Adlercreutzia equolifaciens gen. nov., sp. nov., an equol-producing bacterium isolated from human faeces, and emended description of the genus *Eggerthella*

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Nine strains capable of metabolizing isoflavones to equol were isolated from human faeces. Four of the strains were characterized by determining phenotypic and biochemical features and their phylogenetic position based on 16S rRNA gene sequence analysis. These strains were related to *Eggerthella sinensis* HKU14^T with about 93 % 16S rRNA gene sequence similarity; they were asaccharolytic, obligately anaerobic, non-spore-forming, non-motile and Gram-positive coccobacilli. In enzyme activity tests, arginine dihydrolase, arginine and leucine arylamidases were positive but nitrate reduction, urease and β -glucosidase were negative. The major menaquinone was DMMK-6 (dimethylmenaquinone-6), while that of members of the genus *Eggerthella* was MMK-6 (methylmenaquinone-6). Furthermore, the cell-wall peptidoglycan type of these strains was A1 γ , while that of members of the genus *Eggerthella* was A4 γ . On the basis of these data, a new genus, *Adlercreutzia* gen. nov., is proposed with one species, *Adlercreutzia equolifaciens* sp. nov. The type strain of *Adlercreutzia equolifaciens* is FJC-B9^T (=JCM 14793^T =DSM 19450^T =CCUG 54925^T).

Isoflavones are flavonoids present in various plants, especially in the legume family. Isoflavones exist predominantly as glycoside forms in some plants, mainly as daidzin and genistin. After ingestion, daidzin and genistin are converted to daidzein and genistein by both intestinal mucosal and bacterial β -glucosidases (Day *et al.*, 2000; Setchell *et al.*, 2002). Daidzein is further metabolized via dihydrodaidzein to O-desmethylangolensin (O-DMA) or equol by intestinal bacteria (Chang & Nair, 1995; Joannou *et al.*, 1995). Equol has a stronger oestrogenic activity than daidzein and O-DMA (Sathyamoorthy & Wang, 1997; Schmitt *et al.*, 2001). However, only 30–50 % of the human population can produce equol from daidzein (Arai *et al.*, 2000; Setchell *et al.*, 2003). Although extensive research has been performed to search for a single bacterium capable of

producing equol from isoflavones, there have been only a few reports to date (Wang *et al.*, 2005; Minamida *et al.*, 2006). We isolated nine strains capable of metabolizing isoflavones to equol from human faeces. Seven of these strains could metabolize daidzein via dihydrodaidzein to equol, while the other two isolates could metabolize only dihydrodaidzein to equol. These strains were divided into four groups by 16S rRNA gene sequence analysis. Representative strains selected from each group were asaccharolytic, obligately anaerobic, non-spore-forming, non-motile and Gram-positive coccobacilli. Although these strains were genetically related to the genus *Eggerthella*, they did not belong to the genera *Eggerthella*, *Slackia* or *Denitrobacterium* from 16S rRNA gene sequence analysis. The presence of dimethylmenaquinone-6 (DMMK-6) as the predominant menaquinone of these strains is unique. Furthermore, the cell-wall peptidoglycan type of these strains was A1 γ , while that of members of the genus *Eggerthella* was A4 γ .

Strains FJC-A10, FJC-A161, FJC-B9^T, FJC-B12, FJC-B15, FJC-B19, FJC-B20, FJC-D47 and FJC-D53 were cultivated for 3 days at 37 °C on BL agar (Nissui) with 5 % (v/v) horse blood. *Coriobacterium glomerans* JCM 10262^T was cultivated for 2 days at 30 °C on GAM agar (Nissui).

Abbreviations: O-DMA, O-desmethylangolensin; FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains FJC-A10, FJC-B9^T, FJC-B20 and FJC-D53 are AB306660–AB306663.

Detailed menaquinone and fatty acid profiles of representative strains and related type strains and minimum-evolution and maximum-parsimony 16S rRNA gene sequence-based trees are available as supplementary material with the online version of this paper.

Olsenella profusa JCM 14553^T and *Slackia faecicanis* JCM 14555^T were cultivated for 2 days at 37 °C on Columbia agar (Oxoid) with 10 % (v/v) sheep blood. *Eggerthella lenta* JCM 9979^T, *Eggerthella hongkongensis* JCM 14552^T, *Eggerthella sinensis* JCM 14551^T, *Slackia exigua* JCM 11022^T, *Slackia heliotrinireducens* JCM 14554^T, *Collinsella aerofaciens* JCM 10188^T, *Collinsella intestinalis* JCM 10643^T, *Collinsella stercoris* JCM 10641^T, *Atopobium fossor* JCM 9981^T, *Atopobium minutum* JCM 1118^T, *Atopobium parvulum* JCM 10300^T, *Atopobium rimae* JCM 10299^T and *Olsenella uli* JCM 12494^T were cultivated for 2 days at 37 °C on Eggerth–Gagnon (EG) agar (Merck) with 5 % (v/v) horse blood. All strains were cultivated in anaerobic jars with 100 % CO₂.

All isolates were assayed for their ability to metabolize the isoflavones daidzin, daidzein and dihydrodaidzein to equol, while other related strains were assayed for the ability to metabolize daidzein and dihydrodaidzein only. All strains were subcultured in GAM broth (pH 7.0) or GAM plus 0.5 % arginine broth (three strains in the genus *Slackia*). Subcultures were inoculated into fresh GAM medium containing each isoflavone at 50 µM. The test culture was incubated for 3 days at 37 °C in an anaerobic jar with 100 % CO₂. An aliquot (1.2 ml) of the bacterial cultures was centrifuged at 15 000 r.p.m. for 5 min and 1 ml supernatant was loaded onto a preconditioned Sep-Pak C18 cartridge (Waters). The cartridge was washed with 6 ml 15 % methanol, and isoflavones were eluted with 2 ml 95 % methanol. The eluate was suitably diluted for LC/MS/MS analysis. LC/MS/MS analysis was performed in a system consisting of a Tosoh HPLC system and Finnigan LCQ DUO mass spectrometer. Chromatography was carried out on a Unison UK-18 reversed-phase column (3.0 × 150 mm; Imtakt) at 40 °C. The mobile phase was 30 % acetonitrile in 20 mM ammonium acetate with a flow rate of 0.3 ml min⁻¹ (isocratic analysis). Selected reaction monitoring (SRM) was used to perform mass spectrometric quantification. The SRM analysis was conducted by monitoring the precursor ion to product ion transitions from *m/z* 253/253 (daidzein), 255/149 (dihydrodaidzein), 241/121 (equol) and 257/239 (O-DMA) using electrospray ionization in negative mode.

Fermentation tests were performed using pre-reduced, anaerobically sterilized sugars according to the methods of Holdeman *et al.* (1977). Metabolic end products were prepared as described previously (Holdeman *et al.*, 1977) and were analysed by GLC (GLC-7A; Shimadzu) using a 2.1 m glass column (2.8 mm, FAL-M 25 %, Chromosorb W, 80/100 mesh, AW-DMCS H₃PO₄; Shimadzu). Enzyme profiles were generated with the Rapid ID32A anaerobe identification kit (bioMérieux) according to the manufacturer's instructions. Chromosomal DNA was isolated by the method of Saito & Miura (1963) with some modifications. The 16S rRNA genes were analysed as described previously (Sakamoto *et al.*, 2002). Sequence similarity searches were performed with the BLAST program, and sequences of strains of closely related species were retrieved

from DDBJ, EMBL and GenBank nucleotide sequence databases. Sequence data were aligned with CLUSTAL_X (version 1.83) and corrected by manual inspection. Alignment gaps and ambiguous bases were removed prior to phylogenetic analysis using MacClade (version 4.03) (Maddison & Maddison, 2002). Neighbour-joining and minimum-evolution phylogenetic trees were inferred using the software package MEGA version 4.0 (Tamura *et al.*, 2007), according to the Kimura two-parameter model and Kimura model (Kimura, 1980), respectively. Parsimony analysis was carried out with maximum-parsimony implemented in the PAUP version 4.0b10 software package (Swofford, 2000). Maximum-parsimony trees were obtained by a heuristic search and by selecting the tree bisection/reconnection branch-swapping option (Dauga, 2002). The topology of phylogenetic trees was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The DNA G+C content was determined by using the HPLC method of Tamaoka & Komagata (1984) with some modification. Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and were analysed by HPLC with a Cosmosil 5C₁₈ column (4.6 × 150 mm; Nacalai Tesque). For identification of the main peaks, isoprenoid quinones were eluted from the HPLC column and analysed by MS. Cell-wall peptidoglycan was prepared and hydrolysed by the methods of Kawamoto *et al.* (1981) and amino acid composition was analysed with an automatic amino analyser. The isomers of diaminopimelic acid in the cell walls were determined by the method of Stanek & Roberts (1974). The neutral amino acid fraction was reacted with a chiral reagent [(+)-1-(9-fluorenyl)ethyl chloroformate] and applied to HPLC as described by Einarsson *et al.* (1987). Fatty acid methyl esters (FAMES) were obtained from about 40 mg wet cells by saponification, methylation and extraction, using minor modifications (Kuykendall *et al.*, 1988) of the method of Miller (1982). Bacteria were cultured anaerobically in GAM broth supplemented with arginine at 37 °C and harvested by centrifugation at the late exponential phase. Cellular fatty acid profiles were determined by using the MIDI microbial identification system (Microbial ID).

Strain FJC-A10, FJC-A161, FJC-B9^T, FJC-B12, FJC-B15, FJC-B19, FJC-B20, FJC-D47 and FJC-D53 were obligately anaerobic, non-spore-forming, non-motile, Gram-positive coccobacilli arranged in chains. Cells on BL agar were 0.6–0.7 × 1.5–2.7 µm. Colonies were 1–2 mm in diameter, greyish white, circular, entire, slightly convex and smooth on BL agar plates. Strains FJC-A10 and FJC-A161 were isolated from a healthy man (29 years old), FJC-B9^T, FJC-B12, FJC-B15, FJC-B19 and FJC-B20 from a healthy woman (25 years old) and FJC-D47 and FJC-D53 from another healthy man (47 years old). Approximately 500 bp of the 16S rRNA gene sequence was determined for each of the isolates. From these sequences, the nine isolates were divided into four groups; group 1 comprised FJC-A10 and FJC-A161, group 2 comprised FJC-B12, FJC-B15 and

FJC-B20, group 3 comprised FJC-B9^T and FJC-B19 and group 4 comprised FJC-D47 and FJC-D53. Approximately 1500 bp of the 16S rRNA gene sequence was determined for a representative strain selected from each group. For the phylogenetic analysis, 1334 bp (positions 105–1469; *Escherichia coli* numbering system) of each sequence were used. Analysis of 16S rRNA gene sequences revealed that the four strains were members of the family *Coriobacteriaceae*. The phylogenetic positions of the four strains among representative members of the family *Coriobacteriaceae* are shown in Fig. 1 and in Supplementary Figs S1 and S2 available in IJSEM Online. All phylogenetic trees showed clearly that strains FJC-A10, FJC-B9^T, FJC-B20 and FJC-D53 formed a single cluster and had a distinct line of descent with the genera *Eggerthella*, *Slackia* and *Denitrobacterium*. In addition to the isolates, two unidentified strains SNU-Julong732 and Gram-positive bacterium do03 were included in this cluster. The next closest phylogenetic relatives of strains FJC-A10, FJC-B9^T, FJC-B20 and FJC-B53 were *Denitrobacterium detoxificans* NPOH1^T and *Eggerthella hongkongensis* HKU10^T. 16S rRNA gene sequence similarities of the four isolates with the two unidentified strains in the same cluster, *Denitrobacterium detoxificans* NPOH1^T and *Eggerthella hongkongensis* HKU10^T were respectively 99.4–99.9, 89.3–89.5 and 92.0–92.4%. Because members of the genus *Eggerthella* had the highest sequence similarity (90.8–92.9%) with the four isolates among members of the family *Coriobacteriaceae*, excluding the two unidentified strains, we used three strains from the genus *Eggerthella* as reference strains in the following physiological and biochemical tests.

Table 1. Differential characteristics of *Adlercreutzia equolifaciens* gen. nov., sp. nov. and related *Eggerthella* species

Species: 1, *Adlercreutzia equolifaciens* gen. nov., sp. nov. (data for four strains from this study); 2, *E. lenta* (unless indicated, data from Wade *et al.*, 1999); 3, *E. hongkongensis* (Lau *et al.*, 2004); 4, *E. sinensis* (Lau *et al.*, 2004). +, Positive; –, negative; v (–); variable, type strain negative.

Characteristic	1	2	3	4
DNA G + C content (mol%)	64.1–66.5	63.8*	61.1*	64.9*
β-Glucosidase	–	–	+	–
Nitrate reduction	–	+	–	–
Arginine arylamidase	+	–	v (–)	+
Leucine arylamidase	+	–	v (–)	–

*Data obtained in this study for the respective type strains.

The four selected isolates did not produce acid from any of the sugars tested in this study and did not exhibit gas formation. Results of enzyme activity tests of the four isolates and three strains from the genus *Eggerthella* using Rapid ID 32A are shown in Table 1. The four isolates gave positive reactions for arginine dihydrolase and arginine and leucine arylamidases but negative reactions for nitrate reduction, urease and β-glucosidase. The DNA G + C contents of the isolates were 64.1–66.5 mol%. These values are almost the same as that of *Eggerthella lenta* JCM 9979^T (63.8 mol% in this study) and *Eggerthella sinensis* JCM 14551^T (64.9 mol%), while they were slightly higher than

Fig. 1. Phylogenetic tree showing the relationship between members of *Adlercreutzia equolifaciens* gen. nov., sp. nov. (in bold) and some related taxa. The tree was constructed by using the neighbour-joining method based on 16S rRNA gene sequences. Bootstrap values (>50%) based on 1000 replications are listed as percentages at branching points. Bar, 0.02 substitutions per nucleotide position. GenBank accession numbers are given in parentheses.

that of *Eggerthella hongkongensis* JCM 14552^T (61.1 mol%; Table 1).

Because it has been reported that *Eggerthella lenta* contained methylated menaquinone in addition to normal menaquinone, the menaquinone compositions of the isolates and members of the genus *Eggerthella* were analysed (Fernandez & Collins, 1987). The menaquinone compositions of the strains studied are shown in Supplementary Table S1. The major menaquinones of the isolates were DMMK-6 (70–96 %) and methylmenaquinone-6 (MMK-6) (1–29 %). On the other hand, strains of the genus *Eggerthella* contained MMK-6 (52–66 %), menaquinone-6 (MK-6) (11–47 %) and DMMK-6 (2–28 %). The observation that *Eggerthella lenta* has almost the same contents of MK-6 and MMK-6 (45.1 and 52.0 %, respectively) supports the result reported in a previous paper (Fernandez & Collins, 1987). Although a minor peak was detected in the novel isolates with a retention time between those of MK-5 and MK-6, we could not identify this menaquinone because of its low abundance (Supplementary Table S1; MK-X).

The cellular fatty acid profiles of the four representative isolates and three *Eggerthella* type strains are shown in Supplementary Table S2. The predominant fatty acid in all four novel isolates was C_{18:1} *cis*9 FAME (24–35 %). Furthermore, relatively large amounts of C_{18:0} dimethyl acetyl (DMA) (11–19 %), C_{16:0} DMA (11–15 %), C_{16:0} FAME (7–13 %) and C_{18:1} *cis*9 DMA (8–12 %) were detected in the isolates. The cellular fatty acid compositions of the three *Eggerthella* type strains were almost the same. Their predominant fatty acid was C_{16:0} DMA (29–38 %). Although they also possessed a significant amount of C_{18:1} *cis*9 FAME (15–24 %), C_{18:1} *cis*9 DMA was not detected in the *Eggerthella* type strains. Therefore, C_{18:1} *cis*9 DMA is a key fatty acid capable of differentiation between the taxon represented by the novel isolates and the genus *Eggerthella*.

Structures of the cell-wall peptidoglycan were determined for the four representative isolates and three type strains of the genus *Eggerthella*. All four novel isolates possessed the same peptidoglycan type, which contained, in addition to muramic acid and glucosamine, the amino acids glutamic acid, diaminopimelic acid and alanine at a molar ratio of 1.0:1.0:1.5. Diaminopimelic acid occurred in the *meso* configuration. Therefore, the structural type was suggested to be A1γ, (L-Ala)–D-Glu–*m*-Dpm. On the other hand, the strains from the genus *Eggerthella* possessed a peptidoglycan which contained, in addition to muramic acid and glucosamine, the amino acids glutamic acid, diaminopimelic acid and alanine at a molar ratio of 1.0:0.9:0.5. Glutamic acid and diaminopimelic acid occurred in the *D*- and *meso* configurations, respectively. Therefore, the structural type was suggested to be A4γ, (L-Ala)–D-Glu–*m*-Dpm–D-Glu, with an interpeptide bridge consisting only of D-Glu.

Finally, the isoflavone-metabolizing activities of all strains were assayed. Two of the nine strains (FJC-A10 and FJC-A161) could metabolize only dihydrodaidzein to

equol, in common with SNU-Julong732, an equol-producing bacterium isolated from human faeces (Wang *et al.*, 2005). The other seven isolates could metabolize daidzein via dihydrodaidzein to equol, in common with Gram-positive bacterium do03, another equol-producing bacterium, isolated from rat caecal contents (Minamida *et al.*, 2006). None of the related strains could metabolize daidzein or dihydrodaidzein to equol, although *Slackia exigua* JCM 11022^T metabolized daidzein to O-DMA. These results suggest that all of the strains included in this new cluster have equol-producing activity. However, we have already isolated some strains that belong to this cluster but have no equol-producing activity from faeces of other healthy humans (data not shown). Therefore, it is suggested that there is a comparatively wide diversity in equol-producing activity within this cluster.

To date, eight genera have been classified in the family *Coriobacteriaceae* (Haas & König, 1988; Collins & Wallbanks, 1992; Kageyama *et al.*, 1999a; Nakazawa *et al.*, 1999; Wade *et al.*, 1999; Anderson *et al.*, 2000; Dewhirst *et al.*, 2001). As shown in Table 2, almost no phenotypic characters have been determined for all genera. However, we confirmed that not only the isolated strains but also three species in the genus *Eggerthella* have unique predominant menaquinones DMMK-6 and MMK-6 (Supplementary Table S1 and Table 2). Therefore, the major menaquinone is a potentially valuable chemotaxonomic marker in this family (Table 2). On the basis of these data, strain FJC-B9^T should be classified within a novel genus and species, for which we propose the name *Adlercreutzia equolifaciens* gen. nov., sp. nov. The nine isolates appear from their phenotypic and phylogenetic features to be members of a single species. However, whether all nine isolates should be included in the same species will have to await further analysis including DNA–DNA hybridization.

Description of *Adlercreutzia* gen. nov.

Adlercreutzia [Ad.ler.creut'zi.a. N.L. fem. n. *Adlercreutzia* named after H. Adlercreutz (Emeritus Professor, University of Helsinki, Finland), for his contributions to research on the effects of phyto-oestrogens on human health].

Cells are Gram-positive, obligately anaerobic, non-spore-forming, non-motile coccobacilli, 0.6–0.7 × 1.5–2.7 µm. Colonies on BL agar plates are 1–2 mm in diameter, grey to off-white–grey, circular, entire, slightly convex and smooth. Asaccharolytic. No metabolic end product is detected in peptone-yeast extract medium supplemented with glucose. Arginine dihydrolase and arginine and leucine arylamidases are present. Growth is stimulated by arginine. The cell wall contains A1γ-type peptidoglycan with an (L-Ala)–D-Glu–*m*-Dpm peptide subunit. The principal respiratory quinone is menaquinone DMMK-6. Major cellular fatty acid is C_{18:1} *cis*9. The DNA G+C

Table 2. Differential characteristics of *Adlercreutzia equolifaciens* gen. nov., sp. nov. and other genera of the family *Coriobacteriaceae*

Taxa: 1, *Adlercreutzia equolifaciens* gen. nov., sp. nov.; 2, *Eggerthella*; 3, *Slackia*; 4, *Denitrobacterium*; 5, *Cryptobacterium*; 6, *Collinsella*; 7, *Coriobacterium*; 8, *Olsenella*; 9, *Atopobium*. Chemotaxonomic characteristics summarized by Dewhirst *et al.* (2001) were updated in this study. +, Positive; –, negative; v, variable; ND, no data available; br, branched.

Characteristic	1	2	3	4	5	6	7	8	9
Glucose fermentation	–	–	–	–	–	+	+	+	+
Metabolic end products*	None	(a, l, s)†	(a, iv)	ND	None	a, f, H, l	a, l	a, L, (s)	a, L, (s)
Growth stimulated by:									
Tween 80	–	–	–	ND	–	+	ND	+	+
Arginine	+	+	+	ND	+	ND	ND	–	–
Growth in 20 % bile	–	+†	–	ND	–	ND	ND	v	–
Nitrate reduction	–	v	v	v	–	–	ND	–	–
Hydrogen production	–	–	ND	+	ND	+	+	v	–
Peptidoglycan type	A1 γ	A4 γ	ND	ND	ND	A4	A4 α	ND	A3 γ , A4 α , A4 β
Major cellular fatty acid(s)	C _{18:1} cis9	C _{14:0} br, C _{15:0} br†‡	C _{18:0} br, C _{18:1}	C _{14:0} br, C _{16:0} DMA	ND	ND	ND	C _{18:1} cis9	C _{18:1} cis9
Major menaquinones§	mmk-6, DMMK-6	mk-6, MMK-6, dmmk-6	ND	ND	ND	ND	ND	ND	ND
DNA G+C content (mol%)	64.1–66.5	61.1–64.9	60–64	56–60	50–51	60–65	60–61	63–64	35–46
Source(s)	Human and rat intestine	Human intestine and blood culture	Human oral cavity and dog intestine	Bovine rumen	Human oral cavity	Human intestine	Red soldier beetle intestine	Human oral cavity	Mammals

*a, Acetic acid; f, formic acid; h, hydrogen; iv, isovaleric acid; l, lactic acid; s, succinic acid. Capital letters indicate major products. Products in parentheses indicate strain variation.

†Determined for *E. lenta* but not for *E. hongkongensis* or *E. sinensis*.

‡In this study, this is C_{16:0} br.

§DMMK-6, Dimethylmenaquinone-6; MK-6, menaquinone-6; MMK-6, methylmenaquinone-6. Capital letters indicate the predominant menaquinone.

content is 64–67 mol%. The genus currently comprises a single species, *Adlercreutzia equolifaciens*, which is the type species.

Description of *Adlercreutzia equolifaciens* sp. nov.

Adlercreutzia equolifaciens (e.quo.li.fa'ci.ens. N.L. n. *equolis* equol; L. part. adj. *faciens* making; N.L. part. adj. *equolifaciens* equol-producing).

Displays the following properties in addition to those given in the genus description. The principal respiratory quinone is DMMK-6 (70–96%); MMK-6 (1–29%) is a minor component. The DNA G+C content of the type strain is 64 mol%.

The type strain is strain FJC-B9^T (=JCM 14793^T =DSM 19450^T =CCUG 54925^T), which was isolated from faeces of a healthy human.

Emended description of the genus *Eggerthella* Wade *et al.* 1999

The description is as given previously (Moore *et al.*, 1971; Kageyama *et al.*, 1999b; Wade *et al.*, 1999) with the following modification. Growth is stimulated by arginine. The cell wall contains A4γ-type peptidoglycan with an (L-Ala)-D-Glu-*m*-Dpm-D-Glu peptide subunit with an interpeptide bridge that consists only of D-Glu. The DNA G+C content is 61–65 mol%.

Emended description of *Eggerthella lenta* Wade *et al.* 1999

The description is as given previously (Moore *et al.*, 1971; Kageyama *et al.*, 1999b; Wade *et al.*, 1999) with the modification that the cell wall contains A4γ-type peptidoglycan as specified in the emended genus description.

Emended description of *Eggerthella hongkongensis* Lau *et al.* 2004

The description is as given previously (Lau *et al.*, 2004) with the following modifications. The cell wall contains A4γ-type peptidoglycan as specified in the emended genus description. The principal respiratory quinone is menaquinone MMK-6. Minor menaquinones are MK-6 and DMMK-6. The DNA G+C content is 61 mol%.

Emended description of *Eggerthella sinensis* Lau *et al.* 2004

The description is as given previously (Lau *et al.*, 2004) with the following modifications. The cell wall contains A4γ-type peptidoglycan as specified in the emended genus description. The principal respiratory quinone is menaquinone MMK-6. Minor menaquinones are MK-6 and DMMK-6. The DNA G+C content is 65 mol%.

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