Amycolivicoccus subflavus gen. nov., sp. nov., an actinomycete isolated from a saline soil contaminated by crude oil

Ya-Nan Wang,^{1,2} Chang-Qiao Chi,¹ Man Cai,¹ Zhi-Ying Lou,¹ Yue-Qin Tang,¹ Xiao-Yang Zhi,³ Wen-Jun Li,³ Xiao-Lei Wu¹ and Xun Du²

Correspondence

Xiao-Lei Wu

xiaolei_wu@pku.edu.cn

¹Department of Energy and Resources Engineering, College of Engineering, Peking University, Beijing 100871, PR China

²Key Laboratory of Microbial Engineering at the Institute of Biology, Henan Academy of Sciences, Zhengzhou 450008, PR China

³The Key Laboratory for Microbial Resources of the Ministry of Education, Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, PR China

Two novel actinomycetes, designated DQS3-9A1^T and DQS3-9A2, were isolated from a saline soil contaminated with crude oil in the Shengli Oilfield in China. On the basis of 16S rRNA gene sequence analysis, the two strains were most closely related to *Mycobacterium* species (92.7–94.9 % similarities), and formed a distinct lineage in the suborder *Corynebacterineae*. In addition, the major sugars in the cell wall, arabinose and galactose, supported the affiliation of strain DQS3-9A1^T with members of the family *Mycobacteriaceae*. However, strain DQS3-9A1^T did not contain mycolic acids and MK-8 (85.5 %) was the major menaquinone for both isolates. The major cellular fatty acids for strain DQS3-9A1^T were C_{16:0} (20.5 %), 10-methyl C_{17:0} (19.3 %), 10-methyl C_{18:0} (16.1 %), summed feature 3 (11.4 %), C_{15:0} (11.3 %), C_{17:0} (5.0 %) and C_{17:1ω8c} (5.0 %). The polar lipids of strain DQS3-9A1^T consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and an unknown glucosamine-containing phospholipid. These chemotaxonomic data indicated that strain DQS3-9A1^T differs from the present members of the suborder *Corynebacterineae*. Therefore, the creation of *Amycolivicoccus subflavus* gen. nov., sp. nov. is proposed, with DQS3-9A1^T (=DSM 45089^T=CGMCC 4.3532^T) as the type strain.

The suborder *Corynebacterineae* was first described by Stackebrandt *et al.* (1997) and comprises seven families, *Nocardiaceae*, *Gordoniaceae*, *Tsukamurellaceae*, *Dietziaceae*, *Corynebacteriaceae*, *Mycobacteriaceae* (Stackebrandt *et al.*, 1997) and *Segniliparaceae* (Butler *et al.*, 2005). Each family encompasses one genus, except *Nocardiaceae*, which contains the genera *Nocardia* and *Rhodococcus*, and *Gordoniaceae*, which contains the genera *Gordonia*, *Millisia* (Soddell *et al.*, 2006) and *Skermania*. Currently, the suborder *Corynebacterineae* comprises these 10 genera and another genus, *Williamsia* (Kämpfer *et al.*, 1999). Members of these genera form distinct lineages in the phylogenetic tree based on 16S rRNA gene sequences. Almost all members of the suborder *Corynebacterineae* contain mycolic acids, except for *Corynebacterium amycolatum* (Collins *et al.*, 1988), *Corynebacterium atypicum*

(Hall *et al.*, 2003), *Corynebacterium caspium* (Collins *et al.*, 2004), *Corynebacterium ciconiae* (Fernández-Garayzábal *et al.*, 2004), *Corynebacterium kroppenstedtii* (Collins *et al.*, 1998), *Corynebacterium minutissimum*, *Corynebacterium striatum* and *Corynebacterium xerosis* (Wauters *et al.*, 1996).

Strains DQS3-9A1^T and DQS3-9A2 were isolated from a saline soil contaminated with crude oil in the Shengli Oilfield of eastern China by 10-fold dilution plating on agar plates containing only oil-produced water at 30 °C for 7 days. The two isolates were purified and incubated on plates of artificial seawater (ASW) agar (1⁻¹: peptone, 5 g; yeast extract, 1 g; Na₂SO₄, 4 g; KCl, 0.68 g; KBr, 0.1 g; H₃BO₃, 0.025 g; MgCl₂·H₂O, 5.4 g; CaCl₂·2H₂O, 1.5 g; SrCl₂·6H₂O, 0.024 g; NaHCO₃, 0.2 g; Na₂HPO₄, 0.04 g; NH₄Cl, 0.5 g; NaF, 0.002 g; pH 8.0) with 2.4 % NaCl (Eguchi *et al.*, 1996) for 2 days at 30 °C. Growth was assessed at different temperatures (5–50 °C), pH values (2.0–12.0) and salinity (0–20 %, w/v, NaCl). The pH tolerance was examined in ASW medium containing 5 %

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains DQS3-9A1^T and DQS3-9A2 are EF564379 and FJ882017, respectively.

(w/v) NaCl at 30 °C. The NaCl concentration required for growth was investigated between 0 and 20 % (w/v) in ASW medium at pH 7.0 and 30 °C.

Morphological characteristics of strain DQS3-9A1^T were examined using scanning electron microscopy. Denitrification was assessed using a previously described method (Zumft, 1991). Hydrolysis of starch, gelatin and Tween 80, along with urease activity and carbon source utilization, were examined according to Williams *et al.* (1983) on ASW medium after 5–7 days incubation at 30 °C. Cellular fatty acid methyl esters were prepared and analysed using gas chromatography according to the instructions of the Microbial Identification System (MIDI). Fatty acid analysis was performed using the Sherlock system (Microbial ID). Isoprenoid quinones were analysed using HPLC with a reversed-phase column (Shim-pack, VPODS; Shimazu) as described by Komagata & Suzuki (1987). Polar lipids were extracted and examined by one- and two-dimensional TLC on Merck silica gel 60 F254 aluminium-backed thin-layer plates according to the procedures described by Kates (1986) and Collins *et al.* (1980).

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by Wang *et al.* (2007). The G+C content of genomic DNA was determined by thermal denaturation (Marmur & Doty, 1962) with DNA from *Escherichia coli* K-12 as a control. DNA–DNA hybridization was carried out in triplicate following the methods of De Ley *et al.* (1970) and Huß *et al.* (1983). The 16S rRNA gene sequence was compared to sequences obtained from public databases (GenBank/EMBL/DDBJ) to find the most closely related species. Phylogenetic analysis was performed using the software package MEGA version 3.1 (Kumar *et al.*, 2004) after multiple sequence alignment with CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic tree was constructed with the neighbour-joining method (Saitou & Nei, 1987) based on a distance matrix that was corrected by Kimura's two-parameter model (Kimura, 1980). Bootstrap analysis with 1000 resamplings was used to evaluate the robustness of the tree's topology (Felsenstein, 1985).

Both strain DQS3-9A1^T and strain DQS3-9A2 were Gram-positive cocci. The cells of strain DQS3-9A1^T were 0.7–0.9 × 0.9–1.05 µm without a flagellum (Fig. 1). Neither strain formed spores. The colonies of the two strains were dry, opaque and light-yellowish white on ASW agar. Their growth occurred at 15–42 °C, pH 6.0–11.0 and in 1–12 % (w/v) NaCl. Tests of strains DQS3-9A1^T and DQS3-9A2 for hydrolysis of starch, gelatin, Tween 80 and casein, production of indole and DNase, aerobic nitrite reduction and anaerobic nitrate reduction were negative, whereas tests for hydrogen sulfide production and aerobic nitrate reduction were positive. The utilization pattern of sole carbon and nitrogen sources of strains DQS3-9A1^T and DQS3-9A2 was identical. Detailed physiological characteristics of isolate DQS3-9A1^T are given in the species description.

Fig. 1. Scanning electron micrograph of negatively stained cells of strain DQS3-9A1^T.

The almost-complete 16S rRNA gene sequences of strains DQS3-9A1^T and DQS3-9A2 were obtained. In the phylogenetic tree (Fig. 2), the two strains clustered in a distinct clade that was strongly supported by the high bootstrap value (100 %), and the clade could be equated with branches corresponding to recognized genera. The strains tested were most closely related to recognized *Mycobacterium* species (92.7–94.9 % similarities). Additionally, the major sugars in the cell wall of the two strains were arabinose and galactose, also supporting the affiliation of the isolates to the family *Mycobacteriaceae*. The predominant menaquinones of strain DQS3-9A1^T were MK-8 (85.5 %) and MK-7 (8.0 %), and the major cellular fatty acids were C_{16:0} (20.5 %), 10-methyl C_{17:0} (19.3 %), 10-methyl C_{18:0} (16.1 %), summed feature 3 (11.4 %), C_{15:0} (11.3 %), C_{17:0} (5.0 %) and C_{17:1}ω8c (5.0 %). The patterns of the predominant menaquinones and major cellular fatty acid composition of strain DQS3-9A2 were identical to those of strain DQS3-9A1^T. The polar lipids of strain DQS3-9A1^T consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and an unknown glucosamine-containing phospholipid. Mycolic acid, which is the most important character shared by genera in the *Corynebacterineae*, could not be detected in strain DQS3-9A1^T. The above chemical profiles therefore served to distinguish the tested strains from members of all genera in the *Corynebacterineae* (Table 1). Furthermore, distinctions could be made between strains DQS3-9A1^T and DQS3-9A2 and other members of the family *Mycobacteriaceae* in some signature nucleotide sites (Table 2). The DNA G+C content of strain DQS3-9A1^T was 60.04 mol%. Other biochemical characteristics of isolate DQS3-9A1^T are given in the genus description.

As a result, based on 16S rRNA gene sequence analysis, phenotypic characteristics and chemotaxonomic prop-

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequence analysis, reconstructed from evolutionary distances by using the neighbour-joining method, showing the phylogenetic positions of strains DQS3-9A1^T and DQS3-9A2 within the suborder *Corynebacterineae*. Bootstrap values are shown as percentages of 1000 replicates. Bar, 2 nucleotide substitutions per 100 nt.

erties, it is clear that strains DQS3-9A1^T and DQS3-9A2 represent a novel genus within the family *Mycobacteriaceae* of the suborder *Corynebacterineae*, for which the name *Amycolicoccus subflavus* gen. nov., sp. nov. is proposed.

Description of *Amycolicoccus* gen. nov.

Amycolicoccus [A.my.co.li.ci.coc'cus. Gr. pref. *a-* not; N.L. n. *acidum mycolicum* mycolic acid; N.L. masc. n. *coccus* (from Gr. n. *kokkos*) a grain, berry; N.L. masc. n. *Amycolicoccus* a coccus without mycolic acids].

Gram-positive, aerobic cocci without a flagellum. Spores are not present. Cell wall contains arabinose, galactose, glucose and xylose as major sugars, and alanine, glutamic acid, methionine and histidine as major amino acids. The polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and an unknown glucosamine-containing phospholipid. The predominant menaquinones are MK-8 and MK-7, and the major cellular fatty acids are C_{16:0}, 10-methyl C_{17:0}, 10-methyl C_{18:0}, summed feature 3, C_{15:0}, C_{17:0} and C_{17:1ω8c}. Mycolic acid is absent. The type species is *Amycolicoccus subflavus*.

Description of *Amycolicoccus subflavus* sp. nov.

Amycolicoccus subflavus (sub.flav'vus. L. masc. adj. *subflavus* yellowish).

Displays the following properties in addition to those described for the genus. Cells are 0.7–0.9 × 0.9–1.05 µm without a flagellum and colonies are dry, opaque and light-yellowish white on ASW agar. Growth occurs at 15–42 °C (optimum, 37 °C), pH 6.0–11.0 (optimum, pH 8.0) and in 1–12 % (w/v) NaCl [optimum, 8 % (w/v) NaCl]. Negative for hydrolysis of starch, gelatin, Tween 80 and casein, production of indole and DNase, aerobic nitrite reduction and anaerobic nitrate reduction. Positive for production of hydrogen sulfide and aerobic nitrate reduction. Cellobiose, dextrin, fucose, glucose, inositol, malic acid, maltose, mannose, mannitol, proline, sodium gluconate, sorbitol, sucrose and succinic acid are utilized as sole carbon and nitrogen sources, but alanine, arabinose, arginine, fructose, galactose, lactose, malonic acid, ribose, sorbose and xylose are not. Resistant to gentamicin (10 µg), clindamycin (2 µg), sulfamethoxazole (300 µg), streptomycin (300 µg) and amikacin (30 µg). Sensitive to cephalothin (30 µg),

Table 1. Chemical and morphological properties of strain DQS3-9A1^T and other genera of the suborder *Corynebacterineae*

1, DQS3-9A1^T; 2, *Mycobacterium*; 3, *Corynebacterium*; 4, *Segniliparus*; 5, *Dietzia*; 6, *Tsukamurella*; 7, *Skermania*; 8, *Millisia*; 9, *Gordonia*; 10, *Rhodococcus*; 11, *Nocardia*; 12, *Williamsia*. Data from this study and Butler *et al.* (2005), Chun *et al.* (1997), Kämpfer *et al.* (1999), Li (2007), Rainey *et al.* (1995) and Soddell *et al.* (2006).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Cell morphology	Cocci	Rods, occasionally branched filaments	Pleomorphic rods	Rods	Short rods and cocci	Rods and coccobacilli	Mycelium resembling a pine tree	Characteristic rudimentary right-angled branching	Rods and cocci/moderately branched hyphae	Rods to extensively branched elements that fragment	Mycelium that fragments into rods and cocci	Irregular rods or cocci
Aerial hyphae	Absent	Usually absent	Absent	Absent	Absent	Absent	Present	Absent	Absent	Absent	Present	Absent
Mycolic acid	Absent	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Predominant menaquinone	MK-8	MK-9(H ₂)	MK-8(H ₂)	ND	MK-8(H ₂)	MK-9	MK-8(H ₄ , co-cycl.)	MK-8(H ₂)	MK-9(H ₂)	MK-8(H ₂)	MK-8(H ₄ , co-cycl.)	MK-9(H ₂)
Major polar lipids*	DPG, PE, PG, PC, PI, GluNU	DPG, PE, PG, PI, PIM	DPG, PG	ND	DPG, PE, PG	DPG, PE, PI, PIM	DPG, PE, PI, PIM	DPG, PE, PG, PI, PIM	DPG, PE, PI, PIM	DPG, PE, PIM	DPG, PE, PI, PIM	DPG, PE, PG, PI
Fatty acid composition†	S, U, T, SF3	S, U, T	S, U‡	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T
DNA G+C content (mol%)	60.04	62–70	51–67	68–72	66–73	67–74	67.5	64.7	63–69	67–73	64–72	64–65

ND, No data.

*DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; GluNU, unknown glucosamine-containing phospholipid; PIM, phosphatidylinositol mannosides.

†S, Saturated fatty acids; U, unsaturated fatty acids; T, tuberculostearic acid; SF3, summed feature 3 (16:1 ω 7c/15 iso 2OH).

‡Tuberculostearic acid present in *Corynebacterium ammoniagenes*, *Corynebacterium bovis*, *Corynebacterium minutissimum*, *Corynebacterium urealyticum* and *Corynebacterium variabilis*.

Table 2. Selected 16S rRNA gene signature nucleotides detected for the family *Mycobacteriaceae*, strain DQS3-9A1^T and strain DQS3-9A2

The 16S rRNA gene sequences of all type strains belonging to the family *Mycobacteriaceae* were included in this analysis. The signatures given below for each group were chosen for their presence in more than 95 % of the members of that group.

Position(s)	<i>Mycobacteriaceae</i>	DQS3-9A1 ^T	DQS3-9A2
293–304	G–U	–	G–C
307	A	–	C
316–337	U–G	–	C–G
468	U	–	–
508	U	C	C
586–755	U–G	U–G	U–G
631	G	G	G
661–744	G–C	G–C	G–C
662–743	U–G	C–G	C–G
771–808	A–U	G–C	G–C
824–876	C–G	U–A	U–A
825–875	G–C	A–U	A–U
837–849	G–U	U–A	U–A
843	C	C	C
1059–1198	U–A	C–G	C–G

ciprofloxacin (5 µg), ceftriazone (30 µg), penicillin (10 IU), clarithromycin (15 µg), chloramphenicol (30 µg), erythromycin (15 µg), cefotaxime (30 µg), vancomycin (30 µg) and ampicillin (10 µg). The DNA G+C content is 60.04 mol%.

The type strain, DQS3-9A1^T (=DSM 45089^T=CGMCC 4.3532^T), was isolated from an oil-polluted saline soil in Shengli Oilfield, eastern China. DQS3-9A2, isolated from the same site, is a reference strain.

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References

- Butler, W. R., Floyd, M. M., Brown, J., Toney, S. R., Daneshvar, M., Cooksey, R. S., Carr, J., Steigerwalt, A. & Charles, N. (2005). Novel mycolic acid-containing bacteria in the family *Segniliparaceae* fam. nov., including the genus *Segniliparus* gen. nov., with descriptions of *Segniliparus rotundus* sp. nov. and *Segniliparus rugosus* sp. nov. *Int J Syst Evol Microbiol* 55, 1615–1624.
- Chun, J., Blackall, L. L., Kang, S. O., Hah, Y. C. & Goodfellow, M. (1997). A proposal to reclassify *Nocardia pinensis* Blackall et al. as *Skermania piniformis* gen. nov., comb. nov. *Int J Syst Bacteriol* 47, 127–131.
- Collins, M. D., Goodfellow, M. & Minnikin, D. E. (1980). Fatty acid isoprenoid quinone and polar lipid composition in the classification of *Curtobacterium* and related taxa. *J Gen Microbiol* 118, 29–37.

Collins, M. D., Burton, R. A. & Jones, D. (1988). *Corynebacterium amycolatum* sp. nov., a new mycolic acid-less *Corynebacterium* species from human skin. *FEMS Microbiol Lett* 49, 349–352.

Collins, M. D., Falsen, E., Akervall, E., Sjöden, B. & Alvarez, A. (1998). *Corynebacterium kroppenstedtii* sp. nov., a novel corynebacterium that does not contain mycolic acids. *Int J Syst Bacteriol* 48, 1449–1454.

Collins, M. D., Hoyle, L., Foster, G. & Falsen, E. (2004). *Corynebacterium caspium* sp. nov., from a Caspian seal (*Phoca caspica*). *Int J Syst Evol Microbiol* 54, 925–928.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* 12, 133–142.

Eguchi, M., Nishikawa, T. & Macdonald, K. (1996). Responses to stress and nutrient availability by the marine ultramicrobacterium *Sphingomonas* sp. strain RB2256. *Appl Environ Microbiol* 62, 1287–1294.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.

Fernández-Garayzábal, J. F., Vela, A. I., Egido, R., Hutson, R. A., Lanzarot, M. P., Fernández-García, M. & Collins, M. D. (2004). *Corynebacterium ciconiae* sp. nov., isolated from the trachea of black storks (*Ciconia nigra*). *Int J Syst Evol Microbiol* 54, 2191–2195.

Hall, V., Collins, M. D., Hutson, R. A., Lawson, P. A., Falsen, E. & Duerden, B. I. (2003). *Corynebacterium atypicum* sp. nov., from a human clinical source, does not contain corynomycolic acids. *Int J Syst Evol Microbiol* 53, 1065–1068.

HuB, V. A. R., Festl, H. & Schleifer, K.-H. (1983). Studies on the spectrometric determination of DNA hybridisation from renaturation rates. *Syst Appl Microbiol* 4, 184–192.

Kämpfer, P., Anderson, M. A., Rainey, F. A., Kroppenstedt, R. M. & Salonen, S. M. (1999). *Williamsia muralis* gen. nov., sp. nov., isolated from the indoor environment of a children's day care centre. *Int J Syst Bacteriol* 49, 681–687.

Kates, M. (1986). *Techniques of Lipidology*, 2nd edn. Amsterdam: Elsevier.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16, 111–120.

Komagata, K. & Suzuki, K. (1987). Lipid and cell wall analysis in bacterial systematics. *Methods Microbiol* 19, 161–206.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* 5, 150–163.

Li, W. L. (2007). *Corynebacterineae*. In *Actinomycete Systematic Principles, Methods and Practice*, pp. 323–358. Edited by L. H. Xu, W. L. Li, Z. H. Liu & C. L. Jiang. Beijing: Science Press.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* 5, 109–118.

Rainey, F. A., Klatte, S., Kroppenstedt, R. M. & Stackebrandt, E. (1995). *Dietzia*, a new genus including *Dietzia maris* comb. nov., formerly *Rhodococcus maris*. *Int J Syst Bacteriol* 45, 32–36.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4, 406–425.

Soddell, J. A., Stainsby, F. M., Eales, K. L., Kroppenstedt, R. M., Robert, J., Seviour, R. J. & Goodfellow, M. (2006). *Millisia brevis* gen. nov., sp. nov., an actinomycete isolated from activated sludge foam. *Int J Syst Evol Microbiol* 56, 739–744.

- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997).** Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Wang, Y. N., Cai, H., Yu, S. L., Wang, Z. Y., Liu, J. & Wu, X. L. (2007).** *Halomonas gudaonensis* sp. nov., isolated from a saline soil contaminated by crude oil. *Int J Syst Evol Microbiol* **57**, 911–915.
- Wauters, G., Driessen, A., Ageron, E., Janssens, M. & Grimont, P. A. D. (1996).** Propionic acid-producing strains previously designated as *Corynebacterium xerosis*, *C. minutissimum*, *C. striatum*, and CDC group I and group F coryneforms belong to the species *Corynebacterium amycolatum*. *Int J Syst Bacteriol* **46**, 653–657.
- Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983).** Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.
- Zumft, W. G. (1991).** The denitrifying bacteria. In *The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Application*, 2nd edn, pp. 554–582. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.