'Rothia nasisuis' sp. nov., *'Dermabacter porcinasus'* sp. nov., *'Propionibacterium westphaliense'* sp. nov. and *'Tessaracoccus nasisuum'* sp. nov., isolated from porcine nasal swabs in the Münster region, Germany

A. Schlattmann, K. von Lützau, U. Kaspar and K. Becker

Institute of Medical Microbiology, University Hospital Münster, Münster, Germany

Abstract

We report the core features of *'Rothia nasisuis'* strain 1a5R-CH16 sp. nov., *'Dermabacter porcinasus'* strain 2a11-BL09 sp. nov., *'Propionibacterium westphaliense'* strain 1a7I-CH12an sp. nov. and *'Tessaracoccus nasisuum'* strain 1a6R-CH11an sp. nov. In 2016, these isolates were cultured from porcine nasal swabs taken from healthy pigs from farms in the region around Münster, Germany.

Keywords: *Dermabacter*, microbiota, pig, *Propionibacterium*, *Rothia*, *Tessaracoccus*
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Corresponding author: K. Becker, University Hospital Münster, Institute of Medical Microbiology, Domagkstraße 10, 48149 Münster, Germany.
E-mail: kbecker@uni-muenster.de

Human infections due to livestock-associated methicillin-resistant *Staphylococcus* aureus CC398 have emerged in particular in areas with a high density of pig farming [1,2]. As the nasal cavity of pigs is the primary habitat of livestock-associated methicillin-resistant *S. aureus*, a culturomics project to elucidate the composition of the porcine nasal microbiota commenced in 2015. During the course of this project, four new bacterial species were identified based on 16S rRNA gene sequencing. The isolates in question had failed to be identified using matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany). Intranasal and snout surface swab samples of healthy pigs were acquired in 2016 from two farms in the Münster region located in the northwestern part of the German state North Rhine-Westphalia.

Samples were taken using Transwab transport swabs in Amies medium (Medical Wire & Equipment, Corsham, United Kingdom) and delivered to the Institute of Medical Microbiology within 6 hours. Upon arrival, swabs were stirred in 0.9% saline solution, and serial dilutions with factors 10, 100 and 1000 were prepared. Of each dilution, 100 µL were plated onto Columbia agar with 5% sheep's blood and chocolate agar (both Becton Dickinson, Franklin Lakes, NJ, USA), Columbia CAP agar and MacConkey agar (both Oxoid, Wesel, Germany) and incubated aerobically for 24 hours (chocolate agar, 5% CO_2). Additionally, Schaedler agar, Schaedler + kanamycin/vancomycin agar (both Becton Dickinson), chocolate agar and Columbia CAP agar were inoculated with the same amount for anaerobic incubation for 48 hours. Single colonies were picked after incubation, and after failed MALDI-TOF MS identification applying the Microflex LT system and the Biotyper 2.0 software (Bruker), DNA was isolated using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCRs for 16S rRNA gene amplification were performed using REDTaq ReadyMix (Sigma-Aldrich Chemie, Munich, Germany) with primers SSU-bact-27f (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 16S-5 (5′-AAG GAG GTG ATC CAG CCG CA-3′) [3,4]. Thermal cycling conditions were an initial 94°C for 5 minutes, followed by 28 cycles of 45 seconds at 94°C, 60 seconds at 60°C and 90 seconds at 72°C. Final elongation occurred at 72°C for 10 minutes.

PCR products were purified with the QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions.

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Sequencing was performed using cycle sequencing technology with primers SSU-bact-27f, 16S-2 (5′-CCG TCA ATT CMT TTG AGT TT-3′), 16S-3 (5′-ACT CCT ACG GGA GGC AGC AG-3′), 16S-3R-S (5′-CCC TAC TGC TGC CTC CCG TAG-3′) and 16S-5. Sequences were analysed on an ABI 3730XL sequencing machine (Eurofins Genomics, Ebersberg, Germany).

Sequence homologies to the phylogenetically closest species were <98.7% for all bacterial isolates. Therefore, four new species are proposed to be created according to nomenclature [5].

Strain 1a5R-CH16 was isolated from a swab of the snout surface of a 5-year-old sow held on a breeding farm after 24 hours of aerobic incubation. On blood agar, colonies were white and round, with an entire margin, slightly elevated and 0.5 mm in diameter. No haemolysis was observed; the isolate was catalase-positive but oxidase-negative. Microscopically, the cells were Gram-positive and grew as tetracocci. The phylogenetically closest relative was *Rothia nasimurium* strain CCUG 35957, with 16S sequence identity of 97.5% [5]. Hence, we propose to create a new species, *'Rothia nasisuis'* (na.si.su'is, from *nasus* [L. sg. gen. m.] and *sus* [L. sg. gen. f.], 'from the nose of a pig').

A comprehensive overview of the core features for *'Rothia nasisuis'* strain 1a5R-CH16 sp. nov. can be found in the digital protologue form under http://imedea.uib-csic.es/dprotologue/pdf_entryFormFull.php?form_id=10891&entry_id=685. Its position is shown in Fig. 1.

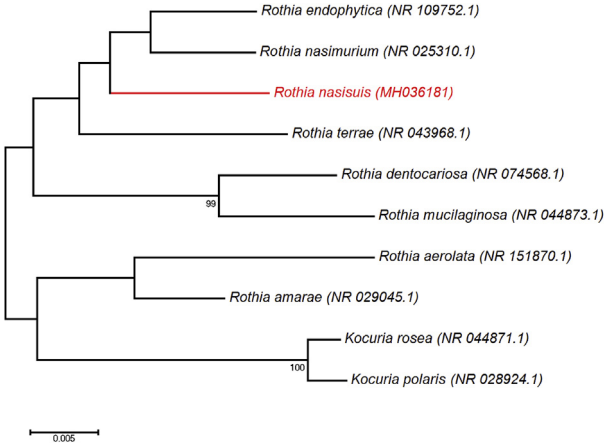

FIG. 1. Position of *'Rothia nasisuis'* strain 1a5R-CH16 (red) in phylogenetic relation to close neighbours. Using MEGA software, CLUSTALW was applied for sequence alignment, and Kimura 2-parameter models using maximum-likelihood method were used for phylogenetic inferences. Numbers at nodes represent bootstrap value percentages generated by 1000 repetitions of analysis to generate majority consensus. Values >95% are shown. Scale bar indicates 0.5% nucleotide sequence divergence.

Colonies of strain 2a1I-BL09 appeared greyish-white, were round with an entire margin and were slightly elevated. Colony diameters were 0.5 mm and no haemolysis was observable on blood agar. The catalase-positive and oxidase-negative bacteria were Gram-positive rods. The bacterium was isolated from a swab of the nasal cavity of a 2-year-old sow held on a breeding farm. The phylogenetically closest relative was *Dermabacter vaginalis* strain AD1-86, with 16S sequence identity of 97.76% [5]. Therefore, we suggest the creation of a new species, *'Dermabacter porcinasus'* (por.ci.na'sus, from *porcus* [L. sg. gen. m.] and *nasus* [L. sg.], 'from a pig's nose').

A comprehensive overview of the core features for *'Dermabacter porcinasus'* strain 2a1I-BL09 sp. nov. can be found in the digital protologue form under http://imedea.uib-csic.es/dprotologue/pdf_entryFormFull.php?form_id=10891&entry_id=686. Its position is shown in Fig. 2.

Strain 1a7I-CH12an grew in whitish, round and elevated colonies with entire margins and a diameter of 0.5 mm on blood agar under anaerobic conditions. Colonies showed no haemolysis, and were delayed catalase-positive and oxidase-negative. Gram-positive short rods were seen under the microscope. The bacterium was isolated from a swab of the nasal cavity of a 4-year-old sow held on a breeding farm.

The phylogenetically closest relative was *Propionibacterium australiense* strain LCDC-98A072, with 16S sequence identity of 95.09% [5]. Hence, our proposition is to create a new species, *'Propionibacterium westphaliense'* (west.pha.li.en'se, N.L. adj., 'of Westphalia,' the name of the region in Germany where the strain was sampled and isolated).

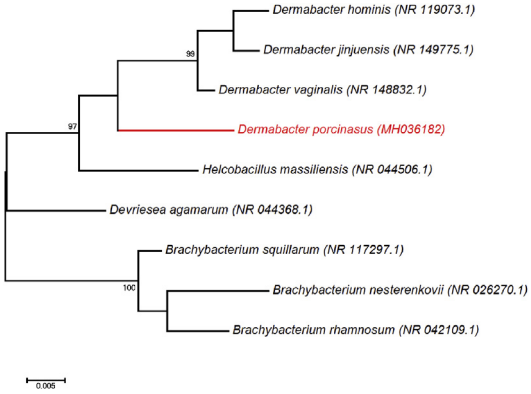

FIG. 2. Position of *'Dermabacter porcinasus'* strain 2a1I-BL09 (red) in phylogenetic relation to close neighbours. Using MEGA software, CLUSTALW was applied for sequence alignment, and Kimura 2-parameter models using maximum-likelihood method were used for phylogenetic inferences. Numbers at nodes represent bootstrap value percentages generated by 1000 repetitions of analysis to generate majority consensus. Values >95% are shown. Scale bar indicates 0.5% nucleotide sequence divergence.

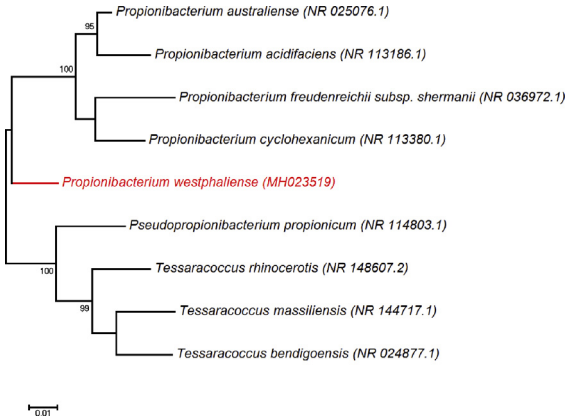

FIG. 3. Position of *'Propionibacterium westphaliense'* strain 1a7I-CH12an (red) in phylogenetic relation to close neighbours. Using MEGA software, CLUSTALW was applied for sequence alignment, and Kimura 2-parameter models using maximum-likelihood method were used for phylogenetic inferences. Numbers at nodes represent bootstrap value percentages generated by 1000 repetitions of analysis to generate majority consensus. Values >95% are shown. Scale bar indicates 1% nucleotide sequence divergence.

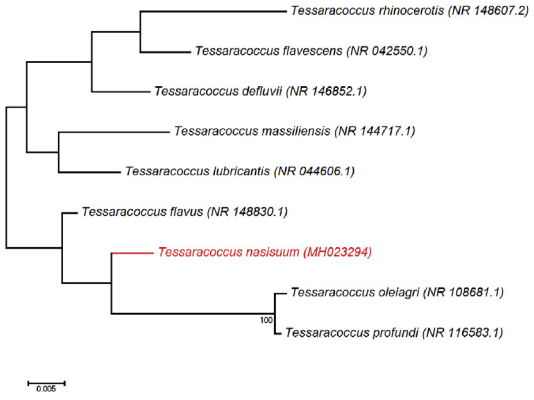

FIG. 4. Position of *'Tessaracoccus nasisuum'* strain 1a6R-CH11an (red) in phylogenetic relation to close neighbours. Using MEGA software, CLUSTALW was applied for sequence alignment, and Kimura 2-parameter models using maximum-likelihood method were used for phylogenetic inferences. Numbers at nodes represent bootstrap value percentages generated by 1000 repetitions of analysis to generate majority consensus. Values >95% are shown. Scale bar indicates 0.5% nucleotide sequence divergence.

A comprehensive overview of the core features for *'Propionibacterium westphaliense'* strain 1a7I-CH12an sp. nov. can be found in the digital protologue form under http://imedea.uib-csic.es/dprotologue/pdf_entryFormFull.php?form_id=10891&entry_id=687. Its position is shown in Fig. 3.

No single colonies were discernable on blood agar after anaerobic incubation for strain 1a6R-CH11an. The conglomerate looked greyish with no haemolytic behaviour, and it was positive for catalase and negative for oxidase. Microscopic observation revealed Gram-positive, club-shaped rods. The bacterium was isolated from a swab of the snout surface of a 3-year-old sow held on a breeding farm.

The phylogenetically closest relative is *Tessaracoccus flavus* strain RP1, with 16S sequence identity of 98.5% [5]. Thus, we propose the creation of a new species, *'Tessaracoccus nasisuum'* (na.si.su'um, from *nasus* [L. pl.] and *sus* [L. pl. gen.], 'from the noses of pigs').

A comprehensive overview of the core features for *'Tessaracoccus nasisuum'* strain 1a6R-CH11an sp. nov. can be found in the digital protologue form under http://imedea.uib-csic.es/dprotologue/pdf_entryFormFull.php?form_id=10891&entry_id=688. Its position is shown in Fig. 4.

Nucleotide sequence accession numbers

Sequences of 16S rRNA genes were deposited in GenBank under the following accession numbers: *'Rothia nasisuis'* strain 1a5R-CH16: MH036181; *'Dermabacter porcinasus'* strain 2a11-BL09: MH036182; *'Propionibacterium westphaliense'* strain 1a7I-CH12an: MH023519; and *'Tessaracoccus nasisuum'* strain 1a6R-CH11an: MH023294.

Deposit in a culture collection

All strains were deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ) under numbers DSM 107101 (*'Rothia nasisuis'* strain 1a5R-CH16), DSM 107102 (*'Dermabacter porcinasus'* strain 2a11-BL09), DSM 107103 (*'Tessaracoccus nasisuum'* strain 1a6R-CH11an) and DSM 107104 (*'Propionibacterium westphaliense'* strain 1a7I-CH12an).

Conflict of interest

None declared.

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