Corynebacterium lubricantis sp. nov., isolated from a coolant lubricant

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Three Gram-positive, rod-shaped, oxidase-negative, non-spore-forming, non-motile bacteria (KSS-3Se^T, KSS-4Se and KSS-10Se) were isolated from a coolant lubricant. 16S rRNA gene sequence analyses revealed almost identical sequences, with only a few (<10 positions) differences for these three isolates. Comparisons showed the highest similarities to *Corynebacterium pilosum* NCTC 11862^T (97.6 % similarity with strain KSS-3Se^T). Similarities with other established *Corynebacterium* species were lower than 97.0 %. Chemotaxonomic data studied for strain KSS-3Se^T [polar lipids – major compounds phosphatidylglycerol and an unknown glycolipid, moderate amounts of phosphatidylinositol and diphosphatidylglycerol; polyamines (small amounts) – major compounds spermidine and spermine; quinones – significant amounts of menaquinones MK-9(H₂), MK-8(H₂) and MK-7(H₂); and major fatty acids – tuberculostearic acid (10-methyl C_{18:0}), C_{16:0} and C_{18:1ω9c}] were congruent with those reported for *Corynebacterium*. The strain showed differences in phenotype from *C. pilosum*. DNA–DNA hybridizations between KSS-3Se^T and *C. pilosum* DSM 20521^T yielded a relatedness of 22.9 % (20.4 % in the reciprocal assay). From these results, it is evident that the organisms represent a novel species, for which the name *Corynebacterium lubricantis* sp. nov. is proposed (type strain KSS-3Se^T = DSM 45231^T = CCUG 56567^T = CCM 7546^T).

The genus *Corynebacterium* currently comprises more than 80 species. The majority of the most recently described *Corynebacterium* species have been isolated from clinical specimens (e.g. Renaud *et al.*, 2001, 2007; Yassin *et al.*, 2002a, b, 2003) or animals (e.g. Fernández-Garayzábal *et al.*, 1998, 2004; Goyache *et al.*, 2003; Collins *et al.*, 2001, 2004).

A study on the diversity of bacteria from coolant lubricants revealed the presence of some yellow-pigmented, rod-shaped isolates that exhibited Gram-positive behaviour and fatty acid profiles consistent with their provisional

assignment to the genus *Corynebacterium*. Strains KSS-3Se^T, KSS-4Se and KSS-10Se were isolated on tryptone soy agar (TSA) at 25 °C. The strains showed yellow colonies on this agar. Subcultivation was done on TSA at 28 °C for 48 h. On this agar, the organisms were able to grow at 10–45 °C, but not at 4 or 50 °C. Growth at 25 °C was also observed on PYE agar (0.3 % peptone from casein, 0.3 % yeast extract, 1.5 % agar, pH 7.2), nutrient agar, MacConkey agar and R2A agar (all from Oxoid). Gram-staining was performed as described by Gerhardt *et al.* (1994) and the KOH-lysis test as described by Moaledji (1986). Cell morphology was observed under a Zeiss light microscope at ×1000, with cells grown for 3 days at 28 °C on TSA. The strains showed a (weak) Gram-positive staining reaction and were not lysed in the KOH test, confirming the results from Gram staining.

The 16S rRNA genes of all three strains were analysed as described by Kämpfer *et al.* (2003). Multiple alignments and phylogenetic analysis were performed using the

Abbreviations: pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains KSS-3Se^T, KSS-4Se and KSS-10Se are FM173119–FM173121, respectively.

An extended neighbour-joining tree and a comparison of biochemical characteristics of the novel species and related members of *Corynebacterium* are available as supplementary material with the online version of this paper.

software MEGA version 3.1 (Kumar *et al.*, 2004). The sequenced length of the 16S rRNA gene was 1379 bp for strain KSS-3Se^T, 1442 bp for strain KSS-4Se and 1407 bp for strain KSS-10Se. Nucleotide sequence comparisons of the three isolates showed only a few (<10 positions) differences between the three isolates. Similarities were 99.7% between KSS-3Se^T and KSS-4Se, 99.6% between KSS-3Se^T and KSS-10Se and 99.9% between KSS-4Se and KSS-10Se. The sequence of strain KSS-3Se^T showed 97.6% similarity with that of *Corynebacterium pilosum* NCTC 11862^T. The similarity was lower than 97.0% compared with the sequences of strains of all other recognized *Corynebacterium* species. A tree based on the neighbour-joining method is shown in Fig. 1, and an extended tree is available as Supplementary Fig. S1 (available in IJSEM Online).

Analyses of cell-wall diamino acid, quinones, polar lipids and polyamines were done with biomass grown on PYE agar. Detection of the diagnostic cell-wall diamino acid was performed by the method of Schleifer (1985). In strain KSS-3Se^T, *meso*-diaminopimelic acid could be identified, which is characteristic for *Corynebacterium* species. Polar lipids and quinones were extracted and analysed as reported by Tindall (1990a, b), Altenburger *et al.* (1997) and Stolz *et al.* (2007). The quinone system of KSS-3Se^T consisted of menaquinones MK-9(H₂), MK-8(H₂) and MK-7(H₂) in the ratio 49:30:21. A complex polar lipid profile was detected in KSS-3Se^T (Fig. 2) consisting of the major compounds phosphatidylglycerol and an unknown, quite hydrophobic glycolipid GL5, moderate amounts of phosphatidylinositol, diphosphatidylglycerol, two unknown glycolipids (GL1, GL3), three unknown amino-lipids (AL1, AL2, AL3) and an unknown polar lipid (L2) and minor amounts of unknown glycolipids GL2 and GL4, aminolipid AL4 and polar lipid L1. Phosphatidylinositol mannosides, reported to be present in several *Corynebacter-*

ium species (Fudou *et al.*, 2002; Yassin *et al.*, 2003; Chen *et al.*, 2004), could not be detected unambiguously. Polyamine analysis was carried out as described by Busse & Auling (1988) and Altenburger *et al.* (1997) using the instrumentation described by Stolz *et al.* (2007). Strain KSS-3Se^T exhibited a polyamine pattern consisting of the major compounds spermidine [0.47 µmol (g dry weight)⁻¹] and spermine [1.16 µmol (g dry weight)⁻¹] and minor amounts of 1,3-diaminopropane [0.01 µmol (g dry weight)⁻¹] and putrescine [0.01 µmol (g dry weight)⁻¹]. This pattern, with rather low polyamine contents and dominated by spermidine and spermine, is in good agreement with those detected in other *Corynebacterium* species (Altenburger *et al.*, 1997). The fatty acid profile of strain KSS-3Se^T studied as described by Kämpfer & Kroppenstedt (1996) is composed mainly of C_{16:0} (40.8%), C_{18:1ω9c} (35.6%) and 10-methyl C_{18:0} (tuberculostearic acid; 19.2%), with smaller amounts of C_{12:0} (0.5%), C_{14:0} (1.2%), C_{16:1ω9c} (1.2%), C_{17:1ω7c} (1.0%) and C_{18:0} (2.6%). It is slightly different from the profile of *C. pilosum* DSM 20521^T (Table 1), which was studied concurrently.

Results of the physiological characterization are given in the species description and in Table 2. Methods used were described previously (Kämpfer *et al.*, 1991). Further differentiating characters of the most similar *Corynebacterium* species (on the basis of 16S rRNA gene sequence similarities) are given in Supplementary Table S1.

DNA–DNA hybridization experiments were performed with strains KSS-3Se^T and *C. pilosum* DSM 20521^T. DNA isolation and DNA–DNA hybridization were done as described previously (Ziemke *et al.*, 1998). Results of the DNA–DNA cross-hybridization yielded a relatedness of 22.9%, whereas reciprocal hybridization resulted in a lower value of 20.4%.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences from the most closely related *Corynebacterium* species available from the EMBL database (accession numbers in parentheses) showing the position of strains KSS-3Se^T, KSS-4Se and KSS-10Se. Multiple alignments, distance calculations (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method were performed by using the software package MEGA version 3.1 (Kumar *et al.*, 2004). Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.005 nucleotide substitutions per nucleotide position. An extended neighbour-joining tree including a wider selection of reference sequences is available as Supplementary Fig. S1.

Fig. 2. Polar lipid profile of strain KSS-3Se^T after two-dimensional TLC and detection with molybdatophosphoric acid. PG, Phosphatidylglycerol; DPG, diphosphatidylglycerol; PI, phosphatidylinositol; AL1–4, unknown aminolipids; L1–2, unknown polar lipids; GL1–5, unknown glycolipids.

From the results of 16S rRNA gene sequencing, DNA–DNA hybridization data and phenotypic analyses (significantly smaller amounts of *C*_{18:1ω9c} and differences in physiological/biochemical traits), it is evident that strain KSS-3Se^T is different from *C. pilosum* and all other *Corynebacterium* species and, hence, a novel species of the genus *Corynebacterium* is proposed.

Description of *Corynebacterium lubricantis* sp. nov.

Corynebacterium lubricantis [lu.bri.can'tis. L. v. *lubricare* to lubricate; N.L. n. *lubricans* -antis (from L. part. adj. *lubricans*) a lubricant; N.L. gen. n. *lubricantis* of/from a (coolant) lubricant].

Table 1. Cellular fatty acid contents of strains KSS-3Se^T, KSS-4Se and KSS-10Se and *C. pilosum* DSM 20521^T

Values are percentages of total fatty acids; minor fatty acids (<0.5%) are not shown. Results were obtained in this study. ND, Not detected.

Fatty acid	KSS-3Se ^T , KSS-4Se and KSS-10Se	<i>C. pilosum</i> DSM 20521 ^T
<i>C</i> _{14:0}	0.9–1.8	ND
<i>C</i> _{16:1ω9c}	1.0–2.8	4.3
<i>C</i> _{17:1ω7c}	1.0–1.9	1.3
<i>C</i> _{16:0}	43.6–49.6	42.9
<i>C</i> _{18:1ω9c}	31.6–45.3	42.5
10-Methyl <i>C</i> _{18:0}	10.8–30.8	7.3
<i>C</i> _{18:0}	1.7–4.3	1.0

Table 2. Differentiating physiological/biochemical properties of the novel strains and *C. pilosum* DSM 20521^T

Characteristic	KSS-3Se ^T , KSS-4Se and KSS-10Se	<i>C. pilosum</i> DSM 20521 ^T
Acid production from:		
Sucrose	–	+*
Mannose	–	+*
Cellobiose	–	+
Aesculin hydrolysis	+	–*
Growth on MacConkey agar	+	–
Nitrate reduction	–	+*
Urease activity	–	+*

*Result in congruence with results reported by Yanagawa & Honda (1978) and Collins & Cummins (1986).

Cells are non-motile, non-spore-forming rods (approx. 1 µm long). Gram-positive, oxidase-negative, showing an oxidative metabolism. Good growth occurs on R2A agar, TSA, nutrient agar and MacConkey agar at 25–30 °C. Yellow, translucent and shiny colonies with entire edges form within 24 h, with a diameter of approximately 0.5 mm. The lipid profile consists of the major compounds phosphatidylglycerol and an unknown glycolipid, moderate amounts of phosphatidylinositol, diphosphatidylglycerol, two unknown glycolipids, three unknown aminolipids and an unknown polar lipid and minor amounts of two glycolipids, an aminolipid and a polar lipid. Polyamines are present in small amounts; the major compounds are spermidine and spermine. The quinone system is composed of significant amounts of menaquinones MK-9(H₂), MK-8(H₂) and MK-7(H₂). The characteristic peptidoglycan diamino acid is *meso*-diaminopimelic acid. The fatty acid profile contains large amounts of *C*_{16:0}, *C*_{18:1ω9c} and 10-methyl *C*_{18:0}. The novel species can be differentiated from *C. pilosum* through its fatty acid profile and by physiological tests (Tables 1 and 2). The following compounds are used as sole sources of carbon: D-fructose, D-glucose, acetate, fumarate and DL-lactate on the basis of the method described by Kämpfer *et al.* (1991). The following compounds are not utilized: *N*-acetylgalactosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, cellobiose, D-galactose, maltose, D-mannose, L-rhamnose, D-ribose, salicin, trehalose, D-xylose, adipate, 2-oxoglutarate, D-gluconate, melibiose, sucrose, adonitol, *myo*-inositol, maltitol, D-mannitol, D-sorbitol, propionate, *cis*- and *trans*-aconitate, 4-aminobutyrate, citrate, glutarate, DL-3-hydroxybutyrate, itaconate, L-malate, mesaconate, pyruvate, L-alanine, β-alanine, L-aspartate, L-leucine, L-ornithine, L-proline, L-serine, putrescine, azelate, suberate, L-histidine, L-phenylalanine, L-serine, L-tryptophan, 3-hydroxybenzoate and phenylacetate. Acid is produced from glucose and trehalose. No acids are produced from sucrose, D-mannitol, dulcitol, lactose, rhamnose, maltose, galactose, salicin, adonitol, inositol, sorbitol, L-arabinose,

raffinose, D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose, D-arabitol or D-mannose. p-Nitrophenyl (pNP) β -D-glucopyranoside, pNP β -D-glucuronide, bis-pNP phosphate and bis-pNP phenylphosphonate are hydrolysed on the basis of the method described by Kämpfer *et al.* (1991). The following compounds are not hydrolysed: pNP α -D-glucopyranoside, pNP β -D-galactopyranoside, pNP β -D-xylopyranoside, bis-pNP phosphorylcholine, L-alanine p-nitroanilide (pNA), L-proline pNA and γ -L-glutamate pNA.

The type strain is KSS-3Se^T (=DSM 45231^T =CCUG 56567^T =CCM 7546^T), isolated in Giessen, Germany, from a coolant lubricant. Strains KSS-4Se and KSS-10Se are additional strains of the species.

Acknowledgements

The study was supported by a grant to P.K. from the Berufsgenossenschaft Metall Nord Süd – BGM (formerly Berufsgenossenschaft Metall Süd).

References

- Altenburger, P., Kämpfer, P., Akimov, V. N., Lubitz, W. & Busse, H.-J. (1997). Polyamine distribution in actinomycetes with group B peptidoglycan and species of the genera *Brevibacterium*, *Corynebacterium*, and *Tsukamurella*. *Int J Syst Bacteriol* **47**, 270–277.
- Busse, H.-J. & Auling, G. (1988). Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* **11**, 1–8.
- Chen, H.-H., Li, W.-J., Tang, S.-K., Kroppenstedt, R. M., Stackebrandt, E., Xu, L.-H. & Jiang, C.-L. (2004). *Corynebacterium halotolerans* sp. nov., isolated from saline soil in the west of China. *Int J Syst Evol Microbiol* **54**, 779–782.
- Collins, M. D. & Cummins, C. S. (1986). Genus *Corynebacterium* Lehmann and Neumann 1986, 350^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1266–1283. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Collins, M. D., Hoyles, L., Foster, G., Sjöden, B. & Falsen, E. (2001). *Corynebacterium capitovis* sp. nov., from a sheep. *Int J Syst Evol Microbiol* **51**, 857–860.
- Collins, M. D., Hoyles, L., Foster, G. & Falsen, E. (2004). *Corynebacterium caspium* sp. nov., from a Caspian seal (*Phoca caspica*). *Int J Syst Evol Microbiol* **54**, 925–928.
- Fernández-Garayzábal, J. F., Collins, M. D., Hutson, R. A., González, I., Fernández, E. & Domínguez, L. (1998). *Corynebacterium camporealensis* sp. nov., associated with subclinical mastitis in sheep. *Int J Syst Bacteriol* **48**, 463–468.
- 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.
- Fudou, R., Jojima, Y., Seto, A., Yamada, K., Kimura, E., Nakamatsu, T., Hirashi, A. & Yamanaka, S. (2002). *Corynebacterium efficiens* sp. nov., a glutamic-acid-producing species from soil and vegetables. *Int J Syst Evol Microbiol* **52**, 1127–1131.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Goyache, J., Ballesteros, C., Vela, A. I., Collins, M. D., Briones, V., Hutson, R. A., Potti, J., García-Borboroglu, P., Domínguez, L. & Fernández-Garayzábal, J. F. (2003). *Corynebacterium spheisci* sp. nov., isolated from wild penguins. *Int J Syst Evol Microbiol* **53**, 1009–1012.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kämpfer, P., Steiof, M. & Dott, W. (1991). Microbiological characterization of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* **21**, 227–251.
- Kämpfer, P., Dreyer, U., Neef, A., Dott, W. & Busse, H.-J. (2003). *Chryseobacterium defluvii* sp. nov., isolated from wastewater. *Int J Syst Evol Microbiol* **53**, 93–97.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Moaledji, K. (1986). Comparison of Gram-staining and alternate methods, KOH test and aminopeptidase activity in aquatic bacteria: their application to numerical taxonomy. *J Microbiol Methods* **5**, 303–310.
- Renaud, F. N. R., Aubel, D., Riegel, P., Meugnier, H. & Bollet, C. (2001). *Corynebacterium freneyi* sp. nov., α -glucosidase-positive strains related to *Corynebacterium xerosis*. *Int J Syst Evol Microbiol* **51**, 1723–1728.
- Renaud, F. N. R., Le Coustumier, A., Wilhelm, N., Aubel, D., Riegel, P., Bollet, C. & Freney, J. (2007). *Corynebacterium hansenii* sp. nov., an α -glucosidase-negative bacterium related to *Corynebacterium xerosis*. *Int J Syst Evol Microbiol* **57**, 1113–1116.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Stolz, A., Busse, H.-J. & Kämpfer, P. (2007). *Pseudomonas knackmussii* sp. nov. *Int J Syst Evol Microbiol* **57**, 572–576.
- Tindall, B. J. (1990a). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Tindall, B. J. (1990b). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Yanagawa, R. & Honda, E. (1978). *Corynebacterium pilosum* and *Corynebacterium cystitidis*, two new species from cows. *Int J Syst Bacteriol* **28**, 209–216.
- Yassin, A. F., Steiner, U. & Ludwig, W. (2002a). *Corynebacterium aurimucosum* sp. nov. and emended description of *Corynebacterium minutissimum* Collins and Jones (1983). *Int J Syst Evol Microbiol* **52**, 1001–1005.
- Yassin, A. F., Steiner, U. & Ludwig, W. (2002b). *Corynebacterium appendicis* sp. nov. *Int J Syst Evol Microbiol* **52**, 1165–1169.
- Yassin, A. F., Kroppenstedt, R. M. & Ludwig, W. (2003). *Corynebacterium glaucum* sp. nov. *Int J Syst Evol Microbiol* **53**, 705–709.
- Ziemke, F., Höfle, M. G., Lalucat, J. & Rosselló-Mora, R. (1998). Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* **48**, 179–186.