Reclassification of *Staphylococcus jettensis* De Bel et al. 2013 as *Staphylococcus petrasii* subsp. *jettensis* subsp. nov. and emended description of *Staphylococcus petrasii* Pantucek et al. 2013

Annelies De Bel,1 Pavel Švec,2 Petr Petrâš,3 Ivo Sedlâček,2 Roman Pantúček,4 Fedoua Echahidi,1 Denis Piérand1 and Peter Vandamme5

1Department of Microbiology and Infection Control, Univeristair Ziekenhuis Brussel, Vrije Universiteit Brussel (VUB), Laarbeeklaan 101, 1090 Brussels, Belgium
2Czech Collection of Microorganisms, Department of Experimental Biology, Faculty of Science, Masaryk University, Kamencice 5, Bld. A25, 625 00 Brno, Czech Republic
3National Reference Laboratory for Staphylococci, National Institute of Public Health, Šrobárova 48, 100 42 Praha 10, Czech Republic
4Division of Genetics and Molecular Biology, Department of Experimental Biology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
5Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

The type and clinical strains of two recently described coagulase-negative species of the genus *Staphylococcus*, *Staphylococcus petrasii* and *Staphylococcus jettensis*, were compared using *dnaJ*, *tuf*, *gap*, *hsp60* and *rpoB* gene sequences, DNA–DNA hybridization, ribotyping, repetitive sequence-based PCR fingerprinting and extensive biochemical characterization. Based on the results, the species description of *S. petrasii* has been emended and *S. jettensis* should be reclassified as a novel subspecies within *S. petrasii* for which the name *Staphylococcus petrasii* subsp. *jettensis* subsp. nov. is proposed. The type strain is SEQ110T (=LMG 26879T=CCUG 62657T=DSM 26618T=CCM 8494T).

Recently two novel coagulase-negative species of the genus *Staphylococcus* were described: *Staphylococcus petrasii* (Pantúček et al., 2013a) and *Staphylococcus jettensis* (De Bel et al., 2013). The description of the former was based on 13 human clinical isolates. Genotypic and phenotypic analyses revealed two closely related subspecies that were named *Staphylococcus petrasii* subsp. *petrasii* (9 isolates) and *Staphylococcus petrasii* subsp. *croceilyticus* (4 isolates). A few months later, the name *S. jettensis* was proposed to accommodate eight human clinical isolates including one, SEQ027, with an aberrant colony colour, biochemical characteristics and a unique position in the *tuf*-based phylogenetic tree (De Bel et al., 2013). In the absence of additional strains representing the same taxon, strain SEQ027 was not given separate taxonomic status (Christensen et al., 2001).

Comparison of *dnaJ*, *tuf*, *gap*, *hsp60* and *rpoB* gene sequences by means of the BioNumerics version 5.1 software package (Applied Maths) consistently revealed very high sequence similarity values among the type strains of *S. petrasii* subsp. *petrasii* (CCM 8418T) and *S. jettensis* (SEQ110T) on the one hand, and between *S. petrasii* subsp. *croceilyticus* CCM 8421T and *S. jettensis* SEQ027, on the other. The similarity levels of the *dnaJ*, *tuf*, *gap*, *hsp60* and *rpoB* gene sequences of *S. petrasii* subsp. *petrasii* CCM 8418T and *S. jettensis* SEQ110T were 99.5, 99.5, 99.7, 97.5 and 99.6 %, respectively; while the corresponding values for *S. petrasii* subsp. *croceilyticus* CCM 8421T and *S. jettensis* SEQ027 were 99.9, 100, 100, 100 and 100 %. *Hsp60* data for strains of the species *S. jettensis* were generated in the present study as described previously (Kwok & Chow, 2003); all other sequences were taken from the reports of Pantúček et al. (2013a) and De Bel et al.

The GenBank/EMBL/DDBJ accession numbers of the *hsp60* sequences of the isolates are KJ540111 (MCC95747), KJ540112 (SEQ036), KJ540113 (SEQ110), KJ540114 (SEQ255), KJ540115 (SEQ256), KJ540116 (SEQ257), KJ540117 (SEQ258), and KJ540118 (SEQ259).

Three supplementary tables and one supplementary figure are available with the online Supplementary Material.
These results indicated that S. petrasii and S. jettensis are representatives of the same species. However, the biochemical characteristics of S. petrasii subsp. petrasii, S. petrasii subsp. croceilyticus, the typical strains of the species S. jettensis and of strain SEQ027 were different. The present study was initiated to clarify the taxonomy of these taxa, and includes strains described previously (Pantůček et al., 2013a; De Bel et al., 2013), as well as one additional human clinical isolate, strain MCC95747, isolated from a case of conjunctivitis.

High-molecular-mass DNA of S. petrasii subsp. petrasii CCM 8418T, S. petrasii subsp. croceilyticus CCM 8421T and S. jettensis SEQ110T (=CCM 84945) was prepared as described by Gevers et al. (2001). Microplate DNA–DNA hybridization was performed according to the method of Ezaki et al. (1989). The hybridization temperature was 34 °C. The hybridization level between these three strains was >81% (Table S1, available in the online Supplementary Material), confirming that they do indeed represent a single species. The name S. petrasii was validated in Validation List no. 152 (Pantůček et al., 2013b), i.e. shortly before the valid publication of the name S. jettensis (De Bel et al., 2013) and, therefore, it takes nomenclatural priority (Euzéby & Tindall, 2004; Tindall et al., 2006).

Automated ribotyping with the EcoRI restriction endonu-
clease of all strains of the species S. jettensis and of strain MCC95747 was performed using the Riboprinter microbial characterization system (DuPont Qualicon) in accordance with the protocol provided by the manufacturer. Ribotype patterns (Fig. 1) separated strains of S. petrasii subsp. petrasii, S. petrasii subsp. croceilyticus and S. jettensis, with the exception of SEQ027, into three homogeneous groups, which clustered at similarity levels of 83.8, 84.5 and 80.2 %, respectively. Strain S. jettensis SEQ027 clustered among the strains of S. petrasii subsp. croceilyticus (Fig. 1). Strain MCC95747 grouped with strains of the species S. jettensis, but occupied a more aberrant position in the dendrogram, along with strain SEQ258. Repetitive sequence-based PCR fingerprinting with the (GTG)₅ primer, performed as described by Švec et al. (2010), confirmed most of these findings (Fig. S1). However, although strains SEQ258 and MCC95747 again clustered together, they now grouped with strains of S. petrasii subsp. petrasii (Fig. S1).

Finally, carriage of the meca gene was detected by multiplex PCR for identification of meticillin-resistant staphylococci (Geha et al., 1994). Phenotypic characteristics of all strains of the species S. jettensis were examined using traditional biochemical tests and the commercial identification kits API Staph, API 50CH and API ZYM (bioMérieux) as described previously (De Bel et al., 2013; Pantůček et al., 2013a) to reassess the biochemical differences reported earlier. The large majority of biochemical test results obtained previously were confirmed in the present analyses. Unlike strains of the species S. petrasii, strains of the species S. jettensis did not exhibit urose, while napthol-AS-BI-phosphohydrolase activity and 5-ketogluconate production were variable.

These strain-dependent characteristics can, therefore, not be retained as part of the general species description of S. petrasii and an emended species description is provided below. Furthermore, we noticed that growth in the presence of 12 % (w/v) NaCl and in a thioglycollate medium, as well as pyrrolidonyl arylamidase and alkaline phosphatase activity and acid production from D-ribose, melezitose, and turanose yielded results that were test-system dependent (Table S2). Finally, three errors that were published in the description of S. jettensis need correction: L-fucose oxidation was tested instead of D-fucose oxidation and strain SEQ27 tested positive for D-fucose oxidation, which confirms its assignment to the subspecies, S. petrasii subsp. croceilyticus, and strain SEQ110T was gentamicin-resistant rather than being susceptible to it. Differential characteristics of S. jettensis and both subspecies of S. petrasii are presented in Table 1.

In conclusion, the present study demonstrated that S. jettensis should be considered a junior heterotypic synonym of S. petrasii. However, genotypic differences, as revealed by automated ribotyping (Fig. 1), (GTG)₅-PCR fingerprinting (Fig. S1) and multiple biochemical differences (Table 1) warrant the reclassification of seven reference strains of the species S. jettensis and of the novel isolate, MCC95747, as a novel subspecies within S. petrasii for which the name Staphylococcus petrasii subsp. jettensis is proposed, with strain SEQ110T as the type strain. Strain S. jettensis SEQ027 should be considered a strain of the subspecies S. petrasii subsp. croceilyticus. The descriptions below are based on our previous studies (Pantůček et al., 2013a; De Bel et al., 2013) and on the results of the present study. Results of acid production from various carbohydrates mentioned in the species description were obtained with API CH50.

**Emended description of Staphylococcus petrasii Pantůček et al. 2013**

Staphylococcus petrasii (pe‘tra.sii.i N.L. masc. gen. n. petrasii of Petras, named in honour of Mr Petr Petráš, a Czech microbiologist, for his contribution to the taxonomy of staphylococci).

The phenotypic characteristics are identical to those of the original species description (Pantůček et al., 2013a), except for the following: urease, 5-ketogluconate and napthol-AS-BI-phosphohydrolase are variable. Alkaline phosphatase activity is variable (mostly weakly positive in API ZYM, while negative in ID32 Staph).

The type strain is CCM 8418T (=CCUG 62727T=NRL/St 10/1050T).

**Description of Staphylococcus petrasii subsp. jettensis subsp. nov.**

*Staphylococcus petrasii subsp. jettensis* (jet.ten’sis. N.L. masc. adj. jettensis from Jette, the municipality of Brussels-Capital region where most of these strains were isolated and where
The medical campus of the Vrije Universiteit Brussel is housed.

The phenotypic characteristics are identical to those in the species description of Staphylococcus petrasii subsp. petrasii (De Bel et al., 2013), except for the following: urease-negative, acid is not produced from D- or L-fucose. All strains produce acid from turanose (weakly). Pyrrolidonyl arylamidase-positive. Variable biochemical reactions are obtained for acid production from D-mannitol (1 out of 8 positive), α-lactose (2 out of 8 positive), melezitose (7 out of 8 positive), D-ribose (5 out of 8 positive) and alkaline phosphatase (6 out of 8 weakly positive). The following additional information is from the present study: acid is not produced from D-arabinose. Weak naphthol-AS-BI-phosphohydrolase activity and α-glucosidase activity, but no lipase (C14) or chymotrypsin activity. Variable biochemical reactions for acid production from 5-ketogluconate (4 out of 8 positive), starch (1 out of 8 positive), β-glucosidase (3 out of 8 positive) and leucine arylamidase (7 out of 8 positive). Acid production characteristics from various carbohydrates mentioned in the subspecies description are from

Table 1. Phenotypic differentiation of subspecies of Staphylococcus petrasii

<table>
<thead>
<tr>
<th>Subspecies</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pale-yellow pigmentation*</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Enzyme activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNase</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>−</td>
<td>+</td>
<td>v</td>
</tr>
<tr>
<td>Acid produced (aerobically) from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L-Fucose</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

*After an overnight incubation.
API CH50, and enzyme activity descriptions are based on API ZYM results. Ability to use carbon sources via respiration, determined in Biolog GP2 MicroPlates, is indicated in Table S3. The presence of the mecA gene is confirmed in all cefoxitin-resistant strains. The type strain dependent test results are as follows: weakly positive for alkaline phosphatase, leucine arylamidase and acid from melezitose. Negative for β-glucosidase and acid from D-ribose, D-mannitol, α-lactose, starch and 5-ketogluconate. Penicillin-, cefoxitin-, gentamicin-, erythromycin- and clindamycin- (inducible, positive D zone-test) resistant.

The type strain, SEQ110T (=LMG 26879T = CCUG 62657T = DSM 26618T = CCM 8494T), was isolated from a human blood culture in Jette, Belgium. The G+C content of the DNA of the type strain is 33.7 mol%.

Acknowledgements

The authors thank Kristof Vandoorslaer, Ina Van Cauwenbergh and Margo Cnockaert for skilled technical assistance. Strain MCC9574 and strain SEQ027 were kindly provided by J. J. Dajcs (Alcon Research, Ltd., Texas, USA) and M. Ieven (University Hospital Antwerpen), respectively. This work was partially supported by the Ministry of Education, Youth and Sports of the Czech Republic project CZ.1.07/2.3.00/20.0183.

References


