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THE MENAQUINONE SYSTEM IN THE CLASSIFICATION OF CORYNEFORM AND NOCARDIOFORM BACTERIA AND RELATED ORGANISMS[1]

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Thirty-nine cultures were examined for the MK system, which belong to the coryneform genera *Corynebacterium*, *Arthrobacter*, *Brevibacterium*, *Microbacterium*, and *Cellulomonas*, and to the actinomycetous genera *Mycobacterium*, *Nocardia*, and *Oerskovia*. The coryneform bacteria were found to have the complex quinone systems composed of MK-8, MK-8 (H_2), MK-8 (H_4), MK-9, MK-9 (H_2), MK-9 (H_4), and MK-11. The actinomycetous organisms represented the systems of MK-8 (H_4), MK-9 (H_2), MK-9 (H_4), and MK-9 (H_6). These results indicate a heterogeneous nature of these organisms. Discussions are made on the classification, especially on several groupings.

Since JENSEN (*1*) designated the organisms that are aerobic, gram-positive, not acid-fast, nonspore-forming, and rod-shaped, "coryneform bacteria," where several genera are taxonomically included such as *Corynebacterium*, *Microbacterium*, *Cellulomonas*, *Arthrobacter*, *Brevibacterium*, and so on, the coryneform group of bacteria has presented a number of unsolved problems in taxonomy and classification. Moreover, the non-pathogenic coryneform bacteria have recently been used in microbial industry, so that their classification has become more confused. On the other hand, human and animal parasites in the genus *Corynebacterium* have some similarity in chemotaxonomic aspects to the actinomycetous genera *Nocardia* and *Mycobacterium*, especially in the presence of arabinose and galactose

[1] This constitutes Part I of a series entitled "Significance of Ubiquinone and Menaquinone System in the Classification of Gram-negative and Gram-positive Bacteria." This work was presented at the Annual Meeting of the Agricultural Chemical Society of Japan, Sapporo, July 24, 1975. The abbreviations used here for menaquinone or vitamin K_2 are: MK, menaquinone; MK-*n* with *n* denoting a specified number of isoprene units in the side chain; MK-*n*(H_m) with *m* indicating the number of hydrogen atoms saturating the isoprenoid chain, *e. g.*, MK-9 or MK-9(H_4), *etc.*

as common cell-wall sugars, a common cell-wall antigen, and mycolic acids (*2–4*).

We previously showed that, in the ubiquinone system of gram-negative, rod-shaped acetic acid bacteria, a specified number of isoprene units in the side chain attached to the quinone ring provided a new criterion with respect to the designation of genus or species group in the genera *Gluconobacter* and *Acetobacter* (*5–7*). Similar results were obtained in the classification of yeasts and yeast-like organisms (*8–12*).

The present paper reports the menaquinone system of the coryneform and nocardioform bacteria and related organisms.

MATERIALS AND METHODS

Microorganisms. All the bacterial strains used in this experiment came from the Central Research Laboratories of Ajinomoto Co., Inc., Kawasaki, by courtesy of Dr. K. Yamada and Dr. M. Takahashi. These strains comprise 39 cultures of the genera *Corynebacterium, Arthrobacter, Brevibacterium, Microbacterium, Cellulomonas, Mycobacterium, Nocardia*, and *Oerskovia*; sources of these strains are listed in Table 1.

Culture media. Stock cultures were generally maintained on glucose-peptone-meat extract-agar slants held at 4°. The agar slants contained (per liter) glucose 10 g, peptone (Kyokuto) 5 g, and meat extract (Kyokuto) 5 g. For the working cultures, modified glucose-peptone-meat extract broth was used (glucose 7 g, peptone 3 g, and meat extract 3 g per liter). Glycerol or brain heart infusion (Eiken) was added, if necessary, to the media in the concentration of 3 or 5 g per liter.

Cultivation. The organisms were shaken in 10 ml of the medium for 48 hr. The cultures were then transferred to 50 ml of the medium and incubated for 24 hr. The final working of cultivation was carried out in 0.5–1 liter of the modified glucose-peptone-meat extract broth, dispensed in a 5-liter conical flask, at 30° for 24 hr on a rotary shaker.

Paper chromatography. To identify the menaquinone system, reversed phase chromatography was made for MK preparations on Toyo No. 50 filter paper, impregnated with 2.5% white petrolatum (w/w, J. P.) in toluene solution, and developed with N, N-dimethylformamide-water, 98: 2, system. After development for about 18 hr, chromatograms were examined under ultraviolet light. The members of menaquinone appeared as brownish spots. Subsequently, this identification was confirmed by dipping the filter paper into 0.2% permanganate solution. Besides the solvent system mentioned above, two other systems were employed to compare mobility by chromatography on paper strips, impregnated with 3% Silicone (w/w, KF–54, Shin'etsu Kagaku); the solvent systems were ethanol-ethyl acetate-water (5: 3: 1) and propanol-water (4: 1).

Mass spectrometry. Mass spectra of MK preparations were recorded with a Hitachi RMU–6M single-focusing mass spectrometer at a chamber temperature of

220°. Samples were vaporized at the ion source with a heated direct inlet system operating at 150°.

RESULTS

Extraction and preparation of menaquinone

Washed cells were immediately lyophilized and stocked in a deepfreezer. The lyophilized cells (1–5 g) were dispersed in 150 ml of a mixture of ether-acetone (1: 3). Extraction was made on a stirrer for about 3 hr. The dispersion was filtered, and this extraction was repeated three times. Subsequently, the combined filtrate was evaporated and dried under a reduced pressure. The residual material in the flask was dispersed again in a small amount of acetone, and the acetonic liquor was filtered.

The lipid extract dissolved in a very small volume of acetone was submitted to thin-layer chromatography. The extract was applied as a streak to a 20×20-cm plate coated with 500-μm layer of silica gel (Kieselgel GF_{254} nach Stahl, Type 60, Merck). Authentic vitamin K_1 (Nakarai) and ubiquinone isolated from *Gluconobacter cerinus* (*13*) were spotted on both sides of the streak to act as a marker. The loaded plate was placed vertically in a closed developing chamber together with a trough containing a solvent system of benzene-hexane-chloroform (1: 1: 1). The development was completed within 30 min. The quinones quench the green fluorescence when the plate is viewed under ultraviolet light (260 nm), and thus appear purple against a green background. A marker of vitamin K_1 gave *Rf* value of 0.8 in this system, but the ubiquinone appeared at 0.3. A yellow band containing MK was found to have almost the same mobility as vitamin K_1, so that the authentic K_1 aided its identification. Thus, menaquinone was separated by the preparative chromatographic technique. The yellow band was scraped from the plate and extracted from the silica gel with acetone.

Identification of menaquinone system

The material obtained was analyzed with a Hitachi mass spectrometer. The mass spectra were found identical in every respect with those of MK, as proposed by BEAU *et al.* (*14*) and by DUNPHY *et al.* (*15*), except for the parent peaks. Two intense fragment peaks were seen at *m/e* 187 and 225, and several peak clusters at *m/e* 715–240, which exhibit successive loss of isoprene units in the side chain. Figure 1 represents the parent peaks of several MKs obtained from coryneform bacteria and related organisms.

Reversed phase chromatography on paper was used for further identification of MK system in these bacterial cells. As described in the previous papers (*6*, *8*), the acetonic solution containing authentic isoprenologs of MK was spotted on both sides of paper strips (12×40 cm), impregnated with petrolatum or Silicone. Portions of the MK preparations were then applied on paper strips inside the authentic spots. These techniques were necessary to ensure accurate identification

Table 1. Menaquinone system in coryneform and nocardioform bacteria and related organisms.

Species and strain	Source	MK system
Corynebacterium		
C. diphtheriae AJ 1414	ATCC 11913	MK-8(H_2)
C. xerosis AJ 1375	ATCC 373	MK-9(H_2)
C. equi AJ 1402	ATCC 6939	MK-8(H_2)
C. fascians AJ 1398	ATCC 12974	MK-8(H_2)
C. flaccumfaciens AJ 1400	ATCC 6887	MK-9
C. poinsettiae AJ 1999	CCM 1587	MK-9
C. glutamicum AJ 1502	ATCC 13032	MK-9(H_2)
C. lilium AJ 1517	NRRL B-2243	MK-9(H_2)
Arthrobacter		
A. globiformis AJ 1422	ATCC 8010	MK-9(H_2)
A. nicotianae AJ 1426	ATCC 15236	MK-8[MK-9]
A. oxydans AJ 1425	ATCC 14359	MK-9(H_2)
A. ureafaciens AJ 1421	ATCC 7562	MK-9(H_2)
A. simplex AJ 1420	ATCC 6946	MK-8(H_4)
A. citreus AJ 1423	ATCC 11624	MK-9(H_2)
Brevibacterium		
B. linens AJ 1520	ATCC 8377	MK-8(H_2)
B. linens AJ 1521	ATCC 9172	MK-8(H_2)
B. lipolyticum AJ 1450	IAM 1398	MK-8(H_4)
B. fuscum AJ 3124	CCEB 277	MK-9[MK-8]
B. helvolum AJ 1445	ATCC 11822	MK-9(H_2)
B. ammoniagenes AJ 1443	ATCC 6871	MK-9(H_2)
B. ammoniagenes AJ 1444	ATCC 6872	MK-9(H_2)
B. sulfureum AJ 1448	IAM 1488	MK-9[MK-10]
B. albidum AJ 1472	IAM 1631	MK-9
B. citreum AJ 1469	IAM 1514	MK-9
B. luteum AJ 1470	IAM 1623	MK-9
B. testaceum AJ 1464	IAM 1537	MK-11
B. aquaticum AJ 1413	ATCC 14665	MK-11[MK-10]
Microbacterium		
M. flavum AJ 1415	ATCC 10340	MK-8(H_2), MK-9(H_2)
Cellulomonas		
C. biazotea AJ 1569	ATCC 486	MK-9(H_4)
C. fimi AJ 1571	ATCC 484	MK-9(H_4)
Mycobacterium		
M. tuberculosis AJ 3368	IMET 1071A	MK-9(H_2)
M. phlei AJ 1574	IFO 3158	MK-9(H_2)
Nocardia		
N. brasiliensis AJ 9141	CCM 165	MK-8(H_4)
N. asteroides AJ 9128	ATCC 19247	MK-8(H_4)
N. asteroides AJ 9169	ATCC 3318	MK-8(H_4)
N. coeliaca AJ 9100	IFM 30	MK-8(H_4)
N. madurae AJ 9136	NRRL B-2127	MK-9(H_6)

(Table 1. continued)

Species and strain	Source	MK system
Oerskovia		
O. turbata AJ 9191	ATCC 25835	MK-9(H_4)
O. xanthinolytica AJ 9194	ATCC 27402	MK-9(H_4)

AJ: The Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan.
ATCC: American Type Culture Collection, Rockville, Maryland, U. S. A.
CCM: Czechoslovak Collection of Microorganisms, J. E. Purkyně University, Brno, Czechoslovakia.
NRRL: Northern Utilization Research and Development Division, Peoria, Illinois, U. S. A.
IAM: Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan.
CCEB: Culture Collection of Entomogenous Bacteria, Department of Insect Pathology, Institute of Entomology, Prague, Czechoslovakia.
IMET: Culture Collection, Institute fuer Mikrobiologie und Experimentelle Therapie, Jena, East Germany.
IFM: Institute for Food Microbiology, Chiba University, Narashino, Japan.

The species are arranged according to the Bergey's Manual of Determinative Bacteriology, 8th Edition.
The parenthesized quinones indicate minor components of MK.

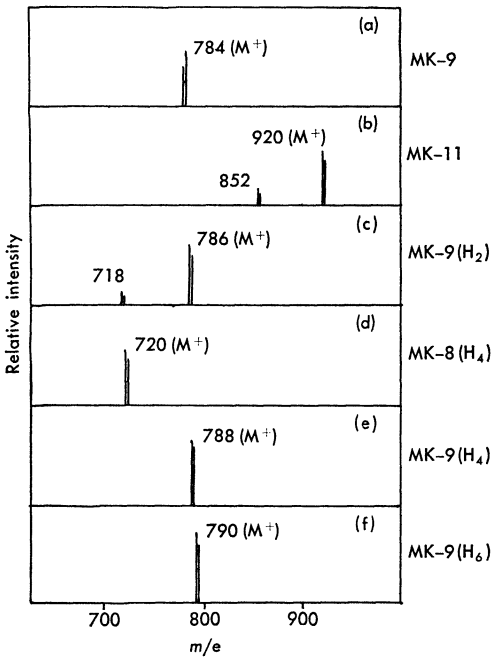

Fig. 1. Mass spectra of menaquinones obtained from coryneform and nocardioform bacteria. These MK preparations exhibited intense fragment peaks at *m/e* 187 and 225. (a) *C. flaccumfaciens* AJ 1400, (b) *B. testaceum* AJ 1464, (c) *A. globiformis* AJ 1422, (d) *B. lipolyticum* AJ 1450, (e) *O. turbata* AJ 9191, (f) *N. madurae* AJ 9136.

of the MK isoprenologs, since interfering substances were present in the preparations. The strips were developed with the three different solvent systems. Hydrogenated menaquinones ran more slowly than normal MKs, when chromatographed on the petrolatum-impregnated paper with the solvent system of N, N-dimethylformamide-water, 98: 2.

The menaquinone system of coryneform and nocardioform bacteria and related organisms

Table 1 shows the menaquinone system of these organisms, which comprises MK-8, MK-8 (H_2), MK-8 (H_4), MK-9, MK-9 (H_2), MK-9 (H_4), MK-9 (H_6), and MK-11. The menaquinone-8 with dihydrogenated isoprene units of the *C. diphtheriae* was in agreement with that reported by SCHOLES and KING (*16*) and BEAU *et al.* (*14*). The MK-9 (H_2) obtained here in *M. phlei* and *M. tuberculosis* coincided with that described by GALE *et al.* (*17*) and BEAU *et al.* (*14*). A unique MK system with tetrahydrogenated isoprene units was found in *A. simplex* and *B. lipolyticum*. In addition, MK-8 (H_4) was distributed in the actinomycetous genus *Nocardia*. However, this genus gave another MK system [MK-9 (H_6)]. The hexahydrogenated MK-9 was reported in a certain member of *Streptomyces* group by PHILLIPS *et al.* (*18*). The genera *Cellulomonas* and *Oerskovia* produced another type of quinone, tetrahydrogenated MK with 9 isoprene units. *Microbacterium flavum* possessed two isoprenologs of quinone [MK-8 (H_2), MK-9 (H_2)]. Menaquinone-11 was found in *B. testaceum* and *B. aquaticum*.

DISCUSSION

Mycobacteria. The two species of mycobacteria gave the system of MK-9 (H_2), which is identical with a large number of coryneform bacteria such as *C. xerosis, C. glutamicum, C. lilium, A. globiformis*, and *B. ammoniagenes* (Table 1). There is no discrepancy in the MK system between "slow growers" and "rapid growers" in the 3 groups of the genus *Mycobacterium* (*19*). The common MK system of MK-9 (H_2) in these organisms reconfirms a closer relationship between the mycobacteria and the corynebacteria.

Nocardioform bacteria. MCCLUNG (*20*) divided the genus *Nocardia* into 3 groups according to morphological differences in the degree of mycelial development. We examined 3 species of the first group, and all of them exhibited a very rare MK system of MK-8 with tetrahydrogenated isoprene units; they are *N. brasiliensis, N. asteroides*, and *N. coeliaca*. Their MK system was, however, in agreement with the two species of coryneform bacteria of *A. simplex* and *B. lipolyticum*.

LECHEVALIER and LECHEVALIER (*21*) established a new genus *Oerskovia*. The two species, *O. turbata* and *O. xanthinolytica*, produced the MK-9 (H_4) system, which is different from the first group of the genus *Nocardia*, but is similar to the

genus *Cellulomonas*. LECHEVALIER and LECHEVALIER (*22*) studied *N. madurae* Blanchard 1896, set up a new genus *Actinomadura*, and placed the bacterium in the genus as *Actinomadura* species. We recognized MK–9 (H_6) in this species, the presence of which had been once reported in a certain member of *Streptomyces* group by PHILLIPS *et al.* (*18*). However, the organism has not been exactly specified as yet, so that its accurate position in the classification is rather obscure. GUINAND *et al.* (*23*) found the occurrence of glycolyl residue in the cell wall of *N. kirovani*. Uchida and Aida (personal communication) recently observed a remarkable distinction in the amount of glycolate in cell-wall components in nocardioform bacteria; they calculated the values in *N. madurae* at 0.4–1.0 nmol/mg of dry cells. In contrast, the members in the first morphological group of nocardiae gave 23.0–51.0 nmol. Further, KOMURA *et al.* (*24*) did not detect phosphatidylethanolamine in *N. madurae* distinct from the other members of the genus *Nocardia*. These results suggest that the organisms classified in these two genera *incertae sedis* may occupy taxonomically particular positions.

Coryneform group of bacteria. As can be seen from the tabulated results (Table 1), the coryneform bacteria contained seven kinds of MK; they are composed of normal and hydrogenated quinones, MK–8, MK–9, MK–11, MK–8 (H_2), MK–8 (H_4), MK–9 (H_2), and MK–9 (H_4). Such a multiplicity in the MK system indicates that this group of bacteria is a very complex and heterogeneous one.

The genus *Corynebacterium* Lehmann and Neumann 1896 nowadays consists of human and animal parasites and pathogens, plant pathogenic and non-pathogenic organisms (*25*). We recognized three types of MK system in this genus; MK–8 (H_2), MK–9, and MK–9 (H_2). This is suggestive of diversity in the genus *Corynebacterium*. Of the three species of animal pathogenic bacteria examined, two types were found; MK–8 (H_2) in *C. diphtheriae* and *C. equi*, and MK–9 (H_2) in *C. xerosis*. Three of the plant pathogenic bacteria produced the quinone system of MK–8 (H_2) [*C. fascians*] and of MK–9 [*C. flaccumfaciens* and *C. poinsettiae*]. The latter two constitute the group 5 (*25*). The non-pathogenic *C. glutamicum* and *C. lilium* had MK–9 with dihydrogenated isoprene units.

Arthrobacter simplex was the only species that has the tetrahydrogenated MK–8 system. Notable is the fact that the normal system of MK–8 [MK–9] was in *A. nicotianae*. All the other members had MK–9 (H_2).

YAMADA and KOMAGATA (*26*) recently classified coryneform bacteria into 7 separate groups in combination with their morphological, cultural, and physiological characteristics and principal amino acids in their cell wall.

Their 1st group is characterized by the snapping type of propagation and DL-diaminopimelate in the cell wall, and the group is considered to correspond to the genus *Corynebacterium* Lehmann and Neumann 1896. The GC content of this group is in the range of 52–70%. Figure 2 shows the relationship between the MK system and %GC of DNA. *Corynebacterium diphtheriae* is clearly distinguishable from the non-pathogenic *C. glutamicum* and *C. lilium*, in spite of their having a very

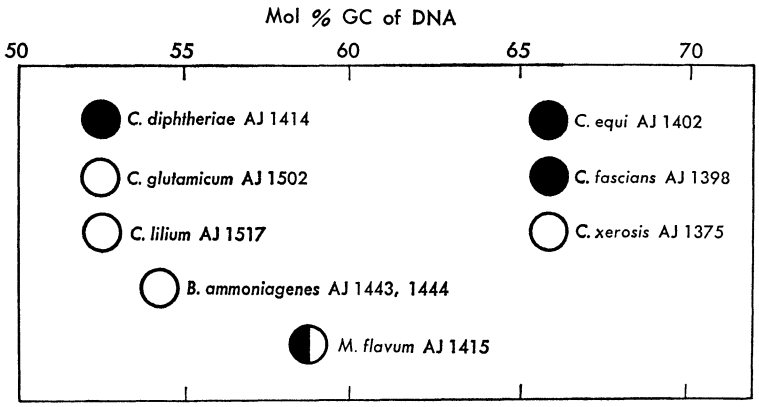

Fig. 2. Menaquinone system in the 1st group or the genus *Corynebacterium* (*26*). The GC values are plotted in combination with the MK system. ● MK–8(H_2), ○ MK–9(H_2), ◐ MK–8(H_2), MK–9(H_2).

close GC value. In a higher GC region of this group, a similar duality exists among the three pathogenic corynebacteria. However, the difference in MK has been found to be reasonable.

Uchida and Aida (personal communication) determined a high concentration of glycolate in the two species, *C. equi* and *C. fascians*, with MK–8 (H_2). In contrast, the remaining *C. xerosis* having MK–9 (H_2) gave a very small amount of glycolyl residue.

KOMURA *et al.* (*24*) found a remarkable distinction in the amount of phosphatidylethanolamine in the members of the genus *Corynebacterium*, which are characterized by the DL-diaminopimelate type of cell wall; a trace amount is distributed in the low GC-content group and a large amount is in the high GC-content one, though they have not yet examined the species, *C. xerosis*, with the high GC content and the MK–9 (H_2) system. The genus *Corynebacterium* is, thus, divided into 4 subgroups, when the MK system is combined with the amounts of phosphatidylethanolamine and glycolate and the GC content of DNA. In the low GC-content region, the 1st subgroup gives the MK–8 (H_2) system [*C. diphtheriae*] and the 2nd one does MK–9 (H_2) [*C. glutamicum* and *C. lilium*], all of which have a trace amount of phosphatidylethanolamine and glycolate. In the high GC-content region, the 3rd one is characterized by the MK–8 (H_2) system and a high concentration of phosphatidylethanolamine and glycolate [*C. fascians* and *C. equi*]. The last subgroup includes *C. xerosis*, which bears the MK–9 (H_2) system and a trace amount of glycolate. The coexistence of two isoprenologs of MK observed in *M. flavum* leads us to the assumption that the bacterium would be a phylogenetical intermediate between the organisms with only the system of MK–8 (H_2) or MK–9 (H_2).

Their 2nd group is characteristic of the bending type of cell division and DL-diaminopimelate in the cell wall. The GC content of DNA is within the range of

61–63%. The group is regarded as the genus *Brevibacterium* Breed 1953 emend. Yamada and Komagata 1972, and the only species *B. linens* remains as the type. We examined two strains and found MK–8 (H_2) (Table 2). The MK system coincided with that of the type species in the genus *Corynebacterium*.

Their 3rd group propagates by the bending type of cell division and possesses lysine in the cell wall. The GC values range from 58 to 66%, and the group corresponds to the genus *Arthrobacter*. The MK system is rather complicated (Fig. 3). This complexity is due to a heterogeneous nature of this group. *Arthro-*

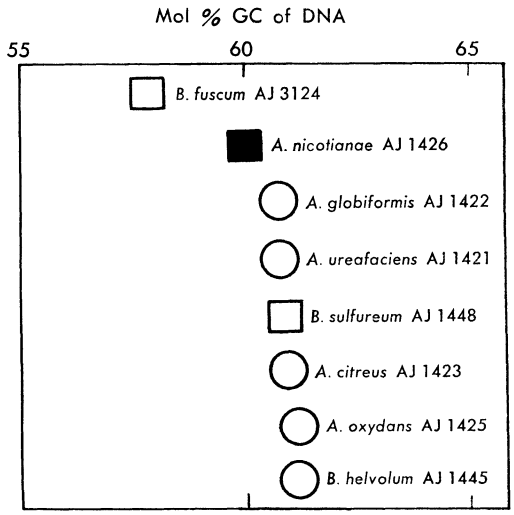

Fig. 3. Menaquinone system in the 3rd group or the genus *Arthrobacter* (*26*). The GC values are plotted. ○ MK–9(H_2), ■ MK–8, □ MK–9.

bacter nicotianae was proposed as a new species by GIOVANNOZZI-SERMANNI (*27*), but the bacterium is now placed in *A. globiformis* as a synonym (*28*) because of similar cell wall composition and GC content. The MK system with normal isoprenologs makes the bacterium separate from *A. globiformis*, and the original species name of *A. nicotianae* should be given. The other two organisms likewise produced a normal MK distinct from the type of this genus.

Their 4th group is of the bending type of propagation and ornithine in the cell wall, and is designated as the genus *Cellulomonas*. The two species tested showed the MK–9 (H_4) system (Table 2), which are identical in MK with the respective species of the genus *Oerskovia*.

YAMADA and KOMAGATA (*26*) further set up two small groups concerning the coryneform bacteria; their 6th group includes the organisms propagating by the bending type of cell division and representing LL-diaminopimelate, and is supposed to be related to the *Streptomyces* group, and the other is the 7th group characterized by the bending type and diaminobutyrate as a cell-wall component. The GC

Table 2. Menaquinone system in the 2nd and 4th groups (*26*).

The 2nd group or the genus *Brevibacterium* Breed 1953 emend. Yamada and Komagata 1972.

Species and strain	GC content of DNA(%)[a]	MK system
B. linens AJ 1520	62.7	MK-8(H_2)
B. linens AJ 1521	63.4	MK-8(H_2)

The 4th group or the genus *Cellulomonas*.

Species and strain	GC content of DNA(%)[a]	MK system
C. biazotea AJ 1569	72.2	MK-9(H_4)
C. fimi AJ 1571	71.7	MK-9(H_4)

[a] The GC content of DNA is cited from YAMADA and KOMAGATA (*29*).

content of DNA is around 70% in both groups. It is of interest that the two species, *A. simplex* and *B. lipolyticum*, classified in the 6th group have a tetrahydrogenated homolog of MK-8, which is common to most members of nocardiae, especially in the morphological 1st group (*20*). Our present finding in the 7th group was a normal MK with eleven isoprene units. From the results obtained in these small groups, it is likely that the coryneform bacteria having such an anomalous MK system may occur taxonomically and phylogenetically in a certain peculiar position (Table 3).

Table 3. Menaquinone system in the 6th and 7th groups (*26*).

The 6th group.

Species and strain	GC content of DNA(%)[a]	MK system
A. simplex AJ 1420	71.7	MK-8(H_4)
B. lipolyticum AJ 1450	70.7	MK-8(H_4)

The 7th group.

Species and strain	GC content of DNA(%)[a]	MK system
B. aquaticum AJ 1413	69.3	MK-11[MK-10]

[a] The GC content of DNA cited from YAMADA and KOMAGATA (*29*).

Their 5th group was postulated as a new genus *Curtobacterium* Yamada and Komagata 1972, into which a number of "motile brevibacteria" (*30*) were placed. This genus agrees in type of cell division and in principal amino acid of cell wall with the 4th group or the genus *Cellulomonas*. However, GC content of DNA in this genus differs from that of the 4th group. As shown in Table 4, all the six species examined gave normal MKs; 5 of them are with 9 isoprene units and the remaining one with 11 units. Their MK systems are quite different from those of any of the 4th group or the genus *Cellulomonas*, which have a tetrahydrogenated MK-9. *Brevibacterium testaceum* with MK-11 has somewhat lower GC value

Table 4. Menaquinone system in the 5th group of the genus *Curtobacterium* (*26*).

Species and strain	GC content of DNA(%)[a]	MK system
B. citreum[b] AJ 1469	70.5	MK-9
C. flaccumfaciens AJ 1400	68.3	MK-9
C. poinsettiae AJ 1999	70.0	MK-9
B. albidum AJ 1472	70.0	MK-9
B. luteum AJ 1470	69.8	MK-9
B. testaceum AJ 1464	65.4	MK-11

[a] The GC content of DNA cited from YAMADA and KOMAGATA (*29*).
[b] This species is the type as *Curtobacterium citreum*.

than the other members (*29*). According to the data from Uchida and Aida (personal communication), this bacterium contains a considerable amount of glycolate in its cell wall, and its quantity is much more than that of all the others. These facts support the postulation that *B. testaceum* should not be included in the genus *Curtobacterium* but transferred to another separate, appropriate new genus.

As has been discussed above, it is obvious that the menaquinone system provides new informations as to the classification of coryneform and nocardioform bacteria and related organisms. On considering the utilization of the MK system, an evidence has been presented as a potential value in the classification, especially in designation for grouping at a genus level, as in the genus *Curtobacterium*.

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