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-3SeT, 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 11862T (97.6 % similarity with strain KSS-3SeT). Similarities with other established Corynebacterium species were lower than 97.0 %. Chemotaxonomic data studied for strain KSS-3SeT [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 C18:0), C16:0 and C18:1ω9c] were congruent with those reported for Corynebacterium. The strain showed differences in phenotype from C. pilosum.

DNA–DNA hybridizations between KSS-3SeT and C. pilosum DSM 20521T 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-3SeT = DSM 45231T = CCUG 56567T = CCM 7546T).

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-3SeT, 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
The sequenced length of the 16S rRNA gene was 1379 bp for strain KSS-3Se\textsuperscript{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\textsuperscript{T} and KSS-4Se, 99.6% between KSS-3Se\textsuperscript{T} and KSS-10Se and 99.9% between KSS-4Se and KSS-10Se. The sequence of strain KSS-3Se\textsuperscript{T} showed 97.6% similarity with that of Corynebacterium pilosum NCTC 11862\textsuperscript{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\textsuperscript{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\textsuperscript{T} consisted of menaquinones MK-9(H\textsubscript{2}), MK-8(H\textsubscript{2}) and MK-7(H\textsubscript{2}) in the ratio 49:30:21. A complex polar lipid profile was detected in KSS-3Se\textsuperscript{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 aminolipids (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 Corynebacterium 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\textsuperscript{T} exhibited a polyamine pattern consisting of the major compounds spermidine [0.47 μmol (g dry weight)\textsuperscript{-1}] and spermine [1.16 μmol (g dry weight)\textsuperscript{-1}] and minor amounts of 1,3-diaminopropane [0.01 μmol (g dry weight)\textsuperscript{-1}] and putrescine [0.01 μmol (g dry weight)\textsuperscript{-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\textsuperscript{T} studied as described by Kämpfer & Kroppenstedt (1996) is composed mainly of C\textsubscript{16}:0 (40.8%), C\textsubscript{18}:1ω9c (35.6%) and 10-methyl C\textsubscript{18}:0 (tuberculostearic acid; 19.2%), with smaller amounts of C\textsubscript{12}:0 (0.5%), C\textsubscript{14}:0 (1.2%), C\textsubscript{16}:1ω9c (1.2%), C\textsubscript{17}:1ω7c (1.0%) and C\textsubscript{18}:0 (2.6%). It is slightly different from the profile of C. pilosum DSM 20521\textsuperscript{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\textsuperscript{T} and C. pilosum DSM 20521\textsuperscript{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%.
From the results of 16S rRNA gene sequencing, DNA–DNA hybridization data and phenotypic analyses (significantly smaller amounts of C18 : 1\(\text{v}9\)c 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 \([\text{lubri}c\text{ant}i\text{s. 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.}]

Cells are non-motile, non-spore-forming rods (approx. 1 \(\mu\)m 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(H\(_2\)), MK-8(H\(_2\)) and MK-7(H\(_2\)). The characteristic peptidoglycan diamino acid is \(\text{meso-diaminopimelic acid.}\

The fatty acid profile contains large amounts of C16 : 0, C18 : 1\(\text{v}9\)c and 10-methyl C18 : 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 Ka¨mpfer et al. (1991). The following compounds are not utilized: N-acetylgalactosamine, N-acetylglucosamine, L-arabinose, L-arbutin, cellobiose, D-galactose, maltose, D-mannose, L-rihanose, D-ribose, salicin, trehalose, D-xylene, adiquote, 2-oxoglutarate, D-gluconate, melibiose, sucrose, adonitol, myo-inositol, maltitol, D-mannitol, D-sorbitol, propionate, cis- and \(\text{trans-}\)aconitate, 4-aminobutyrate, citrate, glutarate, DL-3-hydroxybutyrate, itaconate, L-malate, mesaconate, pyruvate, L-alanine, \(\beta\)-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.

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

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>KSS-3Se(^T), KSS-4Se and KSS-10Se</th>
<th>(C.) (pilosum) DSM 20521(^T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(_{14}) : 0</td>
<td>0.9–1.8</td>
<td>ND</td>
</tr>
<tr>
<td>C(_{16}) : 1(\text{v}9)c</td>
<td>1.0–2.8</td>
<td>4.3</td>
</tr>
<tr>
<td>C(_{17}) : 1(\text{v}7)c</td>
<td>1.0–1.9</td>
<td>1.3</td>
</tr>
<tr>
<td>C(_{16}) : 0</td>
<td>43.6–49.6</td>
<td>42.9</td>
</tr>
<tr>
<td>C(_{18}) : 1(\text{v}9)c</td>
<td>31.6–45.3</td>
<td>42.5</td>
</tr>
<tr>
<td>10-Methyl C(_{18}) : 0</td>
<td>10.8–30.8</td>
<td>7.3</td>
</tr>
<tr>
<td>C(_{18}) : 0</td>
<td>1.7–4.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Result in congruence with results reported by Yanagawa & Honda (1978) and Collins & Cummins (1986).
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 Kämper et al. (1991). The following compounds are not hydrolysed: pNP α-D-glucopyranoside, pNP β-D-galactopyranoside, pNP β-D-xylopyranoside, bis-pNP phosphorlycholine, 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


