Systematic and Applied Microbiology 36 (2013) 90–95

Contents lists available at SciVerse ScienceDirect

Systematic and Applied Microbiology

journal homepage: www.elsevier.de/syapm

Staphylococcus petrasii sp. nov. including *S. petrasii* subsp. *petrasii* subsp. nov. and *S. petrasii* subsp. *croceilyticus* subsp. nov., isolated from human clinical specimens and human ear infections☆

Roman Pantůček[a], Pavel Švec[b], Joseph J. Dajcs[c], Ivana Machová[d], Jitka Černohlávková[b], Ondrej Šedo[e], Tereza Gelbíčová[b], Ivana Mašlaňová[a,b], Jiří Doškař[a], Zbyněk Zdráhal[e], Vladislava Růžičková[a], Ivo Sedláček[b,*]

[a] *Division of Genetics and Molecular Biology, Department of Experimental Biology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic*
[b] *Czech Collection of Microorganisms, Department of Experimental Biology, Faculty of Science, Masaryk University, Tvrdého 14, 602 00 Brno, Czech Republic*
[c] *Alcon Laboratories, Inc., Anti-Infectives Research, 6201 South Freeway, Fort Worth, TX 76134-2099, USA*
[d] *Reference Laboratory for Staphylococci, National Institute of Public Health, Šrobárova 48, 100 42 Praha 10, Czech Republic*
[e] *Core Facility – Proteomics, CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

ARTICLE INFO

Article history:
Received 1 August 2012
Received in revised form 2 November 2012
Accepted 15 November 2012

Keywords:
Coagulase-negative Staphylococci
Staphylococcus petrasii
Otitis externa
Molecular phylogeny
Polyphasic taxonomy

ABSTRACT

Thirteen coagulase-negative, oxidase-negative, and novobiocin-susceptible staphylococci were isolated from human clinical specimens. The isolates were differentiated from known staphylococcal species on the basis of 16S rRNA, *hsp60*, *rpoB*, *dnaJ*, *tuf*, and *gap* gene sequencing, automated ribotyping, $(GTG)_5$-PCR fingerprinting, and MALDI-TOF MS analysis. Phylogenetic analysis based on the 16S rRNA gene sequence indicated phylogenetic relatedness of the analyzed strains to *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus devriesei*, and *Staphylococcus lugdunensis*. DNA–DNA hybridization experiments between representative strains CCM 8418^T, CCM 8421^T, and the closest phylogenetic neighbors confirmed that the isolates represent novel *Staphylococcus* species, for which the name *Staphylococcus petrasii* sp. nov. is proposed. Genotypic and phenotypic analyses unambiguously split the strains into two closely related subclusters. Based on the results, two novel subspecies *S. petrasii* subsp. *petrasii* subsp. nov. and *S. petrasii* subsp. *croceilyticus* subsp. nov. are proposed, with type strains CCM 8418^T (=CCUG 62727^T) and CCM 8421^T (=CCUG 62728^T), respectively.

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Coagulase-negative staphylococci (CoNS) are ubiquitous organisms and common commensals that are isolated mainly from the mammals' skin, mucous membranes, glands, milk, and, less often, from blood cultures. Over the last three decades, the role of CoNS in human infections has been recognized. They are opportunistic pathogens causing mild to fatal infections especially in patients with indwelling devices and immunocompromised individuals [7]. Numerous reports have shown that CoNS are the predominant flora of the outer ear and a leading cause of otic infections [2,18]. The aim of this study was to investigate and clarify the taxonomic position of 13 CoNS isolates from human clinical specimens that could not be identified to the species level by diagnostic techniques.

Eight clinically significant CoNS isolates from human clinical specimens and one isolate from medical equipment recovered in different routine clinical laboratories in the Czech Republic were sent to the Reference Laboratory for Staphylococci (Prague, Czech Republic) for identification. Additional four strains were collected by Alcon Research, Ltd. from infected and healthy ears of human study subjects throughout the United States of America (Table 1). The phylogenetically related reference type strains used for comparative studies in all performed analyses were obtained from the Czech Collection of Microorganisms (http://www.sci.muni.cz/ccm).

Gram staining and phase-contrast and electron microscopy showed that cells are Gram-positive spherical cocci of typical staphylococcal appearance, 0.8–1.2 μm in diameter and without

☆ The GenBank/EMBL/DDBJ accession numbers for sequences of strains CCM 8418^T, CCM 8419, CCM 8420, NRL/St 06/029, NRL/St 07/045, NRL/St 08/280, NRL/St 11/077, NRL/St 12/012, NRL/St 12/015, MCC10046^T, CCM 8422, MCC11690, and MCC10692 are respectively JX139845–JX139853, AY953148, JX139856, JX139854, and JX139855 for 16S rRNA gene, JX139857–JX139869 for *hsp60* gene, JX139870–JX139878, EF423990, and JX139879–JX139881 for *rpoB* gene, and JX139882–JX139894 for *dnaJ* gene; sequences of strains CCM 8418^T and CCM 8421^T for *gap* gene are JX139895 and JX139897 and for *tuf* gene JX139896 and JX139898; sequences of strain *Staphylococcus devriesei* CCM 7896^T for *dnaJ* and *gap* are JX174277 and JX174278.

* Corresponding author at: Masaryk University, Faculty of Science, Department of Experimental Biology, Czech Collection of Microorganisms, Tvrdého 14, Brno 602 00, Czech Republic. Tel.: +420 549 496 922; fax: +420 549 498 289.
E-mail address: ivo@sci.muni.cz (I. Sedláček).

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http://dx.doi.org/10.1016/j.syapm.2012.11.004

Table 1
Origin of the strains under study.

Strain	Date	Locality	Specimen	Sex	Age (years)	Diagnosis/source
NRL/St 06/029	January 2006	Nové Město na Moravě, CZ	Medical equipment swab	NA	NA	NA
NRL/St 07/045	January 2007	Strakonice, CZ	Nasal swab	F	31	Healthy woman screened for bacterial carriage
NRL/St 08/280	April 2008	České Budějovice, CZ	Wound pus	M	6	Leg wound infection
CCM 8420 (=NRL/St 08/738)	September 2008	Hořovice, CZ	Ear swab	F	0	Neonatal bacterial sepsis
CCM 8418^T (=CCUG 62727^T = NRL/St 10/1050^T)	December 2009	Trutnov, CZ	Blood	M	59	Cerebral hemorrhage
CCM 8419 (=NRL/St 10/670)	July 2010	Strakonice, CZ	Blood	F	87	PACU patient
NRL/St 11/077	February 2011	Klatovy, CZ	Blood	F	60	Abdominal pain
NRL/St 12/012	January 2012	Ústí nad Labem, CZ	Blood	F	0	Low birth weight
NRL/St 12/015	January 2012	Kladno, CZ	Blood	F	78	Cerebrovascular accident
CCM 8421^T (=CCUG 62728^T = MCC10046^T)	May 1998	Texas, USA	Ear swab	M	5	Acute otitis externa
MCC11690	October 1998	Texas, USA	Ear swab	M	1	Acute otitis externa
MCC10692	October 1998	Texas, USA	Ear swab	F	7	Acute otitis externa
CCM 8422 (=MCC57032)	October 2001	Texas, USA	Ear swab	F	32	Healthy ear

CZ = Czech Republic; NA = not applicable; PACU = Post Anesthesia Care Unit; NRL/St = Czech National Reference Laboratory for Staphylococci, MCC = Microbiology Culture Collection, Alcon Labs.

flagella (Fig. S1), occurring predominantly in pairs and clusters. Colony size, morphology and pigment production were determined on Nutrient agar CM3 (Oxoid) after incubation for 24 h at 37 °C and further 96 h at room temperature. Haemolysis was examined on Columbia blood agar (Oxoid) supplemented with 7% sheep blood at 37 °C (pH 7.2) after 24 h of incubation in a synergy test with a β-haemolysin-producing strain (*Staphylococcus pseudintermedius* CCM 4710). The phenotypic characteristics of the isolates were initially determined using two commercial identification kits STAPHYtest 24 (Erba Lachema) and API Staph (bioMérieux) according to the manufacturers' instructions. In addition to the tests included in the commercial kits, supplemental physiological and biochemical tests were performed and susceptibility to lysostaphin and lysozyme was tested as described previously [4,9]. All 13 strains were catalase positive, able to grow in the presence of 10% NaCl, resistant to bacitracin (0.2 IU) and lysozyme (400 mg L^{-1}), but susceptible to furazolidone (100 μg). Based on these results, all isolates were identified as members of the genus *Staphylococcus* which was further confirmed by genotyping. Subsequently, the characteristics covered by the API 50CH and API ZYM kits (bioMérieux) and Biolog Identification System were determined to obtain extended phenotypic data. Antimicrobial susceptibility profiles of the strains under study are given in Table S1. Analyzed strains were biochemically similar to *Staphylococcus warneri*, but differed from it mainly in the activity of pyrrolidonyl arylamidase, nitrate reduction and no anaerobic growth in a thioglycollate medium. Basic tests distinguishing novel isolates from the other staphylococci are shown in Table 2. The detailed phenotypic characterization of all isolates is given in the species and subspecies descriptions below.

An almost complete fragment of the 16S rRNA gene, and partial fragments of the *hsp60*, *rpoB*, *dnaJ*, *gap*, and *tuf* housekeeping genes were amplified and sequenced in the Eurofins MWG Operon sequencing facility (Ebersberg) as described previously [8,10,11,16,21,25] and compared with those of other taxa of the genus *Staphylococcus* available in the GenBank database. Genetic distances were corrected using Kimura's 2-parameter model and phylogenetic trees were reconstructed according to the neighbor-joining, maximum-parsimony and maximum-likelihood methods using bootstrap values based on 1000 replications with the MEGA software version 5 [24]. The neighbor-joining tree constructed with 16S rRNA gene sequences (Fig. 1) showed that the analyzed strains belonged to a novel cluster whose phylogenetic position was close to *Staphylococcus haemolyticus* (99.3% similarity), *Staphylococcus hominis* (99.1%), *Staphylococcus devriesei* (98.8%), and *Staphylococcus lugdunensis* (98.8%). No significant differences between neighbor-joining tree topology of this group and that constructed using the maximum-likelihood or maximum-parsimony algorithms could be detected (data not shown). However, bootstrap values <70% do not reliably indicate the specific relatedness between the above mentioned species and the novel isolates.

Multilocus gene sequence analysis and neighbor-joining trees individually constructed with *hsp60*, *rpoB*, *dnaJ*, *gap*, and *tuf* gene sequences (Figs. S2–S6) confirmed clustering of novel isolates as well as the presence of the two specific lineages represented by the Czech and US isolates. Within each of these subtypes, the strains shared the same or highly similar (99.5–100%) gene nucleotide sequences. On the other hand, the Czech and US isolates differed from each other in 16S rRNA (99.7% similarity), *hsp60* (94.9%), *rpoB* (98.7%), *dnaJ* (96.1%), *gap* (98.2%), and *tuf* (97.1%) gene sequences and the differences were close to those observed between the known staphylococcal subspecies. The maximum-likelihood tree

Table 2
Key tests for phenotypic differentiation of *Staphylococcus petrasii* sp. nov. from phylogenetically related and phenotypically similar *Staphylococcus* spp.

Characteristic	Species							
	1	2[a]	3[a]	4[a]	5[a]	6[b]	7[a]	8[a]
Clumping factor	–	–	–	–	–	–	–	+
Novobiocin resistance (5 μg)	–	–	–	–	–	–	+	–
Thioglycolate (growth)	–	(+)	+	–w	+	+	–w	+
Ornithine decarboxylase	–	–	–	–	–	–[c]	–	+
Pyrrolidonyl arylamidase	+	+	–	–	–	–	–	+
Urease	+	–	+	+	+	d	+	d
Acid from trehalose	+	+	+	d	+	+	–	+

Species: 1, *S. petrasii* sp. nov.; 2, *Staphylococcus haemolyticus*; 3, *Staphylococcus warneri*; 4, *Staphylococcus hominis* subsp. *hominis*; 5, *Staphylococcus pasteuri*; 6, *Staphylococcus devriesei*; 7, *Staphylococcus hominis* subsp. *novobiosepticus*; 8, *Staphylococcus lugdunensis*.
Symbols: +, 90% or more strains positive; –, 90% or more strains negative; d, 11–89% strains positive; (+), delayed reaction; w, weak reaction; in the case of *S. petrasii* sp. nov. the test results are: +, 100% strains positive; –, 100% strains negative.
[a] Data from Schleifer and Bell [19].
[b] Data from Supré et al. [22].
[c] Result of type strain (our unpublished result).

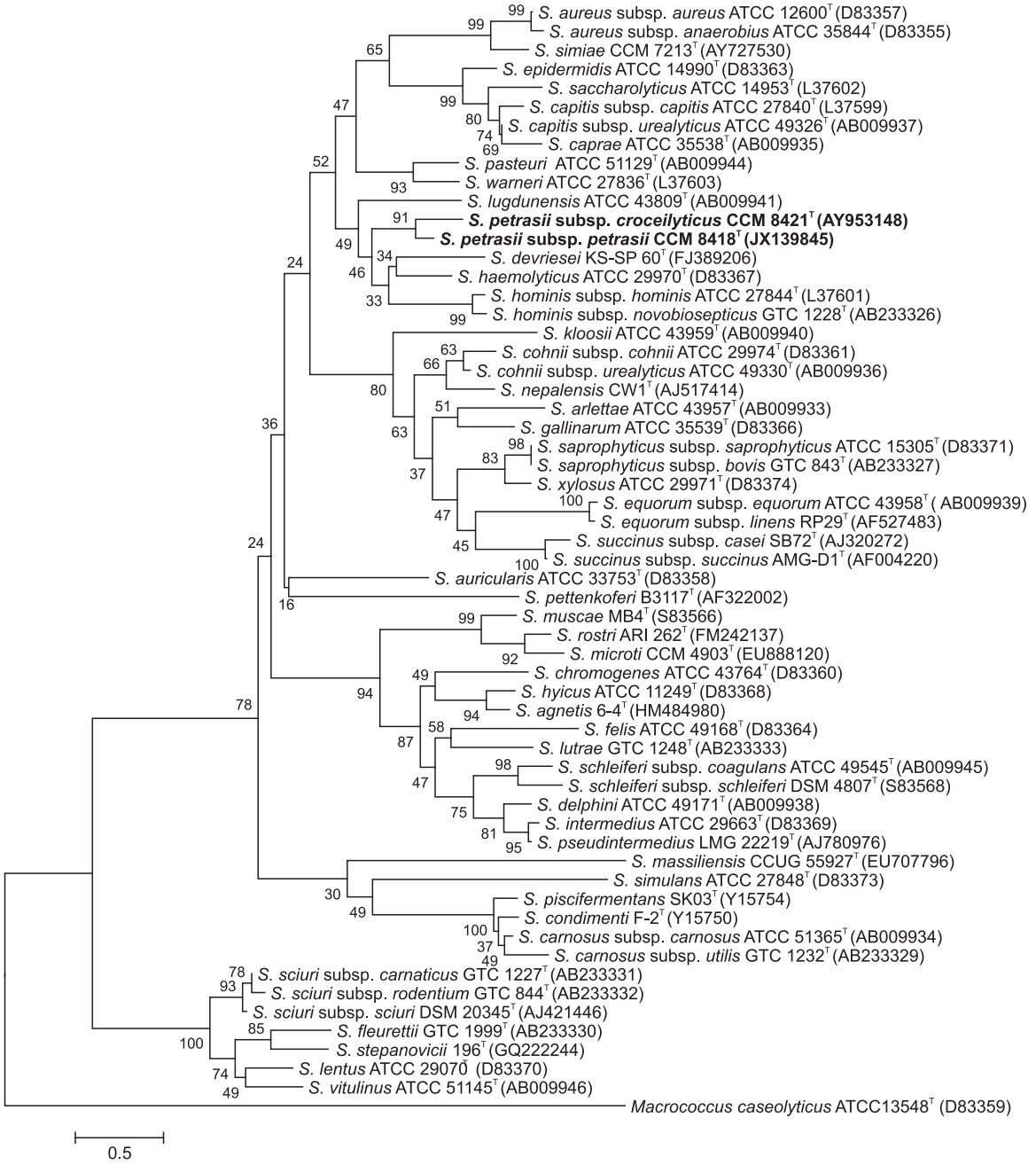

Fig. 1. Unrooted neighbor-joining tree based on 16S rRNA gene sequence comparison, showing the phylogenetic relationships of type strains *Staphylococcus petrasii* subsp. *petrasii* subsp. nov. CCM 8418^T, *S. petrasii* subsp. *croceilyticus* subsp. nov. CCM 8421^T, and reference type strains of other staphylococcal taxa. *Macrococcus caseolyticus* ATCC 13548^T sequence was used as an outgroup. Bootstrap probability values (percentages of 1,000 tree replications) are indicated at branch points. The bar represents the number of base substitutions per 100 nucleotide positions.

based on concatenated amino acid sequences of 5 house-keeping genes is shown in Fig. S7. The amino acid sequence alignments showed the following differences between the Czech and US strains in partial protein sequences: one to four residues in Hsp60, complete conservation of the RpoB, two residues in DnaJ, seven residues in Gap protein, and two amino acid residues in EF-Tu. In contrast, the calculated housekeeping gene sequence similarities between the isolates and remaining *Staphylococcus* spp. were below the cut-off values used previously for species identification, which suggests that the isolates represent a novel *Staphylococcus* species.

Automated ribotyping with the *Eco*RI restriction endonuclease was performed using the RiboPrinter microbial characterization system (DuPont Qualicon) in accordance with the protocol provided by the manufacturer. Ribotype patterns separated the Czech and US strains into two homogeneous groups clustered at similarity levels of 84 and 80%, respectively, and differentiated both clusters from all entries included in the reference database DUP 2011. Fig. S8 shows ribotype profiles of investigated strains and demonstrates their separation from selected phylogenetically related and phenotypically similar *Staphylococcus* spp.

Repetitive sequence-based PCR fingerprinting with the $(GTG)_5$ primer was performed as described by Švec et al. [23]. The numerical analysis of the resulting fingerprints clustered the isolates into two separated homogeneous groups at similarity levels of 78 and 94%, respectively. Both clusters were clearly differentiated from all staphylococci included in the in-house CCM database. Fig. S9 shows

Table 3
DNA–DNA similarity values between strains CCM 8418^T and CCM 8421^T, and the type strains of the phylogenetically closest *Staphylococcus* spp.

	CCM 8418^T	CCM 8421^T	CCM 2737^T	CCM 3474^T	CCM 4064^T	CCM 7896^T
S. petrasii subsp. *petrasii* CCM 8418^T	100					
S. petrasii subsp. *croceilyticus* CCM 8421^T	78[a]/70[b]	100				
S. haemolyticus CCM 2737^T	36/28	36/25	100			
S. hominis subsp. *hominis* CCM 3474^T	32/24	34/25	31/25	100		
S. lugdunensis CCM 4064^T	29/17	30/17	27/13	29/20	100	
S. devriesei CCM 7896^T	30/26	32/27	31/22	33/24	38/15	100

[a] DNA–DNA similarity values obtained with the hybridization temperature 34 °C.
[b] DNA–DNA similarity values obtained with the hybridization temperature 45 °C.

the $(GTG)_5$-PCR fingerprints obtained from the analyzed strains and their separation from the type strains representing selected phylogenetically related and phenotypically similar *Staphylococcus* spp.

Whole genomic DNA fingerprinting by PFGE using *Sma*I restriction endonuclease (Roche Diagnostics) was performed to analyze intraspecific diversity as described by Pantůček et al. [15]. *Sma*I macrorestriction pattern analysis differentiated unambiguously between the two groups represented by Czech and US isolates (Fig. S10). The average genome size is 2650 ± 85 kb for the Czech isolates and 2610 ± 115 kb for the US strains.

Whole-cell protein fingerprinting was performed as described previously [17]. Cluster analysis grouped all but one strain into a single cluster separated from phylogenetically related and phenotypically similar *Staphylococcus* spp. at the similarity level of 59% (Fig. S11). Strain NRL/St 07/045 revealed aberrant protein profile and was separated from the remaining isolates included in the analysis. This strain strongly produced a non-specified slim-like extracellular substance, which might interfere the analysis and affect obtained results.

Strains were analyzed using a MALDI-TOF mass spectrometer (Microflex LT, Bruker Daltonics) in an automatic acquisition mode. Mass spectra were processed using the Flex Analysis (version 3.3) and BioTyper (version 3.0) software. As input data, signals present in at least nine out of the total of 12 replicate analyses of each sample were taken into account. All strains yielded MALDI-TOF MS profiles containing signals in the mass range 2–15 kDa. The profiles did not show significant similarity (BioTyper log(score) > 1.7) to any of the reference entries belonging to *Staphylococcus* species with valid names included in the BioTyper database version 3.2.1.0. The MALDI-TOF MS profiles also clearly separated the investigated isolates from other related *Staphylococcus* type strains as demonstrated by Fig. S12.

The total hydrolysates (4 N HCl, 16 h, 100 °C) of the peptidoglycan of strains CCM 8418^T and CCM 8421^T contained the amino acids Lys, Ala, Ser, Gly, and Glu in the molar ratios 1.0:2.2:0.4:3.7:1.0 and 0.9:2.0:0.4:3.7:1.0, respectively. 2D-TLC of the partial hydrolysate (4 N HCl, 0.75 h at 100 °C) revealed the presence of the following peptides: L-Ala-D-Glu, D-Ala-Gly, L-Lys-Gly, D-Ala-L-Lys-Gly, oligo-Gly as determined by the DSMZ Identification Service (Braunschweig, Germany). Because of the occurrence of serine and the reduced amount of glycine it is most likely that glycine is partially substituted by serine in the interpeptide bridge. It was concluded from these data that the peptidoglycan type of strains CCM 8418^T and CCM 8421^T is A3α L-Lys-Gly_{3-4}(Ser) according to Schumann [20].

Fatty acid methyl esters studies of all isolates were also performed by DSMZ-identification service. Our study showed that the fatty acids consisted of major amounts of saturated and methyl branched *iso* and *anteiso* fatty acids of between 13 and 20 carbons. All 13 tested strains produced as the major fatty acids $C_{15:0\ iso}$ (10.3 ± 1.5%), $C_{15:0\ anteiso}$ (38.9 ± 4.1%), $C_{17:0\ anteiso}$ (9.7 ± 3.1%), $C_{18:0}$ (16.1 ± 3.1%) and $C_{20:0}$ (9.3 ± 3.7%). The higher amount of $C_{16:0}$ (3.2 ± 0.9%) and $C_{17:0\ iso}$ (4.7 ± 1.6%) were found as well. Unsaturated and hydroxy fatty acids were absent in all cases. The detection of various saturated and branched fatty acids in the strains is in a good agreement with the results of previous study concerned on staphylococci [14].

Quinones and polar lipids were extracted and analyzed as described previously [13]. Both strains CCM 8418^T and CCM 8421^T showed similar quinone systems with predominant menaquinone MK-7 (99.0% and 85.2%, respectively) and minor amount of MK-8 (1.0% and 14.8%, respectively). The polar lipid profiles of both type strains were identical with small quantitative differences. Major detected polar lipids were phosphatidylglycerol (PG) and unknown glycolipid (GL1). Moderate amount of diphosphatidylglycerol (DPG), aminolipid (AL) and minor amount of a second glycolipid (GL2) and an unknown polar lipid (L1) were found (Fig. S13). Similar quinone systems and polar lipid profiles have been reported for several other staphylococci, including related *S. haemolyticus* and *S. hominis* [12].

Representatives of each of the gene sequence subtypes, strains CCM 8418^T and CCM 8421^T, and the most closely related species *S. haemolyticus*, *S. hominis* subsp. *hominis*, *S. lugdunensis*, and *S. devriesei* were used for the DDH analysis. The total high molecular weight genomic DNA extraction protocol for DDH was based on the method described by Gevers et al. [5]. DDH experiments were performed using the microplate method [3] and were carried out according to the protocols described previously [1,6]. The hybridization reactions were performed at 34 °C as calculated from the G+C content of the analyzed strains according to Goris et al. [6] and at stringent conditions at 45 °C as proposed by Ezaki et al. [3]. The DDH results differentiated strains CCM 8418^T and CCM 8421^T from the phylogenetically closest relatives (Table 3). The DDH levels between these two strains imply that they constitute a single species, although the obtained similarity values were close to the 70% cut-off point generally accepted for bacterial species delineation.

On the basis of the obtained data, the two groups of isolates can be differentiated one from another and at the same time they can be differentiated from other *Staphylococcus* spp. The overall results summarized above indicate that both analyzed bacterial groups constitute a single species for which we propose the name *Staphylococcus petrasii* sp. nov.; however, significant phenotypic (Table 4) and genotypic differences obtained in all fingerprinting and gene sequence analyses imply that they are members of two subspecies for which we propose the names *Staphylococcus petrasii* subsp. *petrasii* subsp. nov. and *Staphylococcus petrasii* subsp. *croceilyticus* subsp. nov.

Description of *Staphylococcus petrasii* sp. nov. *Staphylococcus petrasii* (pe'tra.si.i N.L. masc. gen. n. *petrasii*, of Petras, named in honor of Mr Petr Petráš, a Czech microbiologist, for his contribution to the taxonomy of staphylococci).

Cells are Gram-positive spherical cocci, occurring predominantly in pairs and clusters, nonsporeforming and non-motile. Colonies on Nutrient agar are circular, whole margin, convex, smooth, shiny, 2–4 mm in diameter, and aerobic. Weak haemolytic activity (production of δ-haemolysin). Growth in the presence of 10% NaCl, at 15 °C (weak) and 45 °C but not at 4 °C. No growth in the presence of 12% and more NaCl

Table 4
Phenotypic differentiation of *Staphylococcus petrasii* sp. nov. subspecies.

Characteristic	*S. petrasii* subsp. *petrasii* subsp. nov.	*S. petrasii* subsp. *croceilyticus* subsp. nov.
Deoxyribonuclease	w	–
β-Glucuronidase	–	+
Acid from		
Mannose	+	–
D-Arabinose	–	+
L-Fucose	–	+
Pale yellow pigment[a]	–	+

Symbols: +, positive; –, negative; w, weak reaction.
[a] Determined on R2A agar after 24 h at 37 °C.

and in a thioglycollate medium. Catalase, urease, pyrrolidonyl arylamidase, arginine dihydrolase, and Voges–Proskauer test (acetoin) positive. Coagulase, clumping factor, hyaluronidase, thermonuclease, oxidase, and ornithine decarboxylase negative. Susceptible to novobiocin (5 μg). Hydrolysis of esculin, Tween 80, and gelatine negative. Esterase (C 4) and esterase lipase (C 8) positive. Valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-Bi-phosphohydrolase, α-galactosidase, β-galactosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase negative. Acid is produced from glycerol, D-glucose, D-fructose, maltose, sucrose, trehalose, D-turanose (weak), and 5 keto-gluconate (weak). Acid is not produced from erythritol, L-arabinose, D-xylose, L-xylose, adonitol, β-methyl-D-xyloside, sorbose, rhamnose, dulcitol, inositol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside, N-acetyl glucosamine, amygdaline, arbutine, salicin, cellobiose, melibiose, inulin, D-raffinose, glycogen, xylitol, β-gentiobiose, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, gluconate, and 2 keto-gluconate. The ability of *S. petrasii* to use carbon sources via respiration determined in Biolog GP2 MicroPlate is issued in Table S2.

The peptidoglycan type is A3α L-Lys-Gly_{3-4}(Ser). The major fatty acids are $C_{15:0\ iso}$, $C_{15:0\ anteiso}$, $C_{17:0\ anteiso}$, $C_{18:0}$ and $C_{20:0}$. Quinone system contains the major component menaquinone MK-7 and minor component MK-8. The polar lipid profile consists of the major compounds phosphatidylglycerol and unknown glycolipid and moderate amount of diphosphatidylglycerol and aminolipid.

Isolated from human clinical material.

Description of *Staphylococcus petrasii* subsp. *petrasii* subsp. nov. The phenotypic characteristics are similar to those of species description except following. Colonies on R2A agar are nonpigmented. Susceptible to lysostaphin (200 mg L^{-1}). Nitrate reduction positive and weak DNase activity. β-Glucuronidase negative. Acid is produced from D-mannose. Acid is not produced from D-arabinose, melezitose, and L-fucose. The variable biochemical reactions were obtained for acid production from ribose (5 of 9 positive), galactose (2 of 9), lactose (2 of 9), mannitol (8 of 9), starch (1 of 9), lipase (C14) (1 of 9), leucine arylamidase (6 of 9), chymotrypsin (4 of 9), and α-glucosidase (1 of 9). The ability of *S. petrasii* subsp. *petrasii* to use carbon sources via respiration determined in Biolog GP2 MicroPlate is issued in Table S2.

Type strain is CCM 8418^T (=CCUG 62727^T = NRL/St 10/1050^T). The DNA G+C content of strain CCM 8418^T is 34.9 mol% (HPLC).

Most characteristics of the type strain CCM 8418^T are in agreement with the subspecies description. The strain dependent test results are as follows: positive leucine arylamidase, chymotrypsin (weak), and acid from ribose, mannitol (delayed), and starch. Negative lipase (C 14), α-glucosidase and acid from galactose and lactose.

Description of *Staphylococcus petrasii* subsp. *croceilyticus* subsp. nov. *Staphylococcus petrasii* subsp. *croceilyticus* (cro.ce.i.ly'ti.cus. L. mascc. adj. croceus, saffron-colored, yellow, golden; N.L. adj. lyticus -a -um (from Gr. adj. lutikos -e -on), able to loose, able to dissolve; N.L. masc. adj. croceilyticus, yellow and able to dissolve host cells).

The phenotypic characteristics are similar to those of species description except following. Colonies on R2A agar are pale creamy-yellow pigmented. Slightly resistant to lysostaphin (200 mg L^{-1}). Leucine arylamidase and β-glucuronidase positive. Lipase (C 14) and hydrolysis of DNA negative. Acid is produced from D-arabinose (weak), ribose (weak), and L-fucose. Acid is not produced from galactose, D-mannose, mannitol, lactose, and starch.

The variable biochemical reactions were obtained for acid production from melezitose (2 of 4 positive), chymotrypsin (2 of 4), α-glucosidase (2 of 4), and nitrate reduction (3 of 4). Basic phenotypic tests distinguishing *S. petrasii* subsp. *croceilyticus* from the nominal subspecies are shown in Table 4. The ability of *S. petrasii* subsp. *croceilyticus* to use carbon sources via respiration determined in Biolog GP2 MicroPlate is issued in Table S2.

The type strain is CCM 8421^T (=CCUG 62728^T = MCC10046^T). The DNA G+C content of strain CCM 8421^T is 35.5 mol% (HPLC).

Most characteristics of the type strain CCM 8421^T are in agreement with the subspecies description. The strain dependent test results are as follows: positive α-glucosidase (weak) and nitrate reduction. Negative chymotrypsin and acid from melezitose.

Acknowledgements

This work was supported by the Internal Grant Agency of the Ministry of Health of the Czech Republic (NT12395-5/2011), the Czech Science Foundation (310/09/0459), the European Regional Development Fund project CEITEC (CZ.1.05/1.1.00/02.0068), and the Operational Programme Education for Competitiveness project CEB (CZ.1.07/2.3.00/20.0183). The authors are grateful to Dr. H.-J. Busse for giving us the opportunity to perform menaquinone and polar lipid analysis in his laboratory. We thank Eva Kodytkova for valuable help.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.syapm.2012.11.004.

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