergey's Manual of Determinative Bacteriology, 7th edition (1). However, since the generic concepts and the interrelation of these bacteria were not clearly established, the identification of this group of bacteria has been actually very difficult.

In the course of taxonomic studies on coryneform bacteria, the present authors (2–5) have reported the following results: (a) The coryneform bacteria were distinguished from the true bacteria by the mode of cell division. The former develops by the snapping or bending type of cell division, and the latter propagates by simple type of cell division. (b) The coryneform bacteria were divided into five types by the principal amino acid in the cell wall, DL- and LL-diaminopimelic acid (DAP), lysine, ornithine, and α, γ-diaminobutyric acid (DAB). (c) The guanine-cytosine content (GC content) in DNA of coryneform bacteria was distributed from 51 to 78%, and a specific range of GC content which characterizes the genus was not found.

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except in *Cellulomonas*. (d) The coryneform bacteria were classified into seven groups by the combination of the principal amino acid in the cell wall and the morphological, cultural, biochemical, and physiological characteristics.

In this paper, classification and concepts of the genera of coryneform bacteria are discussed on the basis of all the results obtained by the present authors.

**MATERIALS AND METHODS**

Microorganisms and experimental results:
Microorganisms and experimental results described in the previous papers (2–5) were used. The names of strains from culture collections were used according to their designations.

**RESULTS AND DISCUSSION**

I. Grouping of coryneform bacteria

In the preceding work (5), coryneform bacteria was divided into seven groups on the basis of the combination of the principal amino acid in the cell wall, and the morphological, cultural, biochemical, and physiological characteristics. This grouping shows an interesting relation to the mode of cell division (2) and GC content in DNA (3) as illustrated in Fig. 1. Numbers in the figure are the same as those of the grouping reported in the preceding paper (5).

Bacteria of Group 1 containing DL-DAP in their cell wall as the principal amino acid grew by snapping type of cell division and their GC content gave a rather wide range from 51 to 70%. Strains of Group 2 possessing DL-DAP in their cell wall were distinguished from Group 1 by hydrolysis of gelatin and extracellular DNase activity. They grew by bending type of cell division and their GC content ranged from 61 to 63%. All the microorganisms of Group 3 containing lysine in the cell wall grew by bending type of cell division and their GC content was distributed continuously from 58 to 66%. Bacteria of Group 4 possessing ornithine in the cell wall produced acid strongly and rapidly from almost all of sugars tested. They grew by bending type of cell division and their GC content fell to about 73%. Organisms of Group 5 having ornithine in the cell wall differed from Group 4 by weak and slow acid production from a limited number of sugars. They propagated by bending type of cell division and GC content ranged from 65 to 70%. Strains of Group 6 containing LL-DAP in the cell wall exhibited bending type of cell division, and their GC content was restricted to a rather narrow range from 69 to 71%. Microorganisms of Group 7 having DAB in the cell wall developed by bending type of cell division, and their GC content fell into the range from 65 to 69%.

Each group placed in a box as illustrated in Fig. 1 showed taxonomic
interests at the generic level. The properties to differentiate these seven groups are shown in Table 1.

II. Taxonomic position of the seven groups

1) Group 1. Bacteria of this group propagate by snapping type of cell division and contain DL-DAP in the cell wall. The GC content of this group is distributed widely from 51 to 70%. The bacteria are strongly gram-positive and non-motile. They exhibit metachromatic granules but do not show distinctive pleomorphism. Elongated and branching cells frequently appear in the media containing 2% sodium citrate. They neither hydrolyze gelatin nor show the activity of extracellular DNase. The patterns of acid production from sugars and of assimilation of organic acids are not so specific for this group. The members with a low GC content assimilate citrate but those with a high GC content do not. The latter exhibit smaller cell form and a small number of diminutive metachromatic granules. Although GC content of this group varied widely, no difference is observed in morphological, cultural, biochemical, and physiological characteristics, mode of cell division, and type of cell wall. Therefore, these bacteria seem to be included in the same group.
The characteristics of this group agree well with the original description of *Corynebacterium* proposed by LEHMANN and NEUMANN (6), especially in respect to the snapping type of cell division, no motility, and the presence of metachromatic granules. Further, many strains labeled as *Corynebacterium*, for example, *Corynebacterium diphtheriae* ATCC 11913, *Corynebacterium equi* ATCC 6939, *Corynebacterium xerosis* ATCC 373, etc., are included in this group. From these facts, Group 1 is regarded as the genus *Corynebacterium*. All the so-called glutamic acid-producing bacteria tested such as *Corynebacterium callunae* NRRL B-2244, *Corynebacterium herculis* ATCC 13868, *Corynebacterium lilium* NRRL B-2243, *Brevibacterium divaricatum* NRRL B-2312, *Brevibacterium flavum* ATCC 14067, *Brevibacterium lactofermentum* ATCC 13655 and ATCC 13869, and *Micrococcus glutamicus* ATCC 13032 are included in this group. VELDKAMP et al. (7) reported that the so-called glutamic acid-producing bacteria should be named *Arthrobacter globiformis* on the basis of their definite life cycle, cell size, and growth on synthetic media, but the present authors do not support their proposal from the present results and consideration made here.

LEHMANN and NEUMANN defined that the species of *Corynebacterium* are non-motile and pathogenic or at least parasitic to animals. After their proposal, HONING (8), KISSKALT and BEREND (9), TOPPING (10), and JENSEN (11) added morphologically similar motile, plant pathogenic or soil habitat

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<tbody>
<tr>
<td>Type of cell wall</td>
<td>DL-DAP</td>
<td>DL-DAP</td>
<td>Lysine</td>
</tr>
<tr>
<td>Type of cell division</td>
<td>Snapping</td>
<td>Bending</td>
<td>Bending</td>
</tr>
<tr>
<td>GC content (%)</td>
<td>51–70</td>
<td>60–63</td>
<td>58–65</td>
</tr>
<tr>
<td>Gram-stain</td>
<td>Strongly</td>
<td>Strongly</td>
<td>Weakly</td>
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<td></td>
<td>positive</td>
<td>positive</td>
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<td>Cystite:</td>
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<td>Strongly</td>
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<td></td>
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<tr>
<td>Motility</td>
<td>Non-motile</td>
<td>Non-motile</td>
<td>Non-motile</td>
</tr>
<tr>
<td>Metachromatic granule</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pleomorphism</td>
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<td>Not</td>
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<td>distinctive</td>
<td>distinctive</td>
<td>Cystite</td>
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<tr>
<td>Citrate effect</td>
<td>+</td>
<td>±</td>
<td>±</td>
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<td>Acid production from</td>
<td>+ or –</td>
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<td>–</td>
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<tr>
<td>sugars</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Utilization of organic</td>
<td>+</td>
<td>+</td>
<td>+(widely)</td>
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<tr>
<td>acids</td>
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<tr>
<td>Hydrolysis of gelatin</td>
<td>–</td>
<td>+</td>
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<tr>
<td>DNase activity</td>
<td>–</td>
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<tr>
<td>Urease activity</td>
<td>+ or –</td>
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<td>– or +</td>
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seven groups of coryneform bacteria.

<table>
<thead>
<tr>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
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<tr>
<td>Ornithine</td>
<td>Ornithine</td>
<td>LL–DAP</td>
<td>DAB</td>
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<tr>
<td>Bending</td>
<td>Bending</td>
<td>Bending</td>
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<td>66–71</td>
<td>70–72</td>
<td>69–71</td>
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<tr>
<td>Weakly positive</td>
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<td>Weakly positive</td>
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<td>Motile or non-motile</td>
<td>Motile or non-motile</td>
<td>Motile or non-motile</td>
<td>Non-motile or motile</td>
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<td>—</td>
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<tr>
<td>Fairly distinctive</td>
<td>Weakly</td>
<td>Distinctive Cystite elongation branching</td>
<td>Slightly</td>
</tr>
<tr>
<td>Inhibit</td>
<td>+(strongly) (rapidly)</td>
<td>+(weakly) (slowly)</td>
<td>—</td>
</tr>
<tr>
<td>+</td>
<td>+(restricted)</td>
<td>+</td>
<td>—</td>
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<td>+(slowly)</td>
<td>+(slowly)</td>
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<td>+ or —</td>
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<td>+</td>
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species in the genus *Corynebacterium*. From the results obtained by the present authors, the strains originating from soil or plant materials such as *M. glutamicus* ATCC 13032 or *B. flavum* ATCC 14067 were included in this group, but all the motile species or plant pathogenic species except *Corynebacterium fascians* were excluded from Group 1. Therefore, *Corynebacterium* should be followed fundamentally to the original description established by LEHMANN and NEUMANN, but the characteristics of pathogenicity or parasitism to animals do not seem to be critical for the identification of this genus.


* Asterisks indicate the type strain of the species.

2) **Group 2.** Bacteria of this group grow by bending type of cell division and have DL-DAP in the cell wall. GC content in DNA ranges from 61 to 63%. The organisms are strongly gram-positive and non-motile, and do not have metachromatic granules. Pleomorphism is not distinctive. Effect of sodium citrate on cell morphology is not so clear. They hydrolyze gelatin, show the activity of extracellular DNase, and do not produce acid from any sugars tested. They assimilate many kinds of organic acids and grow well in the medium containing 10% NaCl.

BREED (12) proposed the genus Brevibacterium in 1953 for a group of bacteria which are aerobic, gram-positive, rod-shaped, and not active in the production of acid from carbohydrates. He also described without definition of the type of cell division that these bacteria propagate by simple cell division. Some species of Corynebacterium, Flavobacterium, and Bacterium described in Bergey’s Manual, 6th edition (13), were transferred to the genus Brevibacterium on the basis of above-mentioned traits. Since the reliable comparison of these bacteria was not reported, the generic concept of Brevibacterium has been uncertain as SKERMAN (14) pointed out.

From the results obtained here, almost all the strains belonging to the species of Brevibacterium were dispersed and absorbed in various groups. The bacteria belonging to Group 2 consisted of the strains of B. linens. As far as BREED’s opinion is held in esteem, this group is considered to be Brevibacterium because he designated B. linens as the type species of this genus. Furthermore, the co-type strain of this species, B. linens ATCC 9172 (15), was included in this group. Therefore, the present authors regard Group 2 as Brevibacterium. However, as the genus Brevibacterium established by BREED is very vague, the concept should be emended based on the characteristics of Group 2.

DA SILVA and HOLT (16) transferred B. linens to the genus Arthrobacter based on the results of computer analysis, but, the present authors do not support this proposal because the principal amino acid and morphological characteristics of this species do not agree with those of Arthrobacter as will be mentioned below. Further, the tolerance against sodium chloride of this species is exceptionally higher than that of Arthrobacter.

From the results obtained and the consideration mentioned above, the following strains belong to the genus Brevibacterium: B. linens AJ 1520 (ATCC 8377), AJ 1521 (ATCC 9172*), AJ 1522 (ATCC 9174), AJ 1523 (ATCC 9175), and AJ 1540 (CCM 47).

3) **Group 3.** Bacteria of this group multiply by bending type of cell
division and contain lysine in the cell wall. Their GC contents range from 58 to 66%. They are weakly gram-positive and most strains exhibit remarkable pleomorphism. The bacteria show gram-positive, rod-shaped cells in fresh culture (about 6 hr after inoculation), and later, weakly gram-positive or negative short rod or coccoid cells appear. In an old culture, most strains exhibit large spherical bodies, cystites, which are strongly gram-positive. Motile strains are found in this group. Metachromatic granules are not found in the cells. Marked effect of sodium citrate to cell form is not clearly recognized. The bacteria of this group do not produce acid from any sugars tested and assimilate many kinds of organic acids. All the strains hydrolyze gelatin and exhibit extracellular DNase activity.

Pleomorphism, property of gram-stain, no acid production from sugars, and proteolytic activity seem to agree with the generic concept of *Arthrobacter* reported by CONN and DIMMICK (17). Many bacteria named *Arthrobacter* and *A. globiformis* ATCC 8010 (type strain) belong to this group. From these points of view, the present authors consider that Group 3 corresponds to *Arthrobacter*.

CONN and DIMMICK described that the species of *Arthrobacter* are gram-negative rod in young cultures and gram-positive coccoid forms appear in old cultures. However, the present authors observed that the rod-shaped cells in the fresh culture were relatively strongly gram-positive, and that short rods or coccoid cells which formed after one or more days changed to weakly gram-positive or negative, and large spherical bodies which were found among these short rods or coccoid cells were strongly gram-positive. JENSEN (18) pointed out that “since the differences between *Arthrobacter* proposed by CONN and DIMMICK and *Corynebacterium* are largely quantitative, the genus *Arthrobacter* should be kept in abeyance until more information on the rest of the saprophytic coryneforms has accumulated.” However, it seems reasonable to separate *Arthrobacter* from *Corynebacterium* on the basis of the discrepancy of the cell wall composition and biochemical characteristics.

4) **Group 4.** Bacteria of this group exhibit bending type of cell division and their principal amino acid in the cell wall is ornithine. Their GC-content is distributed in a narrow range from 71 to 73%. They are weakly gram-positive or mostly gram-negative. Motile and non-motile strains are found. Metachromatic granules are not recognized. Coccoid cells are found in old cultures, but the pleomorphism is less conspicuous than that of Group 3. Growth is inhibited by the presence of 2% sodium citrate in the medium. These bacteria produce acid fermentatively from a wide variety of sugars. Assimilation of organic acids is restricted in number. The low-molecular organic acids such as pyruvate, acetate, and L-lactate are assimilated. Gelatin is slowly hydrolyzed by these organisms.

This group consisted of the strains labeled as *Cellulomonas* including *Cellulomonas biazotea* ATCC 486 (type strain). This fact may indicate that Group 4 corresponds to the genus *Cellulomonas* as emended by Clark (19). According to the description, cellulolytic activity is adopted in general as a specific trait of this genus, but the tested organisms including several type strains did not show the activity under the present experimental condition. It is thought that the cellulolytic activity had been lost during the preservation under the laboratory conditions as Kellerman (20) pointed out. Consequently, it seems inadequate to use the generic name of *Cellulomonas* as Jensen (18) pointed out, but the present authors hesitate to reject this name because this group is differentiated clearly from other groups by the properties without cellulolytic activity. In *Cellulomonas* strains tested, C. *fimi* ATCC 8183 was excluded from this group and the properties of this strain agreed completely with those of Group 3.

From the results obtained and the consideration mentioned above, the following strains belong to the genus *Cellulomonas*: C. *biazotea* AJ 1569 (ATCC 486*), *Cellulomonas flavigena* AJ 1570 (ATCC 482*), *Cellulomonas gelida* AJ 1567 (ATCC 488*), C. *fimi* AJ 1571 (ATCC 484*), AJ 1572 (ATCC 15724), and *Cellulomonas uda* AJ 1568 (ATCC 491*).

5) **Group 5.** Type of cell division and principal amino acid in the cell wall of this group of bacteria are identical with those of Group 4, but the GC content of the strains of Group 5 is distributed from 66 to 71%, and slightly lower than that of Group 4. The bacteria of this group are weakly gram-positive, and almost all the strains are motile. Metachromatic granules are not found. 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 the effect of this substance to cell form is negligible. These bacteria produce acid slowly and weakly from various sugars, and the number of sugars utilized is less than that of Group 4. Furthermore, microorganisms of this group assimilate a larger number of organic acids than those of Group 4. All the strains slowly hydrolyze gelatin. Most strains of this group are "motile brevibacteria" reported by Komagata and Itzuka (21). The suitable genus is not found for this group of bacteria as far as known. Present
authors propose a new genus “Curtobacterium” for Group 5 on the basis of the characteristics mentioned above.

Genus Curtobacterium can be characterized as follows:

Curtobacterium gen. nov.

Cur. to. bac. te ri. um. L, adj. curtus shortened; bacterium Gr. neut. dim. n. a small rod; M.L. neut. n. Curtobacterium a short rodlet. Small short rods. Coccolid cells are found in old cultures. Weakly gram-positive, frequently old cells lose gram-positivity. Generally motile. Motile species show lateral flagellation. Cells multiply by bending type of cell division. Metachromatic granules are not recognized. Pleomorphism is slightly recognized. Ornithine is found in the cell wall as a principal amino acid. Guanine-cytosine content in DNA is distributed from 66 to 71%. Produces acid slowly and weakly from various sugars. Assimilates various kinds of organic acids in addition to acetate, pyruvate, and lactate. Gelatin is slowly hydrolyzed. Chemoheterotroph. Habitat: Widely distributed in plant materials, soil, etc.

The type species is Curtobacterium citreum (Komagata and Iizuka 1964 (22)) comb, nov.

From the results obtained and the consideration mentioned above, the following strains belong to the genus Curtobacterium: Corynebacterium flaccumfaciens AJ 1400 (ATCC 6887), C. flaccumfaciens subsp. aurantiaca AJ 1412 (ATCC 12813*), Corynebacterium poineettiae AJ 1999 (CCM 1587*), Brevibacterium albidum AJ 1472 (IAM 1631*), B. citreum AJ 1469 (IAM 1514*), and AJ 1471 (IAM 1614), Brevibacterium insectophilum AJ 1477 (IFM AM-23), Brevibacterium luteum AJ 1470 (IAM 1623*), Brevibacterium pusillum AJ 1462 (IAM 1479*), and AJ 1463 (IAM 1480*), Brevibacterium saperdae AJ 3126 (CCEB 366*), Brevibacterium testaceum AJ 1464 (IAM 1537*), B. helvolum AJ 1447 (IAM 1478), AJ 1449 (IAM 1498), AJ 1455 (IAM 1391), and AJ 1458 (IAM 1434).

6) Group 6. Bacteria of this group propagate by bending type of cell division and have LL-DAP as a principal amino acid in the cell wall. Their GC content is around 70%. They are weakly gram-positive, and show elongated or complicated branching cells. Strongly gram-positive large spherical bodies, cystites, frequently appear. Metachromatic granules are not found. Pleomorphism is distinctive. Some strains are motile. Change of cell form by 2% sodium citrate is not distinctive. No acid is produced from any sugars but many kinds of organic acids are assimilated. These bacteria hydrolyze gelatin and show the activity of extracellular DNase.

The bacteria of this group seem to be related to Streptomyces because they exhibit complicated cell form such as elongated and secondary branching cells, and contain LL-DAP and a large amount of glycine in the cell wall. The GC content of this group is similar to that of Streptomyces, 70 to 75%.

The suitable genus is not found for this group of bacteria as far as known. This group is considered to form a biological cluster at a generic level but further studies are required for better understanding, because only a small number of strains were employed.
The following strains belong to Group 6: *Brevibacterium lipolyticum* AJ 1450 (IAM 1398), AJ 1451 (IAM 1413), *Arthrobacter atrocyaneus* AJ 1429 (CCM 1645*), *Arthrobacter simplex* AJ 1420 (ATCC 6946*), *Arthrobacter tumescens* AJ 1424 (ATCC 6947*), *Arthrobacter variabilis* AJ 1434 (CCM 1565*).

7) **Group 7.** Bacteria of this group multiply by bending type of cell division and contain DAB in the cell wall. GC content of these organisms ranges from 69 to 71%. Gram-stain is weakly positive and metachromatic granules are not found. Some strains are motile. Comparatively short rod cells are found. Pleomorphism is slightly recognized. Change of cell form by 2% sodium citrate is not substantially found. No acid is produced from any sugars and some organic acids related to TCA cycle are assimilated.

The present authors consider that the bacteria of this group differ fundamentally from other groups with respect to the type of cell wall, GC content in DNA, and the patterns of acid production from sugars and of assimilation of organic acids. The authors hesitate to propose a new genus for this group at present because only a small number of the strains were employed.

The following strains belong to Group 7: *Corynebacterium aquaticum* AJ 1413 (ATCC 14665), *Corynebacterium michiganense* AJ 1390 (ATCC 492), AJ 1391 (ATCC 4450), AJ 1392 (ATCC 7429), AJ 1393 (ATCC 7430), and AJ 1394 (ATCC 10202).

III. Coryneform bacteria which do not belong to any of these groups

Following strains were not classified into any of the groups in this system. *Corynebacterium bovis* AJ 1407 (ATCC 7715) and AJ 1377, *Corynebacterium hydrocarboclastus* AJ 1379 (IAM 1399) and AJ 1386 (IAM 1484), *Corynebacterium insidiosum* AJ 1409 (ATCC 10253), *Corynebacterium sepedonicum* AJ 1406 (ATCC 9850), *Brevibacterium imperiale* AJ 1446 (ATCC 8365*), *Brevibacterium minutilferula* AJ 1482 (IFM AU-27), and *Microbacterium lacticum* AJ 1416 (ATCC 8180*).

a) *Corynebacterium hydrocarboclastus* AJ 1379 and AJ 1386

These two strains are almost identical. Type of cell wall, GC content, and biochemical and physiological properties of these strains agree with those of Group 1. However, they clearly differ from the members of Group 1 by the type of their cell division and morphological characteristics. They grow by bending type of cell division and show long or often complicated branched cells which then become short rods through fragmentation. From these characteristics, *C. hydrocarboclastus* seems to be related to the species of nocardioform bacteria.


These strains contain lysine in the cell wall as a principal amino acid and propagate by bending type of cell division. Their GC content is distributed in a narrow range from 68 to 70%. From the consideration mentioned above,
it seems that these 6 strains form a biological cluster. However, since their biological and cultural properties, such as the patterns of acid formation from sugars and of assimilation of organic acids, growth rate, etc., are different from one another, further studies are required to decide their correct taxonomic position. Fiedler et al. (23) reported that murein of \textit{M. lacticum} showed unusual amino acid composition.

c) \textit{Corynebacterium insidiosum} AJ 1409

The characteristics of strain AJ 1409 agree well with those of Group 7, but the present authors excluded it from Group 7 because of its extraordinarily high GC content (78.1%).

\textbf{IV. Classification of the genera of coryneform bacteria}

From the results obtained, the genera of coryneform bacteria are differentiated as follows:

\textit{Coryneform bacteria}

Aerobic, strongly gram-positive or weakly gram-positive, non-spore forming, not acid-fast, rod-shaped bacteria.

\textit{Key to the genera of coryneform bacteria}

\textbf{I.} DL-Diaminopimelic acid is found in the cell wall.

- Strongly gram-positive. Non pleomorphic.

\textbf{A.} Snapping cell division is recognized.

- Metachromatic granules are found in the cells.
- Gelatin is not hydrolyzed.
- GC content ranges from 51 to 70%.
- Type species: \textit{Corynebacterium diphtheriae} (Flügge) Lehmann and Neumann.

\textbf{B.} Bending type of cell division is recognized.

- Metachromatic granules are not found in the cells.
- Gelatin is hydrolyzed.
- GC content ranges from 60 to 63%.
- Genus \textit{Brevibacterium} Breed, 1953.
- Type species: \textit{Brevibacterium linens} (Weigmann) Breed.

\textbf{II.} Lysine is found in the cell wall.

- Pleomorphic, weakly gram-positive or gram-negative cells and strongly gram-positive large spherical bodies, cystites, are frequently found in older cultures.
- No acid is produced from any sugars.
- Gelatin is hydrolyzed.
- GC content ranges from 58 to 65%.
- Genus \textit{Arthrobacter} Conn and Dimmick, 1947.
- Type species: \textit{Arthrobacter globiformis} (Conn) Conn and Dimmick.
III. Ornithine is found in the cell wall.
   A. Acids are produced strongly and rapidly from many kinds of sugars.
   Organic acids are not assimilated except acetate, pyruvate, and lactate.
   GC content ranges from 72 to 73%.
   Type species: *Cellulomonas biazotea* (Kellerman *et al.*) Bergey *et al.*
   B. Acids are produced weakly and slowly from some sugars.
   Acetate, pyruvate, lactate, and other organic acids are assimilated.
   GC content ranges from 66 to 71%.
   Genus *Curtobacterium* nov. gen.
   Type species: *Curtobacterium citreum* (Komagata and Iizuka) comb. nov.

IV. LL-Diaminopimelic acid and glycine are found in the cell wall.
Elongated or branched cells are frequently found in ordinary media.
GC content ranges from 70 to 72%.
*Bacteria belonging to Group 6.* (LL-DAP type coryneform bacteria)

V. α,γ-Diaminobutyric acid is found in the cell wall.
GC content ranges from 69 to 71%.
*Bacteria belonging to Group 7.* (DAB type coryneform bacteria)

V. The family to which genera of coryneform bacteria belong
According to Bergey's Manual, 7th edition, the coryneform bacteria are included in the families of Corynebacteriaceae and Brevibacteriaceae. The former includes *Corynebacterium, Arthrobacter, Microbacterium,* and *Cellulomonas,* and the latter, *Brevibacterium.* However, differentiating characteristics of these families are not clear. From the results obtained, all the species of *Brevibacterium* except *B. linens* were dispersed and absorbed in various groups. Further, Group 2 (*Brevibacterium*) did not exhibit fundamental characteristics to separate the family in comparison with the other groups. From these facts, it seems unreasonable that the genus *Brevibacterium* is excluded from Corynebacteriaceae. Consequently, the present authors consider that all the genera included in the category of the coryneform bacteria belong to the family Corynebacteriaceae.

VI. Genus Microbacterium
*Microbacterium* was established in 1919 by ORLA-JENSEN (24) on the basis of production of lactic acid and heat resistance. In the present study, *M. flavum* ATCC 10340 (type strain) and *M. lacticum* ATCC 8010 (type strain) isolated by ORLA-JENSEN were found to differ from each other in respects to type of cell division, type of cell wall, GC content, gram-positivity, and morphological and biochemical properties. The former was included in Group 1, while the latter did not belong to any of the groups mentioned above.
DE WOLFF (25), FUJITA and KODAMA (26), and TASMAN and BRANWIJK (27) showed that *C. diphtheriae* produced lactic acid with other acids
under certain conditions. TANAKA et al. (28) and OKADA et al. (29) reported that glutamic acid-producing bacteria which belonged to Group 1 formed a large amount of lactic acid under limited aeration. From these facts, it is not considered to be appropriate to justify the production of lactic acid as a generic character to differentiate Microbacterium from Corynebacterium as JENSEN (18, 30) pointed out. On the other hand, Doi and KANEKO (31) and TAKAYAMA et al. (32) indicated that M. flavum was not heat resistant. From the consideration mentioned above, the genus Microbacterium seems to be rejected.

CONCLUSION

Recently, VELDKAMP (33) reviewed the taxonomy of coryneform bacteria and pointed out several taxonomic problems of this group of bacteria. From the results obtained by the present authors, it is found that bacterial taxonomists have given different evaluation for criteria presently employed in the classification of coryneform bacteria. This fact may also indicate that the generic concepts of these bacteria have not been established clearly and that the generic concept of Corynebacterium was expanded without extensive taxonomical considerations. The genera had been based mainly on cell morphology, biochemical characteristics, habitat, and pathogenicity or parasitism. These characteristics seem to be subjective or indistinct.

The present authors found that the so-called coryneform bacteria could not be classified or identified by the features commonly employed in bacterial taxonomy. Consequently, they adopted some new characteristics such as the mode of cell division, composition of cell wall, and GC content in DNA which seemed to be fundamental and objective. Composition of cell wall or GC content has been studied by many researchers from the viewpoint of bacterial taxonomy, but the classification and definition of the genera of coryneform bacteria have been difficult merely on the basis of one of these characteristics. However, the present authors recognized seven groups in the coryneform bacteria and clarified the concepts of several genera on the basis of the combination of the type of cell wall and characteristics described above. Further, the scheme for differentiation of the genera of these bacteria was proposed.

It was considered that the type of cell wall is taxonomically more important than GC content in DNA, because the type of cell wall is closely related to other characteristics such as the mode of cell division, cell morphology, biochemical characteristics, etc. The GC content in DNA or type of cell wall are not yet employed widely in the bacterial classification and identification, but hereafter, from the standpont of chemotaxonomy, these characteristics would be accepted more commonly in this field. In future, the study on the structure of murein in the cell wall reported by FIEDLER et al. (23, 34) must give an important information to clarify the bacterial phylogeny.

The results of numerical taxonomy on coryneform bacteria (16, 35–39)
do not always agree with the authors’ results. This may be ascribed to the difference of the principal thought on bacterial taxonomy.

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