



# *Staphylococcus edaphicus* sp. nov., Isolated in Antarctica, Harbors the *mecC* Gene and Genomic Islands with a Suspected Role in Adaptation to Extreme Environments

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**ABSTRACT** Two Gram-stain-positive, coagulase-negative staphylococcal strains were isolated from abiotic sources comprising stone fragments and sandy soil in James Ross Island, Antarctica. Here, we describe properties of a novel species of the genus *Staphylococcus* that has a 16S rRNA gene sequence nearly identical to that of *Staphylococcus saprophyticus*. However, compared to *S. saprophyticus* and the next closest relatives, the new species demonstrates considerable phylogenetic distance at the whole-genome level, with an average nucleotide identity of <85% and inferred DNA-DNA hybridization of <30%. It forms a separate branch in the *S. saprophyticus* phylogenetic clade as confirmed by multilocus sequence analysis of six housekeeping genes, *rpoB*, *hsp60*, *tuf*, *dnaJ*, *gap*, and *sod*. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and key biochemical characteristics allowed these bacteria to be distinguished from their nearest phylogenetic neighbors. In contrast to *S. saprophyticus* subsp. *saprophyticus*, the novel strains are pyrrolidonyl arylamidase and  $\beta$ -glucuronidase positive and  $\beta$ -galactosidase negative, nitrate is reduced, and acid produced aerobically from D-mannose. Whole-genome sequencing of the 2.69-Mb large chromosome revealed the presence of a number of mobile genetic elements, including the 27-kb pseudo-staphylococcus cassette chromosome *mec* of strain P5085<sup>T</sup> ( $\psi$ SCC*mec*<sub>P5085</sub>), harboring the *mecC* gene, two composite phage-inducible chromosomal islands probably essential to adaptation to extreme environments, and one complete and one defective prophage. Both strains are resistant to penicillin G, ampicillin, ceftazidime, methicillin, cefoxitin, and fosfomycin. We hypothesize that antibiotic resistance might represent an evolutionary advantage against beta-lactam producers, which are common in a polar environment. Based on these results, a novel species of the genus *Staphylococcus* is described and named *Staphylococcus edaphicus* sp. nov. The type strain is P5085<sup>T</sup> (= CCM 8730<sup>T</sup> = DSM 104441<sup>T</sup>).

**IMPORTANCE** The description of *Staphylococcus edaphicus* sp. nov. enables the comparison of multidrug-resistant staphylococci from human and veterinary sources evolved in the globalized world to their geographically distant relative from the extreme Antarctic environment. Although this new species was not exposed to the pressure of antibiotic treatment in human or veterinary practice, mobile genetic elements carrying antimicrobial resistance genes were found in the genome. The genomic characteristics presented here elucidate the evolutionary relationships in the *Staphylococcus* genus with a special focus on antimicrobial resistance, pathogenicity, and survival traits. Genes encoded on mobile genetic elements were arranged in unique combinations but retained conserved locations for the integration of mo-

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bile genetic elements. These findings point to enormous plasticity of the staphylococcal pangenome, shaped by horizontal gene transfer. Thus, *S. edaphicus* can act not only as a reservoir of antibiotic resistance in a natural environment but also as a mediator for the spread and evolution of resistance genes.

**KEYWORDS** coagulase-negative staphylococci, methicillin resistance, genomics, polyphasic taxonomy, pathogenicity islands, drug resistance evolution, beta-lactams, chromosomal islands, mobile genetic elements, penicillin-binding proteins, phylogenetic analysis

Members of the genus *Staphylococcus* are widespread in nature and occupy a variety of niches (1). As a result of their ubiquity and adaptability, staphylococci are a major group of bacteria inhabiting the skin, skin glands, and mucous membranes of humans, other mammals, and birds. Most environmental sources also contain small, transient populations of staphylococci, many of which are probably contaminants disseminated by human, other mammal, or bird host carriers (2, 3). Moreover, *Staphylococcus succinus* subsp. *succinus* was isolated from plant and soil inclusions in Dominican amber (4). Recently, *Staphylococcus argensis* was isolated from river sediments (5). A small number of species, such as *Staphylococcus xylosus* and *Staphylococcus sciuri*, have occasionally been isolated from soil, beach sand, and natural waters and also from plants (1, 6, 7). They can grow in habitats containing only an inorganic nitrogen source and, thus, might be capable of a free-living existence.

The ubiquity of *S. xylosus* might be explained by its ability to adapt to different environments. Its capacity to colonize biotic and abiotic surfaces is probably due to its ability to form a biofilm (8) and to genes implicated in ecological fitness (9). It has been considered a nonpathogenic commensal organism (10) and has rarely been reported to be associated with infections (11). On the other hand, *Staphylococcus saprophyticus*, the closest phylogenetic relative of *S. xylosus*, is an important opportunistic pathogen, causing human urinary tract infections, wound infections, and septicemia (12). The host range of *S. saprophyticus* varies from humans to lower mammals and birds (6, 13). It has been isolated from the gastrointestinal tract of both humans and animals, as well as from meat and cheese products, vegetables, and the environment (14).

The aim of this study was to investigate the genomic properties and clarify the taxonomic position of two coagulase-negative staphylococcal isolates belonging to the *S. saprophyticus* phylogenetic clade that could not be identified to the species level by common diagnostic techniques. The isolates are notable due to their origin from the Antarctic environment and resistance to beta-lactam antibiotics encoded by a novel pseudo-staphylococcus cassette chromosome *mec* ( $\psi$ SCC*mec*) element.

## TAXONOMY

*Staphylococcus edaphicus* (e.da'phi.cus. Gr. n. edaphos, soil; N. L. masc. adj. *edaphicus*, belonging to soil).

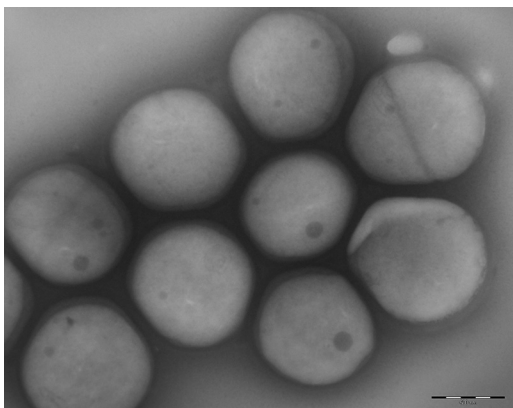
Its cells are Gram stain-positive, spherical or irregular cocci,  $880 \pm 60$  nm in diameter, occurring predominantly in clusters, non-spore forming. Colonies on P agar after 24 h are circular with whole margins, slightly convex, smooth, shiny, whitish, 2 mm in diameter and aerobic. Weak hemolytic activity (production of  $\delta$ -hemolysin) on blood agar. Good growth at 10°C, 42°C, and in the presence of 11% NaCl; weak growth in the presence of 12% NaCl or at 5°C. No growth at 0 or 45°C. No growth in M9 minimal agar. No growth in thioglycolate medium or in the presence of 14% NaCl on a tryptic soy agar (TSA) plate. Catalase, urease, pyrrolidonyl arylamidase, Voges-Proskauer test (acetoin), nitrate reduction, and hydrolysis of Tween 80 positive. Coagulase, clumping factor, hyaluronidase, thermonuclease, oxidase, arginine dihydrolase, ornithine decarboxylase, and arginine arylamidase negative. Susceptible to polymyxin B (300 IU) and furazolidone (100  $\mu$ g), but resistant to bacitracin (0.2 IU) and novobiocin (5  $\mu$ g). Susceptible to lysostaphin (200 mg liter<sup>-1</sup>) and resistant to lysozyme (400 mg liter<sup>-1</sup>). Hydrolysis of esculin, DNA, and gelatin negative. Butyrate esterase, caprylate esterase,

myristate lipase (weak),  $\beta$ -glucuronidase, acid phosphatase (weak), alkaline phosphatase, and naphthol-AS-BI-phosphohydrolase (7-bromo-3-hydroxy-2-naphthoic-*o*-anisidide) activities are present, but not leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin,  $\alpha$ -chymotrypsin,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase, *N*-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -mannosidase, and  $\alpha$ -fucosidase activities. Acid is produced from glycerol, ribose, galactose, *D*-glucose, fructose, mannose, mannitol, sorbitol (weak), *N*-acetylglucosamine, salicin, maltose sucrose, trehalose, and  $\beta$ -gentiobiose, but not from erythritol, *D*-arabinose, *L*-arabinose, *D*-xylose, *L*-xylose, adonitol,  $\beta$ -methyl-*D*-xyloside, sorbose, rhamnose, dulcitol, inositol,  $\alpha$ -methyl-*D*-mannoside,  $\alpha$ -methyl-*D*-glucoside, amygdaline, arbutine, cellobiose, lactose, melibiose, inulin, melezitose, *D*-raffinose, glycogen, xylitol, turanose, *D*-lyxose, *D*-tagatose, *D*-fucose, *L*-fucose, *D*-arabitol, *L*-arabitol, gluconate, 2 keto-gluconate, or 5 keto-gluconate. The test result was strain dependent for acid production from starch (CCM 8730<sup>T</sup> positive). *S. edaphicus* had the ability to utilize the following carbon sources via respiration as determined by the Biolog GEN III MicroPlate test panel: dextrin, *D*-maltose, *D*-trehalose, gentiobiose, sucrose, *D*-turanose,  $\beta$ -methyl-*D*-glucoside, *D*-salicin, *N*-acetyl- $\beta$ -*D*-mannosamine, *N*-acetyl neuraminic acid,  $\alpha$ -*D*-glucose, mannose, *D*-fructose, *D*-galactose, inosine, *D*-mannitol, *D*-arabitol, glycerol, glycy-L-proline, *L*-alanine, *L*-arginine, *L*-aspartic acid, *L*-glutamic acid, *L*-histidine, *L*-serine, pectin, *D*-gluconic acid, *D*-glucuronic acid, glucuronamide, *L*-lactic acid, Tween 40, acetoacetic acid, acetic acid, and formic acid. The negative utilization tests were *D*-cellobiose, stachyose, *D*-raffinose,  $\alpha$ -*D*-lactose, *D*-melibiose, *N*-acetyl-*D*-galactosamine, 3-methyl glucose, *L*-fucose, *D*-sorbitol, *myo*-inositol, *D*-glucose-6-phosphate, *D*-aspartic acid, *D*-serine, gelatin, *L*-pyroglutamic acid, *D*-galacturonic acid, *D*-galactonic acid lactone, mucic acid, quinic acid, *D*-saccharic acid, *p*-hydroxy phenylacetic acid, *D*-malic acid, bromo-succinic acid,  $\gamma$ -amino-butyric acid,  $\alpha$ -hydroxy-butyric acid,  $\beta$ -hydroxy-*D,L*-butyric acid,  $\alpha$ -keto butyric acid, and propionic acid. The strain-dependent tests were *N*-acetyl-*D*-glucosamine, *L*-rhamnose, *D*-serine, *D*-fructose-6-phosphate, rifamycin SV, guanidine HCl, methyl pyruvate, *D*-lactic acid methyl ester, citric acid,  $\alpha$ -keto glutaric acid, and *L*-malic acid (CCM 8730<sup>T</sup> positive in all tests), and *D*-fucose (CCM 8730<sup>T</sup> negative). Chemical sensitivity assays, performed with the Biolog GEN III MicroPlate test panel, exhibited positive growth at pH 6 and in the presence of 1% NaCl, 4% NaCl, 8% NaCl, 1% sodium lactate, nalidixic acid, lithium chloride, potassium tellurite, aztreonam, and sodium butyrate. Negative growth was exhibited at pH 5 and in the presence of fusidic acid, troleandomycin, minocycline, lincomycin, Niaproof 4, vancomycin, tetrazolium violet, tetrazolium blue, and sodium bromate. The tested strains were susceptible to cephalothin, ciprofloxacin, clindamycin, erythromycin, gentamicin, chloramphenicol, imipenem, kanamycin, neomycin, cotrimoxazole, tetracycline, and vancomycin and resistant to penicillin G, ampicillin, ceftazidime, methicillin, cefoxitin, and fosfomycin.

The type strain CCM 8730<sup>T</sup> (= P5085<sup>T</sup> = DSM 104441<sup>T</sup>) was retrieved in January 2013 from fragments of black porous stone at a hill above Cape Lachman, James Ross Island, Antarctica. It had G+C content of 33.3 mol% calculated from whole genomic sequence. The majority of the characteristics of the type strain are in agreement with the general species description; in addition, the CCM 8730<sup>T</sup> strain produces acid from starch.

## RESULTS AND DISCUSSION

Two isolates were obtained as a by-product in the framework of a project monitoring psychrotolerant bacteria of the phylum *Bacteroidetes* from abiotic sources in James Ross Island, Antarctica (CzechPolar2 project). Gram staining and phase-contrast and transmission electron microscopy showed that the cells are Gram-positive spherical cocci with the typical appearance of staphylococci, without flagella (Fig. 1). Strain P5085<sup>T</sup> (= CCM 8730<sup>T</sup>) was isolated from stone fragments sampled from a hill above Cape Lachman (GPS coordinates 63°46'58"S 57°47'11"W), and strain P5191 (= CCM 8731) was isolated from sandy soil in the Panorama Pass locality (GPS coordinates 63°48'51"S 57°50'45"W). The phenotypic characteristics of the new isolates are summarized in the species description above.



**FIG 1** Transmission electron microscopy of type strain *Staphylococcus edaphicus* CCM 8730<sup>T</sup>, performed with Morgagni 268D Philips (FEI Company, USA) electron microscope. Negative staining with 2% ammonium molybdate. Bar represents 500 nm (original magnification,  $\times 10,000$ ).

The preliminary identification by sequencing of the 16S rRNA gene assigned both isolates to the *Staphylococcus saprophyticus* species group defined by Takahashi et al. (15). The whole genomic sequence of the *S. saprophyticus* type strain (16) even exhibited indistinguishability from that of the strain CCM 8730<sup>T</sup> 16S rRNA gene in two *rrn* operons, but there was a 1-residue difference in each of the three other *rrn* operons (positions 190, 278, and 457). However, the phenotypic results obtained, which are listed in the species description given above, did not allow for the classification of isolates into any known staphylococcal species. The key characteristics differentiating the novel taxon represented by strain CCM 8730<sup>T</sup> from the phylogenetically closely related species are shown in Table 1.

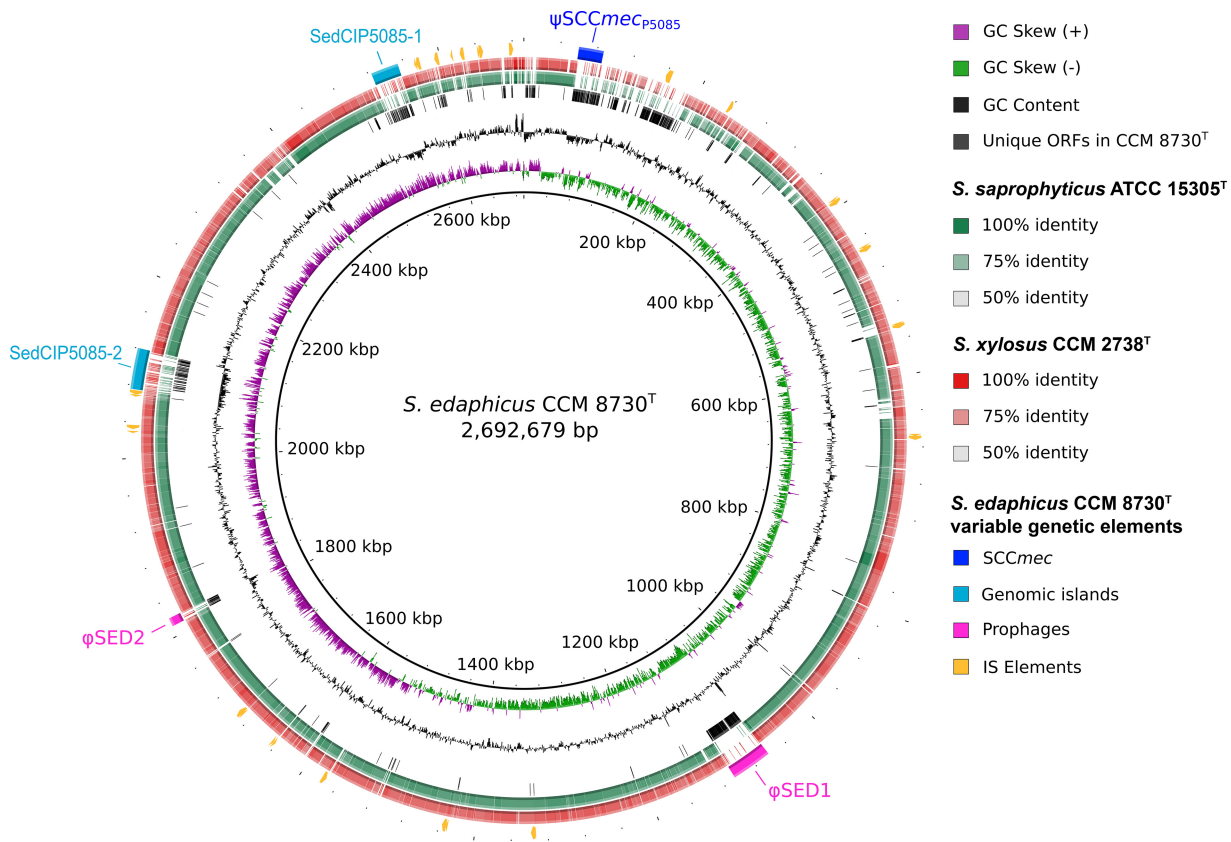
The strains were analyzed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and yielded profiles containing signals in the mass range of 2 to 15 kDa. They did not exhibit significant similarity (BioTyper log score of  $>1.7$ ) to any of the reference entries belonging to *Staphylococcus* species with valid names included in BioTyper database version 5989 (Bruker Daltonics) and proved *S. saprophyticus* to be the closest relative (see Fig. S1 in the supplemental material).

Three DNA fingerprinting techniques were used to show the differences between the isolates and similarities to related taxa. Repetitive sequence-based PCR (rep-PCR)

**TABLE 1** Differentiation of *Staphylococcus edaphicus* sp. nov. from the phylogenetically closest *Staphylococcus* spp.

| Test                           | Result obtained for indicated type strain in this study/from species description <sup>a</sup> : |   |  |   |   |  |   |  |
|--------------------------------|---|---|--|---|---|--|---|--|
|                                | <i>S. edaphicus</i> CCM 8730 <sup>T</sup>   | <i>S. xylosus</i> CCM 2738 <sup>T</sup> | <i>S. saprophyticus</i> subsp. <i>saprophyticus</i> CCM 883 <sup>T</sup> | <i>S. saprophyticus</i> subsp. <i>bovis</i> CCM 4410 <sup>T</sup> | <i>S. succinus</i> subsp. <i>succinus</i> CCM 7157 <sup>T</sup> | <i>S. succinus</i> subsp. <i>casei</i> CCM 7194 <sup>T</sup> | <i>S. equorum</i> subsp. <i>equorum</i> CCM 3832 <sup>T</sup> | <i>S. equorum</i> subsp. <i>linens</i> CCM 7278 <sup>T</sup> |
| Nitrate reduction              | +   | +/D                                     | -/-  | +/+   | -/-   | +/+  | +/+   | +/+  |
| Pyrrolidonyl arylamidase       | +   | +/D                                     | -/-  | W/+   | -/-   | -/-  | -/-   | -/-  |
| Voges-Proskauer test (acetoin) | +   | -/D                                     | +/+  | +/D   | -/-   | -/-  | -/-   | -/-  |
| Esculine hydrolysis            | -   | -/D                                     | -/-  | -/-   | +/+   | +/+  | -/D   | -/-  |
| $\beta$ -Glucosidase           | -   | +/+                                     | -/D  | -/D   | +/-NT   | +/-NT  | +/-NT   | -/-NT  |
| $\beta$ -Glucuronidase         | +   | +/+                                     | -/-  | -/-   | +/+   | +/+  | W/+   | +/+  |
| $\beta$ -Galactosidase         | -   | +/+                                     | +/+  | +/D   | +/+   | +/+  | W/D   | -/-  |
| Acid from mannose              | +   | +/+                                     | -/-  | -/-   | +/D   | +/+  | -/+   | +/+  |

<sup>a</sup>Species description data are from Schleifer and Bell (17). +, positive reaction; W, weakly positive reaction; -, negative reaction; D, variable reaction; NT, not tested.

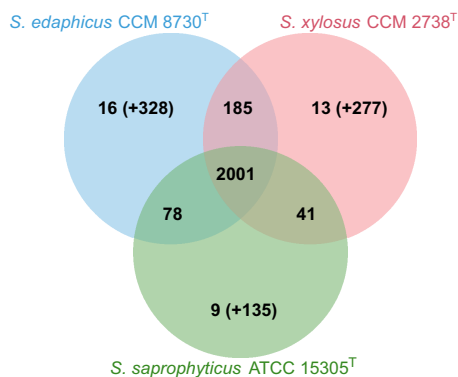


**FIG 2** Circular display of *Staphylococcus edaphicus* CCM 8730<sup>T</sup> genome (GenBank accession number [MRZN000000000](#)) compared to the *Staphylococcus saprophyticus* ATCC 15305<sup>T</sup> (GenBank accession number [NC\\_007350](#)) and *Staphylococcus xylosus* CCM 2738<sup>T</sup> (GenBank accession number [MRZO000000000](#)) genomes. The picture shows (from inner to outer circle) GC skew, mol% G+C, unique regions in CCM 8730<sup>T</sup> genome based on BLASTn analysis, and orthologous regions in *S. saprophyticus* ATCC 15305<sup>T</sup> genome and *S. xylosus* CCM 2738<sup>T</sup> genome based on BLASTn analysis. Outer circle depicts locations of accessory elements in *S. edaphicus* CCM 8730<sup>T</sup> genome.

fingerprinting using the (GTG)<sub>5</sub> primer (Fig. S2) and automated ribotyping with the EcoRI restriction enzyme (Fig. S3) showed high genetic similarity between strains CCM 8730<sup>T</sup> and CCM 8731, because they gave visually identical fingerprints. At the same time, both DNA fingerprinting techniques also clearly differentiated the strains analyzed from the type strains representing the phylogenetically close *Staphylococcus* spp. Strains CCM 8730<sup>T</sup> and CCM 8731 were also undistinguishable by SmaI macrorestriction analysis resolved by pulsed-field gel electrophoresis (Fig. S4).

The fatty acid content of strain CCM 8730<sup>T</sup> corresponded with those of phylogenetically related species (Table S1). The fatty acid compositions of strains CCM 8730<sup>T</sup> and CCM 8731 were found to be very similar. The major fatty acids (>10%) were C<sub>15:0</sub> anteiso (51.4%), C<sub>15:0</sub> iso (13.3%), and C<sub>17:0</sub> anteiso (12.9%). C<sub>15:0</sub> and C<sub>15:0</sub> anteiso were found to be major fatty acids in all members of the *S. saprophyticus* species group. The amount of C<sub>17:0</sub> anteiso differed slightly between members of this group, only reaching the highest value of 10% in strains CCM 8730<sup>T</sup> and CCM 8731 and both subspecies of *S. saprophyticus*. The overall content of other fatty acids was found to be comparable in this clade, with the exception of the *S. succinus* subsp. *succinus* type strain, where C<sub>13:0</sub> iso and C<sub>13:0</sub> anteiso were found to be predominant as well (4).

The genome of strain CCM 8730<sup>T</sup> was shotgun sequenced, and the contigs were compared with *S. saprophyticus* ATCC 15305<sup>T</sup> and *S. xylosus* CCM 2738<sup>T</sup> whole-genome sequences (Fig. 2). The size of the draft genome of CCM 8730<sup>T</sup> is 2.69 Mb, comprised of 45 contigs of >500 bp ( $N_{50}$  = 99,210 bp; 309× mean coverage) with an average G+C content of 33.3 mol%. The sequencing and assembly statistics are shown in Table S2. A total of 2,645 genes were predicted. Comparative analysis of the genes with the

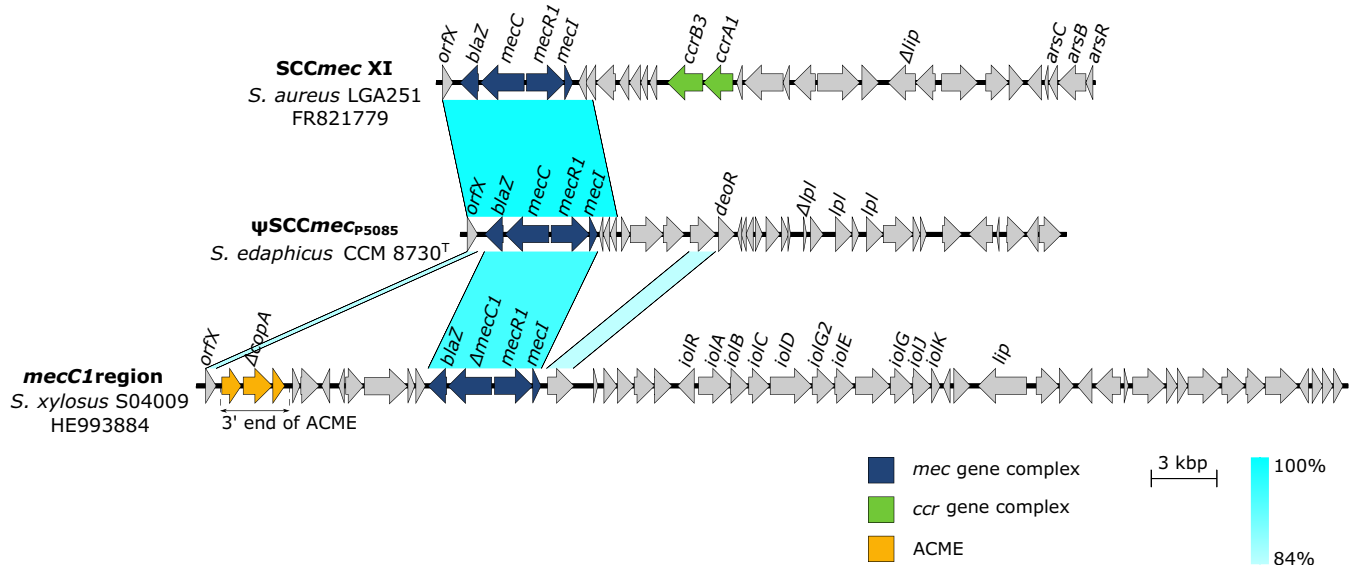


**FIG 3** Venn diagram showing orthologous gene clusters for strain *Staphylococcus edaphicus* CCM 8730<sup>T</sup> (GenBank accession number [MRZN00000000](#)), *Staphylococcus saprophyticus* ATCC 15305<sup>T</sup> (GenBank accession number [NC\\_007350](#)), and *Staphylococcus xylosus* CCM 2738<sup>T</sup> (GenBank accession number [MRZO00000000](#)). The number of singletons specific for each genome is shown in parentheses.

closest relatives identified 2,280 gene clusters and 328 singletons. The majority of the clusters, up to 2,264, are orthologous and shared with either *S. xylosus* or *S. saprophyticus* (Fig. 3). There are 16 unique gene clusters, including 39 open reading frames (ORFs) and 328 singletons specific to the CCM 8730<sup>T</sup> strain, localized mainly on variable genetic elements, such as the pseudo-*Staphylococcus* cassette chromosome *mec* ( $\psi$ SCC*mec*), two composite phage-inducible chromosomal islands (PICIs), and one complete and one defective prophage (Fig. 2). High heterogeneity in the gene composition compared to those of related species was detected in the region following the  $\psi$ SCC*mec*. Genes for a type II restriction modification system similar to Sau3AI were found, as well as a thiamine biosynthesis operon, two predicted protein-coding genes with an LPXTG motif, and genes for a putative PIs cell antiadhesin and cardiolipin synthase. The *pls* gene has been associated with virulence (18), and it has been found adjacent to SCCs present in other staphylococci (19). Cardiolipin synthesis can enhance cell survival under acid or salinity stress conditions (20, 21), and mutations in this gene enhance resistance to daptomycin (22).

The 27,158-bp  $\psi$ SCC*mec* element of strain CCM 8730<sup>T</sup> ( $\psi$ SCC*mec*<sub>P5085</sub>), bordered by imperfectly matched 27-bp direct repeats, CCGCATCACTTGTGATA(C/T)GCTTC(C/T)CCC, was identified inserted between the *rlmH* gene, encoding rRNA-methyltransferase, and the gene for putative threonyl-tRNA synthetase. This  $\psi$ SCC*mec* contains *mec* gene complex class E (*blaZ-mecC-mecR1-mecI*), reflecting the corresponding International Working Group on the Staphylococcal Cassette Chromosome elements (IWG-SCC) designation (23), and it lacks recombinase genes (*ccr*) or their homologues (Fig. 4). The *mecC* gene shares 99% nucleotide identity with that of *Staphylococcus aureus* strain LGA251 (24) and 93% nucleotide identity with the *mecC1* gene of *S. xylosus* strain S04009 (25) and with the *mecC2* of *S. saprophyticus* strain 210 (26). Apart from human and livestock staphylococcal isolates (24), the *mecC* gene was previously identified in isolates from wildlife, including birds and mammals (27–29), and in waste and river waters (30, 31), but to our knowledge, this is the first time the *mecC* gene was identified in an isolate from soil.

Besides the *mecC* gene, very few genes were conserved among  $\psi$ SCC*mec*<sub>P5085</sub> and related SCCs; these were localized mainly in the *mec* gene complex (Fig. 4). This implies that SCC elements might serve for foreign DNA integration and exchange, as suggested for other genomic islands. Downstream from the *mec* gene complex of  $\psi$ SCC*mec*<sub>P5085</sub>, there are 28 predicted ORFs encoding kinase, hydrolase, oxidoreductase, transcriptional regulator, and several hypothetical proteins, whose homologs were identified predominantly in coagulase-negative staphylococcal species. Furthermore, in  $\psi$ SCC*mec*<sub>P5085</sub>, one truncated and other, intact *lpl* genes coding for tandem lipoprotein-like proteins with amino acid similarities of over 89% to those of *S. epidermidis* were identified.



**FIG 4** Comparison of genetic structure of SCCmec element type XI in *Staphylococcus aureus* LGA251 (GenBank accession number FR821779),  $\psi$ SCCmec<sub>P5085</sub> in *Staphylococcus edaphicus* CCM 8730<sup>T</sup>, and *mecC1* region in *Staphylococcus xylosus* S04009 (GenBank accession number HE993884). Arrows indicate ORFs and their orientation on the genome. ACME, arginine catabolic mobile element.

Lipoprotein-like proteins are usually encoded by  $\nu$ Sa $\alpha$  genomic islands (32), and under certain nutrient limitations and environmental conditions, they may be crucial for ion and nutrient transport, allowing growth and survival (33).

Noticeably lower G+C content, below 29 mol%, clearly leads to the identification of two genomic islands (Fig. 2). The genomic analysis revealed a high resemblance of both islands to the *Staphylococcus aureus* pathogenicity islands (SaPIs), the prototypical members of the PICIs (34). The islands of strain CCM 8730<sup>T</sup> carry genes necessary for the transfer and autonomous replication of the element, namely, integrase, primase, small subunit of the phage terminase complex, and transcriptional regulators, and were thus classified as PICIs. However, the two genomic islands are much longer than SaPIs. The first genomic island, designated SedCIP5085-1 and found in CCM 8730<sup>T</sup>, is approximately 45 kb long with more than 70 predicted ORFs. The island is integrated into the transfer-messenger RNA (tmRNA) gene within the attachment sequence site TCCCGC CGTCTCCA(T/C)TATAGAGTCTGCAACC(C/-)AT(T/-)GTGGTTGTGGGCTTTTATTTTTG that corresponds to the attachment site of SaPI<sub>m</sub>/ $\nu$ Sa3 (35). Two additional copies of the *att* site were found in this island, thus dividing the element into three parts. The first part is 17.9 kb long and resembles canonical SaPI ( $\nu$ Sa3) in its gene composition (36). It encodes integrase and contains genes necessary for the transfer, the *fosB* fosfomycin resistance gene homologue (91% amino acid identity with the fosfomycin resistance protein family of *S. saprophyticus*), and several ORFs of unknown function. So far only plasmid-borne fosfomycin resistance genes have been detected in *Staphylococcus* spp., though the possible localization of *fosB* on other mobile genetic elements has been discussed (37). The second part of SedCIP5085-1, 15.4 kb long, is comprised of phage- and SaPI ( $\nu$ Sa2)-related genes, but no integrase has been found. It also carries a remnant of the *fosB* gene and other genes of unknown function. The last part of SedCIP5085-1 is 11.7 kb with an indistinct left border, because the bracketing sequence of the *att* site is absent. This part consists of putative genes encoding resistance to arsenic, cadmium, and bleomycin, hypothetical proteins, and a few phage-related genes, such as a transcriptional regulator from the Cro/cI family, which is known to control the switch between the lytic and lysogenic cycle of bacteriophages. The mosaic organization of islands suggests that more than one integration event occurred in the locus.

The second genomic island, designated SedCIP5085-2, is about 30 kb and

contains more than 45 predicted ORFs. The attachment site TATATTATCCCACT CGAT of SedCIP5085-2 is located within the glutamine-hydrolyzing GMP synthase gene, which matches the attachment site for SaPIbov1/ $\nu$ Sa2 (35, 38). This genomic island probably enhances the resistance and endurance of the host, because it codes for transporters and transcriptional regulators involved in antibiotic resistance, oxidative stress responses, and the synthesis of virulence factors. It also contains an additional copy of the *cspC* gene, coding for cold shock protein C, which has been found to be expressed strongly after antibiotic, arsenate, and peroxide induction (39). In addition, many insertion sequences from the *IS3* and *IS1182* families were found in this genomic island.

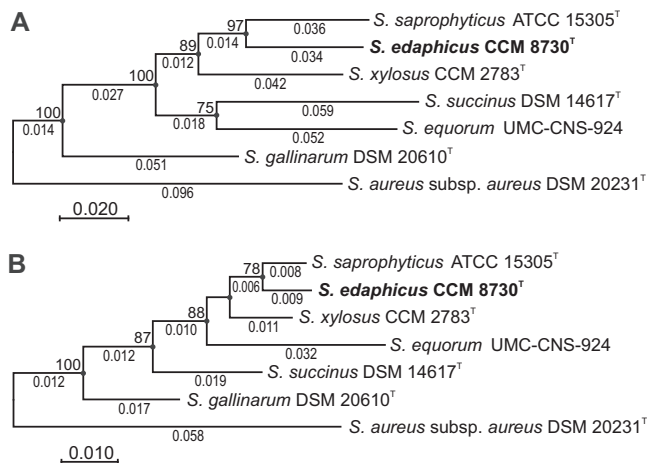
A prophage designated  $\phi$ SED1 ( $\nu$ B\_SedS-P5085-1), located downstream from the tRNA<sup>Leu</sup> gene, was identified. The prophage is 44,424 bp long with an average G+C content of 33.36 mol%, which is comparable to the G+C content of the CCM 8730<sup>T</sup> strain. The direct repeats of the phage *att* site ATCCCGACCACCGGTAT flank 67 ORFs encoding essential phage genes, which cluster together into functional modules corresponding to those in staphylococcal *Siphoviridae* (40). The lysogeny module starts with phage integrase, which has a tyrosine recombinase XerD domain, AP2-like DNA binding domain, and N-terminal S-adenosylmethionine (SAM)-like domain. There are genes in the DNA replication and transcription regulation modules encoding a DNA primase, helicase, HNH endonuclease domain protein, and putative single-stranded binding protein. The DNA packaging and head and tail morphogenesis modules share 78% nucleotide identity with those of an StB20 phage infecting *Staphylococcus capitis* (41). The holin and amidase from the lysis module exhibit high amino acid identities to *S. saprophyticus* prophage holins and amidases, 95 to 99% and 77 to 85%, respectively. The last gene downstream from the lysis module encodes a glycosyltransferase family 2 protein. The prophage does not carry any genes encoding tRNA or virulence factors. However, another, incomplete  $\phi$ SED2 prophage, 9,241 bp long, bordered by TAGTGT CCTGGGAGG direct repeats, was found in the CCM 8730<sup>T</sup> genome. This prophage has an average G+C content of 30.73 mol%, lower than that of its host.

Although the strain CCM 8730<sup>T</sup> comes from a geographically isolated polar environment, it carries genes for a surprisingly high number of antimicrobial resistance factors. Resistance to beta-lactam antibiotics is determined by the class E *mec* complex, coding for a beta-lactamase and alternative penicillin-binding MecC protein that is more stable and active at lower temperatures than the MecA protein (42). This might represent an evolutionary advantage against beta-lactam producers, which are common in a polar environment. McRae et al. (43) documented that penicillia are major decomposers and represent an important element of the terrestrial nutrient cycle of Antarctica, particularly since a limited range of other microbial taxa can tolerate the harsh Antarctic climate. Penicillia producing beta-lactams, such as *Penicillium chrysogenum*, have been isolated from sediments of ponds (44), permafrost (45), subglacial ice (46), and oligotrophic and ornithogenic soil in Antarctica (43, 47). Several studies tested for extrolite production and confirmed the antibacterial activity of *Penicillium* species strains isolated in Antarctica (44, 46, 48). This is consistent with the fact that both localities where the strains were isolated are apical parts of the landscape often visited by skua birds in austral summer, and there is a high probability they are colonized by fungi.

The 16S rRNA analysis had limited discriminatory power for identifying the staphylococcal isolates examined, and therefore, their phylogenetic position was assessed using the concatenated multilocus sequence data of six housekeeping genes, *rpoB*, *hsp60*, *dnaJ*, *tuf*, *gap* and *sod*, that are commonly used in phylogenetic studies of *Staphylococcus*, as well as the amino acid sequences of their protein products. The neighbor-joining and maximum-likelihood phylogenetic trees for the six housekeeping genes were very similar and confirmed that the isolates under study represented a well-delineated group within the *S. saprophyticus* phylogenetic clade, clearly separated from known species (Fig. 5).

To evaluate the intergenomic distances between the genome sequences of strain





**FIG 5** Unrooted maximum-likelihood trees based on concatenated sequences from six multiple loci showing the phylogenetic position of *Staphylococcus edaphicus* sp. nov. The sections of gene sequences used and their protein products correspond to the following gene coordinates of *Staphylococcus aureus* subsp. *aureus* NCTC 8325: 949 to 2748 for *rpoB*, 16 to 1573 for *hsp60*, 1 to 1136 for *dnaJ*, 1 to 1185 for *tuf*, 4 to 1004 for *gap*, and 1 to 597 for the *sod* gene. Bootstrap probability values (percentages of 500 tree replications) greater than 70% are shown at branch points. The evolutionary distances are given as the number of substitutions per site (below the branches). Filled circles indicate that the corresponding nodes are also obtained in the neighbor-joining tree. (A) Evolutionary history inferred from nucleotide sequences by using the maximum-likelihood method based on the Tamura-Nei model. There were a total of 7,175 positions in the final data set. (B) Evolutionary history inferred from amino acid sequences by using the maximum-likelihood method based on the Poisson correction model. There were a total of 2,391 positions in the final data set.

CCM 8730<sup>T</sup> and reference type strains belonging to the phylogenetically closest *Staphylococcus* spp., the average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values were determined (Table S3). The calculated ANI and dDDH values are well below the thresholds of 95 to 96% and 70%, respectively, for species delineation (49). This confirms that strain CCM 8730<sup>T</sup> represents a distinct *Staphylococcus* species.

The data from this study demonstrate that although they are highly similar to *S. saprophyticus*, the two isolates represented by strain CCM 8730<sup>T</sup> belong to a new taxon that can be distinguished both genotypically and phenotypically from established species of the genus *Staphylococcus*. We suggest classifying these isolates as a novel species for which the name *Staphylococcus edaphicus* is proposed, with the strain P5085<sup>T</sup> (= CCM 8730<sup>T</sup> = DSM 104441<sup>T</sup>) as the type strain.

## MATERIALS AND METHODS

**Sampling and cultivation.** Sampling was carried out by dispersing approximately 1 g of stone fragments or soil in 5 ml of sterile saline solution; 100  $\mu$ l of the suspension was spread on an R2A agar plate (Oxoid) and cultivated at 15°C for 5 days. Subsequently, individual morphologically diverse colonies were picked up and purified by repeated streaking on R2A medium at 15°C, and the resulting pure cultures were maintained at -70°C until analyzed. Two Gram stain-positive isolates were obtained. Additional cultivation of these isolates for biotyping and genotyping was performed with cells growing on TSA (Oxoid). The type strains of the closely related staphylococcal taxa were obtained from the Czech Collection of Microorganisms ([www.sci.muni.cz/ccm](http://www.sci.muni.cz/ccm)) and were simultaneously investigated in all tests.

**Phenotypic characterization.** Extensive phenotypic characterization using the commercial kits API 50CH, API ID 32 Staph, and API ZYM (bioMérieux), phenotypic fingerprinting using the Biolog system with the identification test panel GEN III MicroPlate (Biolog), and conventional biochemical, physiological, and growth tests relevant for the genus *Staphylococcus* were done as described previously (50, 51). All phenotypic data presented were from two replicates using commercial kits and three replicates using conventional tests.

**Transmission electron microscopy.** The surface of the plate containing the bacterial culture was washed off and the bacteria resuspended in distilled water. A 200-mesh Formvar-coated grid was placed on a drop of the suspension for 20 min. Bacterial cells located on the grid were negative stained with 2% ammonium molybdate and treated with UV light. A Morgagni 268D Philips (FEI Company) transmission electron microscope was used to visualize bacterial cells.

**Antimicrobial susceptibility tests.** The antibiotic resistance pattern was tested by the disc diffusion method on Mueller-Hinton agar (Oxoid). Commercially prepared plates were dried at 35°C for 15 min before use. The direct colony suspension method was used to make a suspension of the organism in saline to the density of a McFarland 0.5 turbidity standard. The antibiotic discs were applied by a disc dispenser (Oxoid) within 15 min of inoculation. Sixteen discs generally used for Gram-positive cocci were applied: ampicillin (10 µg), ceftazidime (10 µg), ceftiofur (30 µg), cephalothin (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), fosfomicin (50 µg), gentamicin (10 µg), chloramphenicol (30 µg), imipenem (10 µg), kanamycin (30 µg), neomycin (10 µg), novobiocin (5 µg), oxacillin (1 µg), penicillin G (1 IU), co-trimoxazole (25 µg), tetracycline (30 µg), and vancomycin (30 µg). The incubation conditions were 16 to 20 h at 36°C in air. The inhibition zone diameters were measured with a caliper. EUCAST/CLSI standards for reading the inhibition zone diameter and interpreting susceptibility to antibiotics were strictly followed (52, 53).

**MALDI-TOF MS.** For matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), cultures were grown on Columbia blood agar (Oxoid) and transferred with a bamboo toothpick onto an MSP 96 polished steel target plate (Bruker Daltonics). The samples were covered with 1 µl of matrix solution, comprised of a saturated solution of  $\alpha$ -cyano-4-hydroxy-cinnamic acid (Bruker) in 50% acetonitrile (Sigma-Aldrich) and 2.5% trifluoroacetic acid (VWR). Each strain was spotted in 10 replicates. Samples were processed using a MALDI-TOF mass spectrometer (Microflex LT; Bruker Daltonics). The mass range of spectra analyzed was 2,000 to 20,000 *m/z*. Each spectrum was obtained after 300 shots in an automatic acquisition mode. Mass spectra were processed using the software Flex Analysis (version 3.4; Bruker Daltonics). The MALDI-TOF MS-based dendrogram was generated using the software BioTyper (version 3.0; Bruker Daltonics). Signals present in at least 70% of analyses for one sample were transformed into main spectrum projections (MSPs). Afterwards, Pearson coefficients based on the MSPs' similarities were calculated and the dendrogram was generated using the unweighted average linkage algorithm.

**FAME analysis.** The fatty acid methyl ester (FAME) analysis was performed with cells growing on BBL Trypticase soy agar plates (Becton Dickinson) at 28°C  $\pm$  1°C for 24 h. The extraction of fatty acid methyl esters was performed according to the standard protocol of the Sherlock Microbial Identification System (54). Bacterial biomass was harvested from the third sector of growth on agar plates, where the cultures reached the late-exponential stage of growth according to the four-quadrant streak method (54). Cellular fatty acid extracts were analyzed with an Agilent 7890B gas chromatograph using the rapid Sherlock microbial identification system (MIS) (version 6.2B, MIDI database RTSBA 6.21; MIDI Inc.). The quantities of individual fatty acids are given as percentages of all named fatty acids. Strains CCM 8730<sup>T</sup> and CCM 8731<sup>T</sup> and the reference strains were tested three times to evaluate the reproducibility of the method.

**Genotypic analysis by fingerprinting techniques.** Rep-PCR fingerprinting with the (GT)<sub>5</sub> primer was performed as described previously (55). The automatic ribotyping was performed using a RiboPrinter microbial characterization system (DuPont Qualicon) in accordance with the manufacturer's instructions. Numerical analysis of the fingerprints obtained and dendrogram construction were done using the software BioNumerics 7.6 (Applied Maths). The ribotype patterns were imported into the software BioNumerics using the load samples import script provided by the manufacturer. Pulsed-field gel electrophoresis (PFGE) using SmaI macrorestriction pattern analysis was performed as previously described (56).

**Genome sequencing and bioinformatics analysis.** Genomic DNA was extracted using a high pure PCR template preparation kit (Roche) according to the manufacturer's recommendations. The concentration of extracted DNA was estimated with a Qubit 2.0 fluorometer using a Qubit double-stranded DNA (dsDNA) broad-range (BR) assay kit (Invitrogen). The partial 16S rRNA gene was sequenced as described previously (57). Whole-genome shotgun (WGS) sequencing was performed using an Ion Torrent Personal Genome Machine (PGM). The purified genomic DNA was used for preparing a 400-bp sequencing library with an Ion plus fragment library kit (Thermo Fisher Scientific). The sample was loaded onto an Ion 316 chip, version 2, and sequenced using an Ion PGM Hi-Q view sequencing kit (Thermo Fisher Scientific). Quality trimming of the reads was performed with the Ion Torrent Suite software (version 5.0.4) with default settings. The assembly computation and error correction were performed using Assembler SPAdes 3.9 (58) with default parameters for Ion Torrent data and *k*-mer settings of 21, 33, 55, 77, 99, and 127. Contigs were then reordered according to the *S. saprophyticus* ATCC 15305<sup>T</sup> reference genome (GenBank accession number [NC\\_007350](#)) (16) using MauveContigMover (59). Ordering was evaluated using the assembly graph visualized in Bandage (60).

Sequences were manipulated and inspected in the cross-platform bioinformatics software Ugene version 1.23.1 (61). For primary analysis, the genome was annotated using RAST (62). Gene content was further examined by BLASTp (63), ISFinder (64), InterProScan (65), CD-Search (66), and OrthoVenn (67). The complete 16S rRNA gene sequence was extracted from WGS data using RNAmmer version 1.2 (68). Phylogenetic analysis from multilocus sequence data was performed using the software MEGA version 7 (69).

**DNA homology studies.** To calculate the ANI value, the OrthoANI algorithm implemented on the EzBioCloud server (<http://www.ezbiocloud.net/tools/ani>) was used (70). The dDDH values were calculated using the web-based genome-to-genome distance calculator (GGDC) version 2.1 (71), and the recommended formula 2 was taken into account to interpret the results. Whole-genome sequences of related staphylococcal species were obtained from the NCBI database (72–75).

**Accession number(s).** The GenBank accession number for the 16S rRNA genes of isolate CCM 8730<sup>T</sup> is [KY315825](#). The data from WGS of strains *Staphylococcus edaphicus* CCM 8730<sup>T</sup> and *Staphylococcus xylosus* CCM 2738<sup>T</sup> were recorded in the GenBank WGS project under the accession numbers [MRZN00000000](#) and [MRZO00000000](#).

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AEM.01746-17>.

**SUPPLEMENTAL FILE 1**, PDF file, 0.6 MB.

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I.S. isolated and preliminarily identified the strains. I.S., J.K., and P.P. performed phenotypic characterization. Whole-genome sequencing was performed by V.V., and V.K. and A.I. analyzed the data. Phylogenetic analyses were performed by I.M. and V.V. P.S. performed genotyping by fingerprinting techniques and analyzed the data. S.K. performed FAME analysis. P.P. and L.K. performed MALDI-TOF identification and analyzed the data. R.P. and I.S. designed the study, R.P., I.S., A.I., V.V., I.M., and J.D. wrote the manuscript. All the authors approved the final manuscript.

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