THE MENAQUINONE SYSTEM IN THE CLASSIFICATION OF THE GENUS ACTINOMADURA

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The menaquinone system of three species in the genus Actinomadura was determined. Actinomadura madurae and A. pelletieri were found to have the same quinone system comprising MK-9(H₆)[MK-9(H₄)MK-9(H₈)]. In contrast, several strains classified in A. dassonvillei were different in the chain length and the degree of saturation; they had the MK-10MK-10(H₂)[MK-10(H₄)] or MK-10(H₄)MK-10(H₈)[MK-10(H₈)MK-9(H₄)MK-9(H₆)] system. These results obtained are discussed from the taxonomic point of view.

Since the establishment of the actinomycetous genus Actinomadura by LECHEVALIER and LECHEVALIER (1), several species have been described; A. madurae, A. pelletieri, A. dassonvillei, etc. These species are characterized by type III of cell walls, and can be discriminated from the members of the genus Nocardia which have a type-IV cell wall.

In our previous papers (2, 3), we reported that a strain of the type species of the genus Actinomadura, A. madurae, possessed a unique menaquinone system different from the members of the genus Nocardia such as N. asteroides, N. farcinica, and N. brasiliensis. This paper is concerned with the system of menaquinones in the remaining species of the genus Actinomadura.

MATERIALS AND METHODS

Microorganisms. Cultures of A. madurae and A. pelletieri were from the National Collection of Type Cultures, London, England, American Type Culture.

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1 This constitutes Part VII of a series entitled "Significance of the Ubiquinone and Menaquinone Systems in the Classification of Gram-negative and Gram-positive Bacteria." The abbreviations used here for menaquinone or vitamin K₂ are as follows: MK-ₙ(Hₘ) with n and m denoting a specified number of isoprene units in a side chain and the number of hydrogen atoms saturating the isoprenoid chain, respectively, e.g., MK-9(H₄), etc.
Collection, Rockville, Maryland, U. S. A., Institute of Food Microbiology, Chiba University, Narashino, and Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki. Strains of *A. dassonvillei* were kindly sent by Dr. R. E. Gordon, Institute of Microbiology, Rutgers University, New Brunswick, New Jersey, U. S. A. The sources of these cultures are listed in Table 1.

Table 1. The menaquinone system in the species of the genus *Actinomadura*.

<table>
<thead>
<tr>
<th>Species and strain</th>
<th>MK system</th>
<th>Source and remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. pelletieri</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCTC 4162</td>
<td>MK-9(H₅)[MK-9(H₅)MK-9(H₅)]</td>
<td>NCTC (Type strain)</td>
</tr>
<tr>
<td>IFM 111</td>
<td>MK-9(H₅)[MK-9(H₅)MK-9(H₅)]</td>
<td>IFM</td>
</tr>
<tr>
<td>IFM 112</td>
<td>MK-9(H₅)[MK-9(H₅)MK-9(H₅)]</td>
<td>IFM</td>
</tr>
<tr>
<td><strong>A. dassonvillei</strong></td>
<td></td>
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<tr>
<td>IMRU 509</td>
<td>MK-10MK-10(H₅)MK-10(H₅)</td>
<td>R. E. Gordon, 509 (ATCC 23218, NCTC 10488, type strain) (II)</td>
</tr>
<tr>
<td>IMRU 1250</td>
<td>MK-10MK-10(H₅)MK-10(H₅)</td>
<td>R. E. Gordon, 1250 (ATCC 23219, NCTC 10489) (II)</td>
</tr>
<tr>
<td>IMRU 1288</td>
<td>MK-10MK-10(H₅)MK-10(H₅)</td>
<td>R. E. Gordon, 1288 (II)</td>
</tr>
<tr>
<td>IMRU 1289</td>
<td>MK-10MK-10(H₅)MK-10(H₅)</td>
<td>R. E. Gordon, 1289 (II)</td>
</tr>
<tr>
<td>IMRU 794</td>
<td>MK-10MK-10(H₅)MK-10(H₅)</td>
<td>R. E. Gordon, 794 (II)</td>
</tr>
<tr>
<td>IMRU 1236</td>
<td>MK-10MK-10(H₅)MK-10(H₅)MK-10(H₅)</td>
<td>R. E. Gordon, 1236 (II)</td>
</tr>
<tr>
<td>IMRU 575</td>
<td>MK-10(H₅)MK-10(H₅)MK-10(H₅)MK-9(H₅)MK-9(H₅)</td>
<td>R. E. Gordon, 575 (II)</td>
</tr>
<tr>
<td>N 3255</td>
<td>MK-10(H₅)MK-10(H₅)MK-10(H₅)MK-9(H₅)MK-9(H₅)</td>
<td>R. E. Gordon, N 3255 (NCTC 3255) (II)</td>
</tr>
<tr>
<td><strong>A. madurae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 19425</td>
<td>MK-9(H₅)[MK-9(H₅)MK-9(H₅)]</td>
<td>ATCC (NCTC 5654, type strain)</td>
</tr>
<tr>
<td>AJ 9136</td>
<td>MK-9(H₅)[MK-9(H₅)MK-9(H₅)]</td>
<td>AJ (NRRL B-2127)</td>
</tr>
<tr>
<td>AJ 9089</td>
<td>MK-9(H₅)[MK-9(H₅)MK-9(H₅)]</td>
<td>AJ (IFM 10)</td>
</tr>
</tbody>
</table>

Abbreviations: NCTC, National Collection of Type Cultures, London, England, U. K.; IFM, Institute of Food Microbiology, Chiba University, Narashino, Chiba-ken, Japan; IMRU, Institute of Microbiology, Rutgers University, New Brunswick, New Jersey, U. S. A.; ATCC, American Type Culture Collection, Rockville, Maryland, U. S. A.; AJ, Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan; NRRL, Northern Utilization Research and Development Division, Peoria, Illinois, U. S. A.

The menaquinones in the brackets represent minor components of quinones.
a The system of quinones was already reported (2, 3).
b The amount of MK-9(H₅) was greater than that of MK-9(H₅).

**Paper chromatography.** Reversed-phase paper chromatography was used for the determination of the menaquinone system (2). However, the concentration of white petrolatum in toluene solution was changed to 1.25% by weight, because these organisms gave menaquinones with a longer and more saturated isoprenoid
Mass spectrometry. Mass spectra of the menaquinones were recorded with a Hitachi RMU-6M single-focusing mass spectrometer at an 80-eV ionizing potential. The chamber temperature of this spectrometer was retained at 200°. The samples were vaporized at the ion source with a heated direct-inlet system operating at 150° in *A. madurae* and *A. pelletieri* and at 160° in *A. dassonvillei*.

Reagents and chemicals. Silica gel was obtained from E. Merck, Darmstadt (*Kieselgel GF254 nach Stahl, type 60*). Other reagents and chemicals used in this experiment were commercial preparations.

RESULTS AND DISCUSSION

All the microorganisms used in this experiment were grown as shaking cultures in 1 liter of a medium containing glucose 0.7%, glycerol 0.3%, meat extract (*Kyokuto*) 0.3%, peptone 0.3%, and brain-heart infusion (*Nissan*) 0.2%, per liter, dispensed into a 5-liter conical flask, at 30° for 24–30 hr. The cultured media were heated by dipping in a boiling water-bath for 5 min, and the heat-shocked cells were harvested by centrifugation. The packed cells of these nocardioform bacteria were vigorously shaken in a mixture of 50 ml of ethanol and 200 ml of ether for 20 min. The mixture was filtered, and the extraction was repeated three times. The combined filtrate was treated to obtain a menaquinone preparation as described in a previous paper (2).

All the compounds isolated from these nocardioform bacteria showed characteristic absorption peaks at 247, 270, and 332 nm in their ethanol solution, so that they are recognizable as members of the menaquinone group. The menaquinone system of these organisms was determined by reversed-phase paper chromatography and also by mass spectrometry. For example, the menaquinone preparation of *A. pelletieri* NCTC 4162 gave a single spot migrating with the same mobility as the MK–9(H₈) isolated from *Oerskovia turbata* AJ 9191 (4) on Toyo Roshi No. 50 filter paper impregnated with 3% silicone (w/w, KF–54, Shin’etsu Kagaku), when developed with a solvent system of ethanol-ethyl acetate-water (5: 3: 1) (5) at the average *Rf* value of 0.18. However, this preparation exhibited three spots by another chromatography on a paper strip impregnated with 1.25% white petrolatum (w/w, J. P.) with N, N-dimethylformamide-water (98: 2) (2); one larger spot appeared on the chromatogram at the same *Rf* value of 0.21 as found in the authentic MK–9(H₈), and the remaining two smaller spots had the average *Rf* values of 0.28 and 0.14. The mass spectrometric analysis of this menaquinone preparation gave three molecular ions together with two fragment ones at *m/e* 187 and 225; one larger molecular peak was at *m/e* 790, and two smaller peaks with about 50% intensity of the main peak were at *m/e* 788 and 792. These results provided the assignment that the quinone system of *A. pelletieri* NCTC 4162 comprised MK–9(H₈)[MK–9(H₄)MK–9(H₈)]. In the case of *A. dassonvillei* IMRU
three spots were also seen, only when developed with the N, N-dimethylformamide-water system; two larger spots corresponded to MK–10 and MK–10(H₄), and a smaller spot indicated the presence of MK–10(H₃).

As shown in Table 1, these *Actinomadura* species were recognized to have rather complex menaquinone systems distinct from “true nocardiae” such as *N. asteroides* and *N. brasiliensis* (2, 3). *Actinomadura pelletieri* NCTC 4162 has the same quinone system comprising MK–9(H₈)[MK–9(H₆)MK–9(H₈)] as found in the type species of this genus, *A. madurae*. All the strains examined in these two species agreed in this respect with one another. The MK system of *A. dassonvillei* IMRU 509 was clearly distinguishable from the above-mentioned two species; it contained the MK–10MK–10(H₃)[MK–10(H₆)] system. Among the strains classified in this species, another type of the quinone system was found (IMRU 575 and N 3255). Such a duality in the system of quinones from *A. dassonvillei* allows us to predict that this species would be a heterogeneous taxon.

According to Lechevalier and Lechevalier (1), *A. madurae* and *A. pelletieri* are very closely related, but *A. dassonvillei* is different morphologically and chemically. Strains from *A. dassonvillei* produce very long aerial hyphae, and sporulate by first dividing into rather long segments. The vegetative mycelium of this species may fragment unlike *A. madurae* and *A. pelletieri*. Moreover, whole-cell hydrolysates of *A. madurae* and *A. pelletieri* contain madurose. However, *A. dassonvillei* does not have such a characteristic sugar. These heterogeneous natures found in the genus *Actinomadura* have also been reported in spore morphology (6), fatty acid composition (7), and pigmentation (8). From the facts described above, it is reasonable that *A. dassonvillei* has quite a distinct menaquinone system; it differs in the chain length as well as the degree of saturation from those of *A. madurae* and *A. pelletieri*.

In our previous papers (9, 10), we mentioned that the quinone system has a potential value for classifying microorganisms at the genus level. When the genus *Actinomadura* Lechevalier and Lechevalier 1970 is restricted only to members with type III of cell walls and with MK–9(H₈) as a major component of quinones, the strains of *A. dassonvillei* which are characterized by their peculiar MK system can be removed from the genus *Actinomadura*, and a separate appropriate genus may be established for these organisms.

In view of the menaquinone system described above, it was also recognized that a species from the genus *Actinomadura*, *A. dassonvillei*, is composed of two types of organisms. According to Gordon and Horan (11), the strains IMRU 575 and N 3255 were originally described as *N. madurae* and *Nocardia* species, respectively. Previously, we distinguished organisms with a longer isoprenolog of ubiquinone classified in the genus *Acetobacter* as *A. xylinum* apart from *A. aceti*, based on the quinone system (12, 13). Whether the organisms from *A. dassonvillei* possessing more saturated isoprenologs of menaquinones such as the strains IMRU 575 and N 3255 should be classified as a separate species from
A. dassonvillei itself or not is at present uncertain, however, it will be resolved in the near future.

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REFERENCES


Addendum. Very recently, after completion of this manuscript, we found that Collins et al. reported the distribution of menaquinones in actinomycetous bacteria on the basis of the intensity of their parent peaks in mass spectrometry. They observed quinones similar to our MK systems in the three species of the genus Actinomadura [M. D. Collins, T. Piouz, M. Goodfellow, and D. E. Minnikin, J. Gen. Microbiol., 100, 221 (1977)].