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Isoprenoid Quinones of *Campylobacter cryaerophila*, *C. cinaedi*, *C. fennelliae*, *C. hyointestinalis*, *C. pylori*, and "*C. upsaliensis*"

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The isoprenoid quinone contents of *Campylobacter cryaerophila*, *C. cinaedi*, *C. fennelliae*, *C. hyointestinalis*, *C. pylori*, and "*C. upsaliensis*" were determined by reverse-phase thin-layer and high-performance liquid chromatography. All six of these recently named *Campylobacter* species contained menaquinone-6 (MK-6), but only *C. hyointestinalis* and "*C. upsaliensis*" contained 2,[5 or 8]-dimethyl-3-farnesyl-farnesyl-1,4-naphthoquinone (*MK-6), a previously described novel menaquinone of the *Campylobacter* genus. *C. cryaerophila*, *C. cinaedi*, *C. fennelliae*, and *C. pylori* contained an unidentified quinone (Un-MK-6) with a molecular weight of 580 and a base peak ion of *m/e* = 225 by mass spectrometry but with chromatographic properties different from those of MK-6. *MK-6 and Un-MK-6 are important chemotaxonomic markers of *Campylobacter* and *Campylobacter*-like organisms.

In 1984 we reported that all known *Campylobacter* species contain a novel methyl-substituted menaquinone-6 (2,[5 or 8]-dimethyl-3-farnesyl-farnesyl-1,4-naphthoquinone) (*MK-6) that is absent in other bacteria (9). Since that report, several new *Campylobacter* species have been described on the basis of morphological and biochemical characteristics, but their isoprenoid quinones were not determined (5, 7, 10–12, 14). In this study, we report the isoprenoid quinone content of type strains of *Campylobacter cinaedi* (ATCC 35683), *C. cryaerophila* (NCTC 11885), *C. fennelliae* (ATCC 35684), *C. hyointestinalis* (ATCC 35217), *C. pylori* (NCTC 11637), and "*C. upsaliensis*" (NCTC 11541). One to four additional DNA-confirmed strains of each species were also tested.

All strains were grown on 8 to 12 plates (15 by 100 mm) of heart infusion agar with 5% defibrinated rabbit blood for 48 to 72 h at 36°C in an atmosphere of approximately 5% O_2, 10% CO_2, 10% H_2, and 75% N_2. Cells were harvested with sterile water, and the isoprenoid quinones were prepared and analyzed by reverse-phase high-performance liquid chromatography (RPHPLC), reverse-phase thin-layer chromatography, and mass spectrometry (MS) as described previously (6, 8, 9).

The isoprenoid quinone contents of five strains each of *C. hyointestinalis* and "*C. upsaliensis*" were consistent with those reported previously for other *Campylobacter* species (1, 2, 9), since each contained menaquinone-6 (MK-6) and a methyl-substituted menaquinone-6 (*MK-6) as major components (Fig. 1A). MK-6 was also present in *C. cinaedi* (two strains), *C. cryaerophila* (three strains), *C. fennelliae* (three strains), and *C. pylori* (four strains), but each lacked *MK-6 (Fig. 1B). These four species all contained a compound (Un-MK-6) that eluted at 7.5 min by RPHPLC (Fig. 1B). The retention time of Un-MK-6 by RPHPLC matched that of an MK-5 standard, but MK-5 was ruled out by subsequent results from MS and reverse-phase thin-layer chromatography.

Fractions corresponding to each peak in Fig. 1 were collected from RPHPLC and analyzed by MS. The peaks at a retention time of 9.2 min in both chromatograms in Fig. 1 were confirmed as MK-6 by the molecular ion (M^+) of *m/e* = 580, a base peak ion at *m/e* = 225, and other fragment ions at *m/e* = 511, 443, 375, 307, 239, and 187; the base peak ion at *m/e* = 225 and an intense *m/e* = 187 ion indicate the

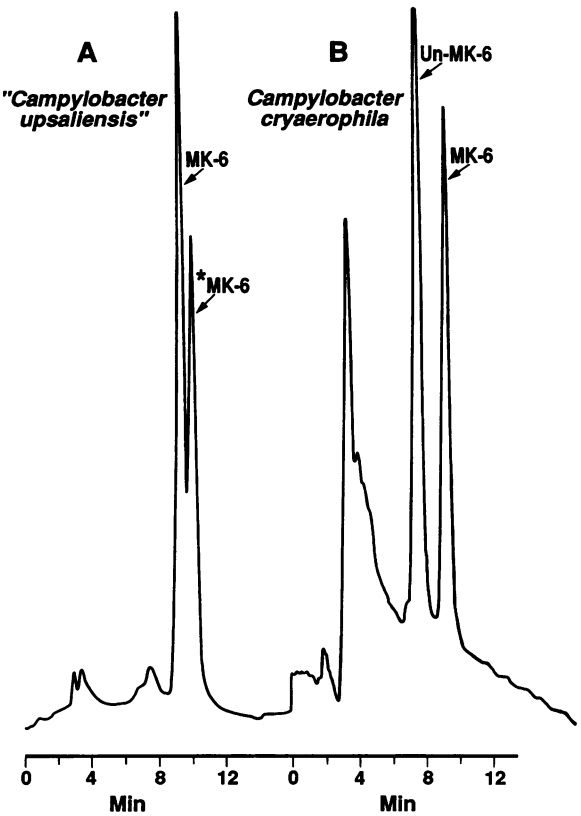

FIG. 1. Reverse-phase high-performance liquid chromatogram of isoprenoid quinones extracted from "*C. upsaliensis*" NCTC 11541 and *C. cryaerophila* NCTC 11885. MK-6, Menaquinone-6 (2-methyl-3-farnesyl-farnesyl-1,4-naphthoquinone); *MK-6, methyl-substituted MK-6 (2,[5 or 8]-dimethyl-3-farnesyl-farnesyl-1,4-naphthoquinone); Un-MK-6, unidentified MK-6.

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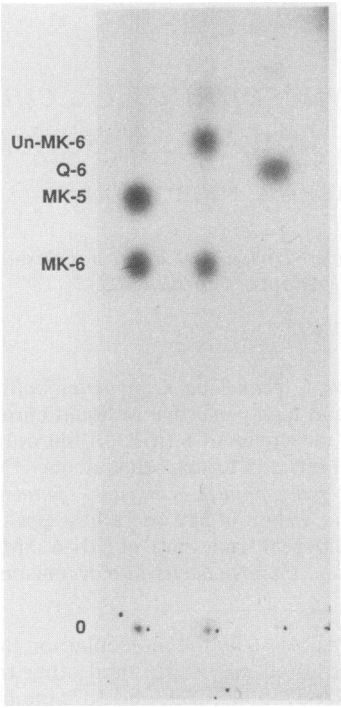

FIG. 2. Reverse-phase thin-layer chromatogram of isoprenoid quinone standards and extracts of *C. cryaerophila*. Lanes (from left to right) show menaquinone standard containing MK-5 and MK-6, *C. cryaerophila* NCTC 11885, and ubiquinone-6 (Q-6) standard. O, Origin. See the legend to Fig. 1 for other abbreviations.

naphthoquinone nucleus (4). *MK-6 at a retention time of 10.0 min was confirmed by M^+ = *m/e* 594, a base peak ion at *m/e* = 239, and an intense ion at *m/e* = 201; the *m/e* = 239 and 201 ions are consistent with the presence of an additional methyl group on the naphthoquinone ring (1, 9).

The MS of the peak at retention time 7.5 min in Fig. 1B had an M^+ ion of *m/e* = 580 and a base peak ion at *m/e* = 225. Since the *m/e* = 225 indicated a naphthoquinone nucleus and the M^+ at *m/e* = 580 is the mass for an unsaturated MK-6 structure, this component was designated Un-MK-6. However, the MS of Un-MK-6 differed from that of MK-6 by a more prominent *m/e* = 307 ion and less intense fragment ions at *m/e* = 511, 443, 375, 239, and 187. Since these ions originate from cleavage of isoprene units in the C-3 prenyl side chain (1, 4), it appears that the polyprenyl side chain of Un-MK-6 is structurally different from that of MK-6. This difference in structure results in a more polar compound, as Un-MK-6 elutes before MK-6 by RPHPLC and also has a higher R_f value by reverse-phase thin-layer chromatography (Fig. 2). Although the chromatographic and MS data indicate that Un-MK-6 is an unusual menaquinone with six isoprene units, further studies are required to elucidate the detailed structure of this compound.

Our results from isoprenoid quinone analysis lend support to recent phylogenetic studies which have shown that *C. cinaedi*, *C. fennelliae*, *C. pylori*, and *C. cryaerophila* are in different rRNA homology groups than *C. fetus*, *C. coli*, *C. jejuni*, *C. laridis*, *C. hyointestinalis*, *C. concisus*, *C. mucosalis*, *C. sputorum*, and "*C. upsaliensis*" (13). The latter nine species, which form rRNA group I and are considered true campylobacters (13), each contain *MK-6 as a major quinone. The absence of *MK-6 and the presence of the unidentified quinone (Un-MK-6) in *C. cinaedi*, *C. fennelliae*, *C. pylori*, and *C. cryaerophila* support their exclusion from the genus *Campylobacter* (13). Three of these species (*C. cinaedi*, *C. fennelliae*, and *C. pylori*) are in rRNA homology group II described by Thompson and co-workers (13). *Wolinella succinogenes* is also included in this rRNA group; its respiratory quinone composition, however, is more like that of the true campylobacters because *W. succinogenes* contains *MK-6 and MK-6 (3; C. W. Moss, unpublished data). A small peak (<15% of the total quinones) at the same retention time as Un-MK-6 was found in *W. succinogenes* as well as several of the *C. hyointestinalis* and "*C. upsaliensis*" strains (Moss, unpublished data); its identity was not determined by MS. Thompson and co-workers did not include *W. recta* and *W. curva* in their rRNA study, but the quinone patterns of these two species are like that of the true campylobacters and *W. succinogenes* because they contains *MK-6 and MK-6 (Moss, unpublished data).

The third rRNA homology group of Thompson et al. contains *C. cryaerophila* and *C. nitrofigilis* (13). Preliminary RPHPLC results from our laboratory show that the quinone pattern of *C. nitrofigilis* is like that of *C. cryaerophila* (as well as *C. cinaedi*, *C. fennelliae*, and *C. pylori*) rather than true campylobacters because *C. nitrofigilis* contains Un-MK-6 and MK-6 and does not contain *MK-6. Although determination of respiratory quinones provides valuable chemical data for defining *Campylobacter* species and related organisms, additional studies are needed to resolve the taxonomic position of these species. Studies are currently in progress to determine the chemical structure of Un-MK-6.

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