**Tessaracoccus lubricantis** sp. nov., isolated from a metalworking fluid

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A Gram-positive-staining, coccoid-shaped, oxidase-negative, non-spore-forming, non-motile bacterium, strain KSS-17Se⁺, was isolated from a metalworking fluid. On the basis of its major fatty acid (ai-C₁₅:₀) and 16S rRNA gene sequence similarity, the strain grouped with *Tessaracoccus bendigoensis* and *Tessaracoccus flavescens*, sharing 95.3 and 97.4 % 16S rRNA gene sequence similarity with the respective type strains. Similarities with other established species of the genera *Luteococcus*, *Propioniferax* and *Granulicoccus* were lower than 95.5 %.

The quinone system was characterized by the major menaquinone MK-9(H₄). In the polar lipid profile, diphosphatidylglycerol, phosphatidylglycerol, an unknown glycolipid and an unknown polar lipid were detected as major compounds. Additionally, three unknown glycolipids and minor amounts of phosphatidylethanolamine, two unknown aminolipids and two unknown polar lipids were detected. Phosphatidylinositol was present only in trace amounts. Predominant polyamines were spermine and spermidine. Ll-Diaminopimelic acid was identified as the diagnostic diamino acid in the cell wall. The strain showed clear differences in phenotype (including chemotaxonomic features) from both *Tessaracoccus* species and members of the other above-mentioned genera.

DNA–DNA hybridization between KSS-17Se⁺ and *T. bendigoensis* Ben-106ᵀ and *T. flavescens* SST-39ᵀ yielded similarities of 15.1 and 21.0 %, respectively. It is evident that the organism represents a novel species, for which the name *Tessaracoccus lubricantis* sp. nov. is proposed. The type strain is KSS-17Se⁺ (≡DSM 19926ᵀ =CCUG 55516ᵀ).

Metalworking fluids are substances that are used for cooling and lubrication when metals are being cut or formed. A distinction is drawn between non-water-miscible and water-miscible metalworking fluids; the latter are products which are mixed with water before use. In 2007, more than 32 000 tons of water-miscible metalworking fluid concentrate were used in Germany. Assuming a 4 % batch, this means an estimated consumption of 800 000 tons of emulsion or solution per year.

The investigations described here were all done with oleaginous, preserved, water-mixed metalworking fluids. In a study on the diversity of bacteria isolated from different metalworking fluids, strain KSS-17Seᵀ was isolated from tryptone soy agar (TSA; Oxoid) at 25 °C. The strain formed very small, yellow colonies on this agar. Subcultivation was done for 24 h on TSA at 28 °C for 48 h. On this agar, the strain was able to grow at 15–36 °C, but not at 10 or 45 °C. Growth at 25 °C was also observed on nutrient agar and R2A agar (both from Oxoid). Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at ×1000, with cells grown for 3 days at 28 °C on TSA. The cells were non-motile, non-spore-forming rods (approx. 0.5–1 μm wide and 0.8–2 μm long). The strain showed a weak Gram-positive reaction.

The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003). Multiple alignment of the sequence and phylogenetic analysis were performed using the software MEGA version 3.1 (Kumar *et al.* 2004). The sequenced length of the 16S rRNA gene was 1393 bp for strain KSS-
17Se\textsuperscript{T}. Nucleotide sequence similarities were 97.4% with *Tessaracoccus flavescens* SST-39\textsuperscript{T}, 95.3% with *Tessaracoccus bendigoensis* Ben 106\textsuperscript{T} and less than 95.5% with strains of all species of the genera *Luteococcus*, *Propioniferax* and *Granulicoccus* with validly published names. A phylogenetic tree resulting from neighbour-joining reconstruction (Saitou & Nei, 1987) is shown in Fig. 1.

The following analytical procedures were performed according to the indicated references: respiratory quinones and polar lipids (Tindall, 1990a, b; Altenburger et al., 1996); polyamines (Busse & Auling, 1988; Busse et al., 1997; Stolz et al., 2007); diagnostic peptidoglycan diamino acid (Bousfield et al., 1985); and fatty acids (Kämpfer & Kroppenstedt, 1996).

The diagnostic diamino acid in the peptidoglycan was determined to be LL-diaminopimelic acid. The polar lipid profile of KSS-17Se\textsuperscript{T} consisted of the predominant compounds diphosphatidylglycerol, phosphatidylglycerol, an unknown glycolipid and an unknown polar lipid. Additionally, minor amounts of three unknown glycolipids, phosphatidylethanolamine, two unknown aminolipids and two unknown polar lipids were detected (Supplementary Fig. S1, available in IJSEM Online). Phosphatidylinositol was present in trace amounts, detectable only after spraying with molybdenum blue and not visible in the complete lipid profile. We might have overlooked this tiny spot without knowledge of its chromatographic behaviour and its presence in *T. bendigoensis* Ben 106\textsuperscript{T}. *T. flavescens* SST-39\textsuperscript{T} has been reported to contain only phosphatidylglycerol and diphosphatidylglycerol (Lee & Lee, 2008). Re-examination of the polar lipid profile of *T. flavescens* SST-39\textsuperscript{T} revealed, in addition to the reported lipids, the presence of unknown glycolipids GL2, comprising a major compound in the profile, and GL3 and unknown polar lipid L1. As in *T. bendigoensis* Ben 106\textsuperscript{T}, we also detected glycolipids in the extract from KSS-17Se\textsuperscript{T}, with one glycolipid found in large amounts. However, the findings of a fourth glycolipid, only trace amounts of phosphatidylinositol and the presence of phosphatidylethanolamine clearly distinguish KSS-17Se\textsuperscript{T} from *T. bendigoensis* Ben 106\textsuperscript{T}. Strain KSS-17Se\textsuperscript{T} can be distinguished from *T. flavescens* SST-39\textsuperscript{T} based on additional glycolipids and an unknown lipid L3. The predominant quinone was MK-9(H\textsubscript{4}) (92%), Additionally, minor amounts of MK-8(H\textsubscript{4}) (2%), MK-9(H\textsubscript{2}) (3%) and MK-9(H\textsubscript{8}) (3%) could be detected. In a second analysis from independently grown biomass, the content of MK-9(H\textsubscript{2}) was considerably higher (approximately 25% of the total quinone content), but MK-9(H\textsubscript{4}) was still predominant. This observation indicates that the relative amounts of minor or moderate quinone components may vary depending on differences in culture media or conditions or the point of harvest of biomass. Analysis of the quinone composition of *T. flavescens* SST-39\textsuperscript{T} confirmed MK-9(H\textsubscript{4}) as the major compound (Lee & Lee, 2008) but, unlike these authors, we did not detect MK-8 or other quinone components in amounts >2%. This result confirms our observation with KSS-17Se\textsuperscript{T} that only the major component is of significance, while other quinone components may be detected in variable amounts depending on the growth conditions.

The cell-wall diamino acid, quinone system and polar lipid profile reflected the close relatedness of KSS-17Se\textsuperscript{T} with *T. flavescens* and *T. bendigoensis*. The polyamine pattern of strain KSS-17Se\textsuperscript{T} consisted of the major compounds spermidine [1.2 \(\mu\)mol (g dry weight)\textsuperscript{-1}] and spermine [1.0 \(\mu\)mol (g dry weight)\textsuperscript{-1}]. Trace amounts of putrescine [0.05 \(\mu\)mol (g dry weight)\textsuperscript{-1}] were detected as well. Although the polyamine pattern has not been reported for *T. bendigoensis*, this pattern is in excellent agreement with those of other members of the family *Propionibacteriaceae* such as *Propionibacterium*, *Propioniferax*, *Microlunatus*, *Friedmanniella* and a representative of the next most closely related genus *Luteococcus* (Busse & Schumann, 1999) as well as *T. flavescens* SST-39\textsuperscript{T}, which was also examined in this study. Its polyamine pattern consisted of spermidine [4.5 \(\mu\)mol (g dry weight)\textsuperscript{-1}], spermine [1.1 \(\mu\)mol (g dry weight)\textsuperscript{-1}], putrescine [0.5 \(\mu\)mol (g dry weight)\textsuperscript{-1}], 1,3-diaminopropane [0.1 \(\mu\)mol (g dry weight)\textsuperscript{-1}] and cadaverine [0.05 \(\mu\)mol (g dry weight)\textsuperscript{-1}]. Although representatives of all genera of the

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**Fig. 1.** Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses). 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, 0.01 substitutions per nucleotide position.
Propionibacteriaceae have not yet been analysed for polyamines, the available data suggest that a common characteristic of the family is a polyamine pattern with the major compounds spermidine and spermine.

The fatty acid profile of strain KSS-17Se\textsuperscript{T} (Table 1) is composed mainly of ai-C\textsubscript{15:0} (83.2\%) with much smaller amounts of i-C\textsubscript{14:0} (2.0\%), i-C\textsubscript{15:0} (3.4\%), i-C\textsubscript{16:0} (3.8\%), ai-C\textsubscript{17:0} (3.8\%), C\textsubscript{14:0} (1.3\%) and C\textsubscript{16:0} (2.6\%). It is clearly different from the profiles of T. bendigoensis Ben 106\textsuperscript{T} and T. flavescens SST-39\textsuperscript{T}. The presence of ai-C\textsubscript{15:0} and also other iso- and anteiso-branched fatty acids is reported for all other members of the genera Tessaracoccus, Luteococcus, Propioniferax and Granulicoccus; however, members of these genera can be differentiated on the basis of levels of these fatty acids and also on the presence of additional compounds.

The results of the physiological characterization are given in the species description and in Table 2. The methods used were described previously (Kämpfer et al., 1991).

DNA–DNA hybridization experiments were performed with strain KSS-17Se\textsuperscript{T} and T. bendigoensis Ben-106\textsuperscript{T} and T. flavescens SST-39\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 similarities of the isolate to these type strains of 15.1 and 21.0\% respectively.

From the results of 16S rRNA gene sequence comparisons and DNA–DNA hybridizations and chemotaxonomic characterization, it is evident that strain KSS-17Se\textsuperscript{T} is different from the two Tessaracoccus species. On the basis of the striking differences in the phylogenetic data and phenotypic features, a novel species of the genus Tessaracoccus is proposed with the name Tessaracoccus lubricantis sp. nov.

**Description of Tessaracoccus lubricantis sp. nov.**

Tessaracoccus 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].

Cells are non-motile, non-spore-forming rods (approx. 0.5–1 µm wide and 0.8–2 µm long). Gram-positive-staining and 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 diagnostic diamino acid in the peptidoglycan is LL-diaminopimelic acid. The quinone system consists mainly of MK-9(H4). The polyamine pattern includes spermidine and spermine as major compounds. Predominant polar lipids are diphosphatidylglycerol, phosphatidylglycerol, an unknown glycolipid and an unknown polar lipid. In addition, minor amounts of phosphatidylethanolamine, three unknown glycolipids, two unknown aminolipids and two unknown polar lipids and only trace amounts of phosphatidylinositol are present. The fatty acid profile contains large amounts of...
Table 2. Comparative phenotypic characters of strain KSS-17SeT and Tessaracoccus type strains

All three strains are Gram-positive-staining, non-motile and non-spore-forming chemo-organotrophs that are oxidase-negative, catalase-positive and urease-negative and do not produce indole. All three strains show MK-9(H4) and MK-7(H4) as the major menaquinones and LL-diaminopimelic acid as the cell-wall diamino acid. The murein type has been determined for T. bendigoensis Ben 106T only, as A3c; Metachromatic polyphosphate granules were reported for T. bendigoensis Ben 106T; their presence has not been investigated in the other strains. All three strains utilize arabinose, fructose, galactose, maltose, ribose and xylose (confirmed in this study for T. bendigoensis Ben 106T using the method of Kämpfer et al., 1991). Data for T. bendigoensis Ben 106T were obtained from Maszenan et al. (1999) and data for T. flavescens SST-39T were taken from Lee & Lee (2008). ND, No data available.

<table>
<thead>
<tr>
<th>Phenotypic character</th>
<th>KSS-17SeT</th>
<th>T. flavescens SST-39T</th>
<th>T. bendigoensis Ben 106T</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2 requirement</td>
<td>Aerobe</td>
<td>Facultative anaerobe</td>
<td>Facultative anaerobe</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>Short rods</td>
<td>Short rods</td>
<td>Cocci (0.5–1.1 μm) arranged in tetrads</td>
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<tr>
<td>Isolation source</td>
<td>Water (metalworking fluid)</td>
<td>Marine sediment sample, Korea</td>
<td>Sewage-treatment plant, Australia</td>
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<td>Growth temperature (°C)</td>
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<tr>
<td></td>
<td>15–36</td>
<td>20–30</td>
<td>20–37</td>
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<td>Growth pH</td>
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<td>5.5–9.3</td>
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<tr>
<td></td>
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<tr>
<td>Polar lipids*</td>
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<td>PG, DPG</td>
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<td>Raffinose</td>
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<tr>
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<tr>
<td>Sucrose</td>
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<td>–†</td>
</tr>
<tr>
<td>Adonitol</td>
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*DPG, Diphosphatidylglycerol; GL, unknown glycolipid(s); L, unknown polar lipid(s); PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid(s).
†Confirmed in this study using the method of Kämpfer et al. (1991).

ai-C15:0 and moderate amounts of i-C14:0, i-C15:0, i-C16:0 and ai-C17:0. Can be differentiated from other Tessaracoccus species by its polar lipid and fatty acid profiles (Tables 1 and 2) and by physiological tests. The following compounds are used as sole sources of carbon: N-acetylgalactosamine, N-acetylglucosamine, L-arabinose, L-arginine, L-arbutin, cellobiose, maltose, ribose and xylose (confirmed in this study for T. bendigoensis Ben 106T using the method of Kämpfer et al., 1991). The following compounds are not utilized: D-glucuronate, α-melibiose, sucrose, adonitol, myo-inositol, maltitol, D-mannitol, D-sorbitol, acetal, propionate, cis-and trans-aconitate, 4-aminobutyrate, citrate, fumarate, 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, lactose, rhamnose, maltose and galactose. No acids are produced from sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, D-xylene, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose, D-arabitol or D-mannose.

The type strain is KSS-17SeT (=DSM 19926T =CCUG 55516T), isolated in Giessen, Germany, from a metalworking fluid.
Acknowledgements

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References


