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

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Abstract

A Gram-stain-positive, aerobic, endospore-forming bacterium isolated from a coolant lubricant was studied for its taxonomic allocation. On the basis of 16S rRNA gene sequences, strain KSS164-79ᵀ shared highest similarity (92.3–92.4 %) to type strains of the species Marinicrinis sediminis, Paenibacillus dongondensis, Paenibacillus abyssi and Paenibacillus motobuensis. In phylogenetic trees based on the 16S rRNA gene, strain KSS164-79ᵀ always formed a distinct cluster with the type strain of M. sediminis. The fatty acid profile supported the grouping of the strain to the genus Marinicrinis. As major fatty acids, anteiso-C₁₅:₀, iso-C₁₅:₀ and iso-C₁₆:₀ were detected. The polar lipid profile contained the major components diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylethanolamine and two unidentified glycolipids. The major quinone was menaquinone MK-7. Hence, KSS164-79ᵀ represents a novel species of the genus Marinicrinis, for which the name Marinicrinis lubricantis sp. nov. is proposed, with KSS164-79ᵀ (=DSM 104943ᵀ=LMG 30062ᵀ=CCM 8749ᵀ=CIP 111345ᵀ) as the type strain.

Recently the novel genus Marinicrinis was established [1] with one species, Marinicrinis sediminis, based on the description of a marine strain. The genus was allocated to the family Paenibacillaceae comprising, at the time of writing, the genera Paenibacillus, Aneurinibacillus, Ammoniphilus, Aneurinibacillus, Brevibacillus, Cohnella, Fontibacillus, Oxalophagus, Saccharibacillus and Thermobacillus (http://www.bacterio.net, January 2018). The family is distributed between two phylogenetic clusters [2]; two newly described genera (Saccharibacillus and Fontibacillus) are grouped with the genera Cohnella and Paenibacillus, while the other genera cluster distantly to members of the genus Brevibacillus. The genus Thermobacillus does not appear as the most deeply branching lineage [3] but as a rapidly evolving lineage within the genus Paenibacillus [4]. Members of the family in general are Gram-stain-positive, -variable or -negative, and sporulate with oval or ellipsoidal spores. The major menaquinone is either MK-7 or MK-6.

Here we report the description of a strain isolated from a coolant lubricant, which shows moderate to high similarities to members of the genera Marinicrinis and Paenibacillus.

Strain KSS164-79ᵀ was isolated from a coolant lubricant in Germany. The strain was initially isolated on nutrient agar (NA; Sigma-Aldrich) at 30 °C and also further maintained and subcultivated on this agar at 30 °C for 48 h. Analyses of the 16S rRNA gene sequence phylogeny, the fatty acid methyl ester composition of whole cell hydrolysates, and further biochemical and physiological features were conducted to characterize the strain.

For phylogenetic identification, the full-length 16S rRNA gene of strain KSS164-79ᵀ was PCR-amplified from a cell lysate generated by three freeze–thaw cycles from two loops of fresh biomass (–20/100 °C, 1 min) using the primer system 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-ACGGCTACCTTGTTACGACTT-3′) [5] and sequenced with the Sanger method using primers 27F (5′-GAGTTTGATCCTGGCTCAG-3′) and E786F (5′-GATTAGATAC CCTGGTAG-3′) [6]. Sequences were manually corrected using MEGA 7 [7] which resulted in a 16S rRNA gene sequence 1463 nt in length spanning gene termini 20–1482 (numbered according to the Escherichia coli rnb gene sequence; [8]). The most closely related type species were determined using the EzBioCloud identifier [9]. Strain KSS164-79ᵀ (accession number MG589643) shared highest 16S rRNA gene sequence similarity of only 92.4–92.5 % with Marinicrinis sediminis NC2-42ᵀ (KR604722) and some Paenibacillus type strains, Paenibacillus dongondensis KUDC0114ᵀ (KF425513), Paenibacillus abyssi SC5017010ᵀ (KC978082) and Paenibacillus motobuensis MC10ᵀ (AY741810). 16S rRNA gene sequence similarities to other type strains were even lower (<92.2 %).
The phylogenetic placement of strain KSS164-79\textsuperscript{T} within the *Paenibacillaceae* was subsequently investigated by the reconstruction of phylogenetic trees using ARB release 5.2 [10] and the 'All-Species Living Tree' Project (LTP; [11]) database release LTP\textsuperscript{s}128 (February 2017). Sequences not included in the database were aligned with the SILVA Incremental Aligner (SINA; v. 1.2.11; [12]) and implemented into the database. The sequence alignment including all *Paenibacillaceae* was checked manually and trimmed for the phylogenetic analysis to the sequence positions between gene termini 101 and 1402 (numbering according to [8]). The sequence region was covered by nearly all *Paenibacillaceae* type strain 16S rRNA gene sequences. Shorter sequences were excluded from analysis (a few *Paenibacillus* type strains). Phylogenetic trees were calculated with the maximum-likelihood method using RAxML v7.04 [13] with GTR-GAMMA as the evolutionary model and rapid bootstrap analysis, the maximum-parsimony method using DNAPARS version 3.6 [14] and the neighbour-joining method using the ARB neighbour-joining tool with the Jukes–Cantor correction as the evolutionary model [15]. Subsequently a consensus tree was reconstructed based on the three individual trees. All phylogenetic trees were calculated with 100 replications (bootstrap analysis; [16]). In addition, the same set of phylogenetic trees was reconstructed by the selection of representative taxa of the family *Paenibacillaceae*. For the genus *Paenibacillus*, type strains of the type species and its' next most closely related species were selected in addition to the type strains of species that shared highest 16S rRNA gene sequence similarity to the novel strain as determined by the EzBioCloud identification. In addition, type strains of *Paenibacillus* species were selected which were placed next to the novel strain based on the phylogenetic analysis, including nearly all members of the *Paenibacillaceae*. Here, gene sequences covering gene termini 72–1402 (numbering according to [8]) were used. Independent of the analysis that was performed, strain KSS164-79\textsuperscript{T} always formed a distinct cluster (mostly supported by high bootstrap values, >70 \%) with the type strain of *M. sediminis*. The two strains formed a distinct branch among members of the genus *Paenibacillus* (Fig. 1).

The genomic G+C content of strain KSS164-79\textsuperscript{T} was determined by the DNA melting temperature methods established by Gonzalez and Saiz-Jimenez [17] as described by Glaeser et al. [18]. High-molecular-weight DNA was therefore extracted according to the method of Pitcher et al. [19]. The genomic G+C content determined for strain KSS164-79\textsuperscript{T} was 62.8 mol\%. This value was slightly higher than the G+C content of 58.11 mol\% determined for *M. sediminis* NC2-42\textsuperscript{T} by Guo et al. [1] by the HPLC method.

All cultural and morphological characteristics were recorded from cultures grown on tryptic soy agar (TSA). Gram staining was performed according to Gerhardt et al. [20] and the motility test was done under a light microscope with cells grown for 3 days in tryptic soy broth (TSB; Oxoid) at 30 °C. Temperature-dependent growth was tested at 4, 10, 15, 20, 30, 36, 40, 45, 50 and 55 °C on TSA. NaCl tolerance was investigated at different concentrations of NaCl [0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 \% (w/v)] in TSB. pH-dependent growth was tested in TSB adjusted with HCl and NaOH to pH values between 4.0 and 12.0. Growth under anaerobic conditions was determined on anaerobic agar (Difco) at 30 °C using a GasPak jar (<1 % O\textsubscript{2}; >13 % CO\textsubscript{2}) (Merck) for 7 days.

Strain KSS164-79\textsuperscript{T} showed a Gram-positive staining behaviour and formed visible (diameter about 2 mm) beige colonies within 48 h at 30 °C. The isolate did not grow below 20 °C or above 50 °C. Strain KSS164-79\textsuperscript{T} grew very slowly at 20 and 50 °C and was able to grow at NaCl concentrations of 0.5–7 \% (w/v).

Colonies showed a beige, slightly glistening appearance. Oxidase activity was positive using Oxidase reagent (bio-Mérieux) according to the instructions of the manufacturer. Cells of the strain were non-motile irregular rods (approx. 1 \µm wide and 2–5.5 \µm long). Subterminal spores were observed. The strain grew well on NA, brain heart infusion agar, R2A agar and TSA.

The strain was physiologically/biochemically characterized using the 96-well plate test system [21] and by some additional biochemical tests: production of hydrogen sulphide using the lead acetate paper and triple-sugar-iron methods, indole reaction with Ehrlich’s and Kovacs’ reagents, activity of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, DNase (Oxoid CM321; supplemented with 0.01 % toluidine blue), \(\beta\)-galactosidase (ONPG) and urease on Christensen’s urea agar [22], and hydrolysis of casein, gelatin (plate method) and starch [23]. The biochemical/physiological data are given in Table 1 and in the species description.

Analysis of cellular fatty acid profiles was performed as described previously [24] using an HP 6890 gas chromatograph with Sherlock MIDI software version 2.11 and TSBA peak naming table version 4.1. Prior to fatty acid extraction, the strains were cultured on TSA at 28 °C for 48 h. The results revealed a *Marinicrinis*-typical profile for strain KSS164-79\textsuperscript{T} with the following most abundant fatty acids: anteiso-C\textsubscript{15:0}, iso-C\textsubscript{15:0} and iso-C\textsubscript{16:0}. However, some differences could be identified in comparison with those of the type strain of *M. sediminis* (Table 2).

Quinones and polar lipids were extracted from biomass that was grown on PYE medium (1.0 % peptone from casein, 1 % yeast extract, pH 7.2) at 28 °C and harvested at the stationary growth phase. Analyses of quinones and polar lipids were carried out according to Tindall [25, 26] and Altenburger et al. [27]. The HPLC equipment applied was as described by Stolz et al. [28]. The quinone system was composed of menaquinones MK-7 (98.6 \%) and MK-8 (1.4 \%). In the polar lipid profile (Fig. 2) diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and two unidentified glycolipids were predominant. In addition, minor to moderate amounts of one unidentified glycolipid and four unidentified lipids lacking a functional group were detected. Glycolipids GL2 and GL3 seem to correspond to the two glycolipids of *M. sediminis* NC2-42\textsuperscript{T}. *M. sediminis*
NC2-42<sup>T</sup> lacks DPG, but it is highly likely that the phospholipid reported for *M. sediminis* NC2-42<sup>T</sup> is DPG. A clear difference is that PN reported for *M. sediminis* NC2-42<sup>T</sup> is not present in strain KSS164-79<sup>T</sup> but an additional glycolipid is found.

Based on the genotypic and phenotypic differences from *M. sediminis*, it is clear that strain KSS164-79<sup>T</sup> (=DSM 104943<sup>T</sup>=LMG 30062<sup>T</sup>) represents a novel species. We allocate this novel taxon on the basis of the data reported in this manuscript to the genus *Marinicrinis*, for which the name *Marinicrinis lubricantis* sp. nov. is proposed.

**DESCRIPTION OF MARINICRINIS LUBRICANTIS SP. NOV.**

*Marinicrinis lubricantis* [lu.bri.can’tis. L. v. lubricare to lubricate; N.L. gen. n. lubricantis of/from a (coolant) lubricant]. Cells show a Gram-stain-positive behaviour. They are non-motile, endospore-forming rods, approx. 1 μm in width and 2–5 μm in length. Aerobic, oxidase-positive and catalase-positive. Good growth occurs after 48 h on NA, brain heart infusion agar, TSA and R2A agar (all Oxoid) at 25–45 °C. Unable to grow below 20 °C or above 50 °C. Optimum temperature is 28–37 °C. Cells grow in the presence of 0.5–7.0 % (w/v) NaCl as an additional ingredient of NA [optimum: 1–2 % (w/v) NaCl]. Colonies on NA produce a beige colour and appear circular and slightly translucent. Acid is produced from D-glucose (weakly). No acid is produced from maltose, trehalose, sucrose, L-arabinose, adonitol, D-arabitol, dulcitol, erythritol, i-inositol, lactose, D-mannitol, melibiose, methyl α-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol or D-xylene. Negative for urease activity, hydrolysis of casein, gelatin, starch, DNA and tyrosine, indole production, hydrogen sulphide production, and activity of arginine dihydrolase, lysine decarboxylase, lysine, ornithine, proline, tyrosine, arginine, ornithine, proline, and citrulline, and activity of adenosine deaminase, cyanide, nitrate, and nitrite reductases.
The G+C content of the type strain of M. sediminis was determined by Guo et al. [1].

Table 2. Cellular fatty acid contents (%) of strain KSS-164-79T and M. sediminis NC2-42T

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>iso-C15:0</td>
<td>3.0</td>
<td>4.7</td>
</tr>
<tr>
<td>C16:0</td>
<td>17.9</td>
<td>51.1</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>46.8</td>
<td>30.4</td>
</tr>
<tr>
<td>C16:1ω7c alcohol</td>
<td>15.7</td>
<td>18.0</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>1.3</td>
<td>7.3</td>
</tr>
<tr>
<td>C16:1ω11c</td>
<td>9.7</td>
<td>9.7</td>
</tr>
<tr>
<td>C16:0</td>
<td>7.1</td>
<td>13.0</td>
</tr>
<tr>
<td>iso-C17:0ω10c</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>3.4</td>
<td>2.2</td>
</tr>
<tr>
<td>anteiso-C17:0</td>
<td>7.1</td>
<td>4.7</td>
</tr>
<tr>
<td>Summed feature 4</td>
<td>2.4</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 comprises iso-C17:1ω1 and/or anteiso-C17:1ω9c.

ornithine decarboxylase and β-galactosidase. The following compounds are weakly utilized as a sole source of carbon: D-glucose, L-arabinose and D-xyllose. The following compounds are not utilized as a sole source of carbon: acetate, propionate, N-acetylglactosamine, N-acetylgalactosamine, cellobiose, D-galactose, gluconate, D-mannose, D-fructose, glycerol, D-mannitol, maltitol, L-ribose, D-ribose, salicin, sucrose, adonitol, L-inositol, D-sorbitol, putrescine, cis-aconitate, trans-aconitate, 4-amino butyrate, adipate, azelate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, 2-oxoglutarate, pyruvate, suberate, citrate, mesaconate, L-alanine, β-alanine, L-ornithine, L-phenylalanine, L-serine, L-aspartate, L-histidine, L-leucine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. The chromogenic substrates p-nitrophenyl-α-D-glucopyranoside and p-nitrophenyl-β-D-glucopyranoside (weak) are hydrolysed. The compounds bis-p-nitrophenyl-phosphate, bis-p-nitrophenyl-phosphonate, bis-p-nitrophenylphosphoryl-choline, 2-deoxymyridine-2′-p-nitrophenyl-phosphosphate, L-alanine-p-nitroanilide, γ-L-glutamate-p-nitroanilide, L-proline-p-nitroanilide, p-nitrophenyl-β-D-galactopyranoside, p-nitrophenyl-β-D-xylopyranoside and p-nitrophenyl-β-D-glucuronide are not hydrolysed. The major cellular fatty acids are anteiso-C15:0, iso-C15:0 and iso-C16:0. The quinone system contains predominantly MK-7. DPG, PG, and PE are major components in the polar lipid profile. In addition, moderate to minor amounts of unidentified lipids are present (four lipids lacking a functional group).
The type strain is KSS164-79T (DSM 104943T=LMG 30062T=CCM 8749T=CIP 111345T) isolated from a coolant lubricant in Germany. The genomic DNA G+C content of the type strain is 62.8 mol%.

References


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