Staphylococcus rostri sp. nov., a haemolytic bacterium isolated from the noses of healthy pigs

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Twenty coagulase-negative *Staphylococcus* strains displaying α -haemolysis (δ -haemolysin) on sheep-blood agar were isolated from the noses of different pigs in Switzerland. The strains were Gram-stain-positive, non-motile cocci, catalase-positive and coagulase-negative. Sequence analysis of the 16S rRNA gene, *sodA*, *rpoB*, *dnaJ* and *hsp60* and phylogenetic characteristics revealed that the strains showed the closest relatedness to *Staphylococcus microti* CCM 4903^T and *Staphylococcus muscae* DSM 7068^T. The strains can be differentiated from *S. microti* by the absence of mannose fermentation and arginine arylamidase and from *S. muscae* by the absence of β -glucuronidase activity and production of alkaline phosphatase. The chosen type strain ARI 262^T shared 20.1 and 31.9% DNA relatedness with *S. microti* DSM 22147^T and *S. muscae* CCM 4903^T, respectively, by DNA–DNA hybridization. iso-C_{15:0}, anteiso-C_{15:0} and iso-C_{17:0} were the most common fatty acids. Cell-wall structure analysis revealed the peptidoglycan type A3 α L-Lys–Gly₂–L-Ser–Gly (type A11.3). The presence of teichoic acid was determined by sequencing the *N*-acetyl- β -D-mannosaminyltransferase gene *tarA*, which is involved in biosynthesis of ribitol teichoic acid. Menaquinone 7 (MK-7) was the predominant respiratory quinone. The G+C content of ARI 262^T was 38.8 mol%. The isolated strains represent a novel species of the genus *Staphylococcus*, for which we propose the name *Staphylococcus rostri* sp. nov. The type strain is ARI 262^T (=DSM 21968^T =CCUG 57266^T) and strain ARI 602 (=DSM 21969 =CCUG 57267) is a reference strain.

In recent years, public health concerns have arisen when pigs were reported to harbour meticillin-resistant *Staphylococcus aureus* (MRSA) in their nasal cavities (de Neeling *et al.*, 2007; Guardabassi *et al.*, 2007; Khanna *et al.*, 2008). MRSA are characterized by the presence of the meticillin resistance gene *mecA*, which confers resistance to all β -lactam antibiotics (Deurenberg & Stobberingh, 2009). They are one of the leading causes of nosocomial and community-acquired infections worldwide (de Lencastre *et al.*, 2007; Deurenberg & Stobberingh, 2009). In general, *S. aureus* and other staphylococci belong to the normal skin flora of the host and have been found in many animal species (Götz *et al.*, 2006). At the time of writing, 51 different *Staphylococcus* taxa have been described.

Abbreviation: MRSA, meticillin-resistant *Staphylococcus aureus*.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *dnaJ*, *hsp60*, *rpoB*, *sodA* and *tarA* gene sequences of strain ARI 262^T are FM242137, FM244714, FM244716, FM242139, FM242138 and FN433126, respectively, and those of strain ARI 602 are AM989462, FM244715, FM244717, AM989464, AM989463 and FN433127, respectively.

Neighbour-joining trees based on partial *sodA*, *rpoB*, *hsp60* and *dnaJ* sequences are available as supplementary material with the online version of this paper.

While MRSA were not found during a surveillance study in Switzerland that screened pigs for the presence of staphylococci (Riesen & Perreten, 2009), a third of the sampled animals were found to carry in their noses bacteria that displayed α -haemolysis on sheep-blood agar. The isolates were Gram-stain-positive cocci, catalase-positive and coagulase-negative. Sequence analysis of the 16S rRNA gene revealed that they belonged to the genus *Staphylococcus*, but differed from the other 51 known *Staphylococcus* species and subspecies (Fig. 1). The isolates were further characterized using 16S rRNA, *sodA*, *rpoB*, *dnaJ* and *hsp60* gene sequence comparison and phenotypic properties. The fatty acid profile, cell-wall structure, menaquinone pattern and DNA G+C content and DNA–DNA hybridization were determined using strains ARI 262^T and ARI 602 following the recommendations for the description of novel staphylococcal species (Freney *et al.*, 1999).

Bacteria were isolated from the nasal cavities of pigs [Swiss Large White (Edelschwein)] using swabs (Oxoid) at the slaughterhouse. Swabs were placed directly into tubes containing Mueller–Hinton II broth (Becton Dickinson) supplemented with 6.5% NaCl and inoculated at 37 °C for 24 h under agitation. From there, a loopful was spread onto tryptone soy agar plates containing 5% sheep blood (TSA-SB; Oxoid). The plates were incubated at 37 °C for 24 h. All strains displaying

Fig. 1. Cluster analysis of partial 16S rRNA gene sequences (1413 bp) of *Staphylococcus rostri* sp. nov. ARI 262^T compared with strains of 51 taxa of the genus *Staphylococcus*. The tree was generated by neighbour-joining [Pairwise (OG: 100 %, UG: 0 %), Gapcost: 0 %] and Jukes–Cantor correction using Bionumerics 5.10 (Applied Maths). Bootstrap values are shown at each node as percentages of 1000 replicates. GenBank/EMBL/DBJ accession numbers are displayed in parentheses.

α - or α - β -haemolysis on TSA-SB plates were spread onto chromagar *S. aureus* ID (SAID; bioMérieux), a selective agar that reveals α -glucosidase activity, allowing the distinction of *S. aureus* from the majority of other staphylococci (Perry *et al.*, 2003). On this agar, *S. aureus* colonies appear green, while other staphylococci display another colour.

Of 54 herds tested, 31 (57.4 %) contained pigs that harboured staphylococci with colonies that displayed α -

haemolysis on TSA-SB and appeared pink on SAID agar. Twenty of these isolates, each originating from pigs of a different herd, were selected randomly and further identified and characterized.

Partial nucleotide sequences of the 16S rRNA gene (1413 nt) were determined for the 20 strains as described previously (Kuhnert *et al.*, 2002). These strains shared identical 16S rRNA gene sequences, indicating that they are

all likely to belong to the same species. DNA sequences were also determined for specific conserved markers present within the genes *sodA* (Poyart *et al.*, 2001), *rpoB* (Drancourt & Raoult, 2002), *hsp60* (Kwok & Chow, 2003) and *dnaJ* (Shah *et al.*, 2007). These markers have been shown to allow good discrimination between *Staphylococcus* species. Sequence alignment of these markers revealed two different subtypes among the 20 strains. The two subtypes shared identical 16S rRNA gene and *sodA* sequences, but differed from each other in their *rpoB* (99.2 % similarity), *dnaJ* (98.8 %) and *hsp60* (98.2 %) sequences. A parallel study that determined the antibiotic resistance profiles and genetic relatedness of 105 isolates of the novel species also revealed the presence of two specific genotypes, as determined by *hsp60* sequence analysis and PFGE (Stegmann & Perreten, 2010). Therefore, one representative of each of these genotypes, strains ARI 262^T and ARI 602, was used for characterization.

DNA sequences of the 16S rRNA, *rpoB*, *sodA*, *dnaJ* and *hsp60* genes showed that the novel strains also differ from *Staphylococcus microti*, a bacterium originally isolated from voles (Nováková *et al.*, 2010), and *Staphylococcus muscae*, originally isolated from flies (Hájek *et al.*, 1992), which shared the highest sequence identity. The 16S rRNA gene sequences of strains ARI 262^T and ARI 602 showed 99.6 % identity to the sequence of *S. microti* CCM 4903^T and 96.2 % identity to the sequence of *S. muscae* MB4^T (Fig. 1). *sodA* fragments from strains ARI 262^T and ARI 602 showed 91.8 % sequence identity to the sequence of *S. microti* CCM 4903^T and 87.6 % identity to the sequence of *S. muscae* CIP 103641^T (Supplementary Fig. S1, available in IJSEM Online). Sequenced *rpoB* fragments of strains ARI 262^T and ARI 602^T showed 94.1 and 93.8 % identity, respectively, to that of *S. microti* CCM 4903^T and 91.3 and 91.1 % identity, respectively, to that of *S. muscae* CIP 103641^T (Supplementary Fig. S2). The sequenced *hsp60* fragments of strains ARI 262^T and ARI 602 displayed 91.1 and 91.3 % identity, respectively, to that of *S. microti* CCM 4903^T and 87.7 and 87.9 % identity, respectively, to that of *S. muscae* DSM 7068^T (Supplementary Fig. S3) and the sequenced *dnaJ* fragments of strains ARI 262^T and ARI 602 displayed 88.2 and 88.5 % identity, respectively, to that of *S. microti* CCM 4903^T and 84.3 and 84.4 % identity, respectively, to that of *S. muscae* GTC 704^T (Supplementary Fig. S4).

The novel strains also differed from the type strains of *S. microti* and *S. muscae* by DNA–DNA hybridization, as determined by the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany). DNA–DNA hybridization was performed with DNA isolated using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite as described previously (Cashion *et al.*, 1977). DNA–DNA hybridization was carried out at 63 °C as described by De Ley *et al.* (1970) under consideration of the modifications described by Huß *et al.* (1983) using a model Cary 100 Bio UV/Vis spectrometer equipped with a

Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian). A threshold value of 70 % DNA–DNA relatedness for the definition of bacterial species was considered by the ad hoc committee (Wayne *et al.*, 1987). Strains ARI 262^T and ARI 602 belong to the same species, with a DNA–DNA relatedness of 83.4 %. Neither strain belongs to the species *S. microti* (20.1 and 30.0 % relatedness, respectively, to *S. microti* CCM 4903^T) or *S. muscae* (31.9 and 36.8 % relatedness to *S. muscae* DSM 7068^T), which are the closest relatives by 16S rRNA, *sodA*, *dnaJ* and *hsp60* sequence comparison. The novel strains shared only 19 % DNA–DNA relatedness with *Staphylococcus hyicus* DSM 20459^T, another pig strain.

DNA G + C contents, menaquinone profile, peptidoglycan structure and fatty acid composition were determined at the DSMZ. DNA G + C contents of strains ARI 262^T and ARI 602 were 38.8 and 39.0 mol%, respectively, as determined by HPLC. The G + C contents are within the range reported for other *Staphylococcus* species (33–40 mol%; Götz *et al.*, 2006). The fatty acid composition was determined by gas chromatography (<http://www.midi-inc.com>). Fatty acids iso-C_{15:0}, anteiso-C_{15:0} and iso-C_{17:0} were the most common fatty acids in strains ARI 262^T and ARI 602 (Table 1). The fatty acid composition was similar to that of *S. microti* 4005-LJ(m)^T and differed from that of *S. muscae* MB4^T, in which iso-C_{15:0}, C_{16:0} and C_{18:0} were the most prevalent (Table 1). The peptidoglycan structure of strains ARI 262^T and ARI 602 was determined as

Table 1. Fatty acid compositions of strains ARI 262^T and ARI 602, *S. microti* 4005-LJ(m)^T and *S. muscae* MB4^T

Data for *S. microti* 4005-LJ(m)^T and *S. muscae* MB4^T were taken from Nováková *et al.* (2010) and Hájek *et al.* (1992), respectively. Values are percentages of total fatty acids. NR, Not reported; –, not detected.

Fatty acid	ARI 262 ^T	ARI 602	<i>S. microti</i> 4005-LJ(m) ^T	<i>S. muscae</i> MB4 ^T
iso-C _{13:0}	0.72	0.80	0.5	1.1
iso-C _{14:0}	0.38	0.71	–	NR
C _{14:0}	1.39	3.15	0.6	3.7
iso-C _{15:0}	43.65	36.79	51.7	40.9
anteiso-C _{15:0}	19.20	15.36	12.6	1.0
C _{15:0}	0.21	0.53	–	NR
iso-C _{16:0}	0.70	0.95	0.5	NR
C _{16:0}	6.24	9.11	3.7	14.8
iso-C _{17:0}	8.04	8.43	13.7	5.8
anteiso-C _{17:0}	5.70	5.69	3.9	1.9
C _{17:0}	0.36	0.49	–	NR
iso-C _{18:0}	0.07	–	–	NR
C _{18:0}	7.50	9.08	4.4	10.7
iso-C _{19:0}	0.76	1.07	1.1	1.0
anteiso-C _{19:0}	0.41	0.57	–	NR
C _{19:0}	0.39	0.50	–	NR
C _{20:0}	4.26	6.77	1.2	10.1

described by Schleifer (1985) and Schleifer & Kandler (1972) with the modification that TLC on cellulose was applied instead of paper chromatography. Quantitative analysis of amino acids was performed after derivatization by GC and GC/MS according to MacKenzie (1987).

Total hydrolysates (4 M HCl, 16 h, 100 °C) of the peptidoglycan contained the amino acids Lys, Ala, Gly, Ser and Glu in molar ratios of about 1.0:1.8:3.1:0.9:1.0 (ARI 262^T) and 1.1:1.7:3.2:1.1:1.0 (ARI 602). Partial hydrolysates (4 M HCl, 0.75 h, 100 °C) of the peptidoglycan of the two strains contained (in addition to the amino acids) the peptides L-Ala→D-Glu, Gly→Gly, D-Ala→Gly, Gly→L-Lys, L-Lys→D-Ala and Gly→L-Lys→D-Ala. From these data, it was concluded that strains ARI 262^T and ARI 602 show the peptidoglycan type A3 α L-Lys-Gly₂-L-Ser-Gly (type A11.3) according to the system proposed by Schleifer & Kandler (1972) for the characterization and representation of peptidoglycan types (http://www.dsmz.de/microorganisms/main.php?content_id=35).

The presence of teichoic acid in the novel strains was determined by sequencing the *N*-acetyl- β -D-mannosaminyltransferase gene *tarA*, which is involved in ribitol teichoic acid biosynthesis (Qian *et al.*, 2006). The *tarA* gene was amplified from strains ARI 262^T and ARI 602 by PCR and subsequently sequenced using degenerate primers tarA-F-univ (5'-AAITTTTITIGTACIGCIAAIC-CIGA) and tarA-R-univ (5'-TTCIAIIGAICCCICACI-CCCAT) designed from the consensus sequence of aligned *tarA* genes from *S. aureus* MRSA252 (GenBank accession no. BX571856), *Staphylococcus capitis* SK14 (ACFR01000002), *Staphylococcus warneri* L37603 (ACPZ01000027), *Staphylococcus hominis* SK119 (ACLP01000017), *Staphylococcus epidermidis* ATCC 12228 (AE015929), *Staphylococcus haemolyticus* JCSC1435 (AP006716), *Staphylococcus saprophyticus* subsp. *saprophyticus* ATCC 15305^T (AP008934) and *Staphylococcus carnosus* subsp. *carnosus* TM300 (AM295250). PCR was performed using *Taq* polymerase following the manufacturer's instructions (Roche Diagnostics). PCR conditions consisted of an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 37 °C for 30 s and elongation at 72 °C for 50 s. PCR products were sequenced on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

Respiratory lipoquinones were extracted from 100 mg freeze-dried cell material based on the two-stage method described by Tindall (1990a, b). Respiratory quinones were extracted using methanol/hexane (Tindall, 1990a, b). Respiratory lipoquinones were separated into their different classes (menaquinones and ubiquinones) by TLC on silica gel (Macherey-Nagel art. no. 805 023), using hexane/*tert*-butyl methyl ether (9:1, v/v) as solvent. UV-absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and analysed further by HPLC. This step was carried out on an LDC Analytical HPLC (Thermo Separation Products) fitted with a

reversed-phase column (Macherey-Nagel; 2 × 125 mm, 3 μ m, RP18) using methanol as the eluent. Respiratory lipoquinones were detected at 269 nm. Strains ARI 262^T and ARI 602 contained menaquinone 7 (MK-7) as the major component (79 %) and smaller proportions of MK-6 (4 and 7 %, respectively), as well as an unidentified menaquinone (possible dimethylmenaquinone 7). Similar menaquinone profiles have been reported in *S. microti* and *S. muscae* (Nováková *et al.*, 2010).

Phylogenetic relationships of the novel strains and other staphylococcal species were determined using Bionumerics 5.10 (Applied Maths), analysing sequences of the 16S rRNA gene (Drancourt & Raoult, 2002), *sodA* (Poyart *et al.*, 2001), *rpoB* (Drancourt & Raoult, 2002), *dnaJ* (Shah *et al.*, 2007) and *hsp60* (Kwok & Chow, 2003). The trees were generated by neighbour-joining [Pairwise (OG: 100 %, UG: 0 %); Gapcost: 0 %] using the Jukes–Cantor correction. Bootstrap values were determined from 1000 replications. The phylogenetic trees showed that the novel strains cluster within the *S. hyicus*–*S. intermedius* group (Shah *et al.*, 2007) and are related to *S. microti* and *S. muscae* (Fig. 1 and Supplementary Figs S1–S4).

Phenotypic characterization of strains ARI 262^T and ARI 602, *S. microti* CCM 4903^T and *S. muscae* DSM 7068^T was achieved using ID 32 STAPH (bioMérieux) following the manufacturer's recommendations. Contradictory results to those published for *S. microti* CCM 4903^T by Nováková *et al.* (2010) were obtained for β -galactosidase and arginine arylamidase using the same strain and the same system. *S. microti* CCM 4903^T appeared to be negative for the production of these two enzymes in our tests. These criteria cannot be used to differentiate between these two species. However, strains ARI 262^T and ARI 602 can be differentiated from *S. microti* CCM 4903^T by the absence of mannose fermentation and alanine arylamidase. Strains ARI 262^T and ARI 602 can be differentiated from *S. muscae* DSM 7068^T by the absence of β -glucuronidase and production of alkaline phosphatase (Table 2). To confirm the results obtained with ID 32 STAPH (bioMérieux), mannose fermentation was additionally tested in bromothymol blue broth containing 0.5 % mannose; *S. microti* CCM 4903^T was positive, whereas strains ARI 262^T and ARI 602 remained negative after 18 h of incubation at 37 °C. β -Galactosidase activity was tested on Mueller–Hinton plates containing 40 μ g 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside (X-Gal) ml⁻¹. Alanine arylamidase was tested using Vitek 2 (bioMérieux). β -Glucuronidase activity was tested on glucuronidase chromagar CPS ID3 (bioMérieux); colonies of the novel strains appeared white, whereas colonies of *S. muscae* DSM 7068^T appeared red. Alkaline phosphatase was confirmed on Mueller–Hinton agar containing 0.01 % phenolphthalein diphosphate tetrasodium salt and exposing colonies to ammonia vapour (Holding & Collee, 1971). Colonies that produced phosphatase turned pink. Aesculin hydrolysis was determined on aesculin agar (Bio-Rad). Catalase activity was determined by direct application of a single

Table 2. Biochemical characteristics of the novel strains (*Staphylococcus rostri* sp. nov.), *S. microti* CCM 4903^T and *S. muscae* DSM 7068^T

Novel strains: 1, ARI 262^T; 2, ARI 311; 3, ARI 411; 4, ARI 522; 5, ARI 731; 6, ARI 1211; 7, ARI 2162; 8, ARI 2231; 9, ARI 2701; 10, ARI 2981; 11, ARI 602; 12, ARI 161; 13, ARI 791; 14, ARI 841; 15, ARI 921; 16, ARI 991; 17, ARI 1052; 18, ARI 1101; 19, ARI 1301; 20, ARI 2491. Data for *S. microti* CCM 4903^T were taken from Nováková *et al.* (2010). Strains 1–10 are related to type strain ARI 262^T and strains 10–20 are related to ARI 602, as determined by *hsp60*, *dnaJ* and *rpoB* sequencing. +, Positive; ±, weakly positive reaction (may appear negative when determined with ID 32 STAPH); (–), negative in this study, in contrast to results of Nováková *et al.* (2010); –, negative. All strains were negative for oxidase, urease and ornithine decarboxylase activities, novobiocin resistance and acid production from maltose, D-mannitol, raffinose, D-ribose, cellobiose, turanose and L-arabinose. All strains were positive for reduction of nitrate, pyrrolidonyl arylamidase activity and acid production from D-glucose, D-fructose and sucrose.

Characteristic	<i>S. rostri</i> sp. nov.																				<i>S. muscae</i> DSM 7068 ^T	<i>S. microti</i> CCM 4903 ^T
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
Arginine dihydrolase	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	–	+	+	+	+	–	–
Hydrolysis of aesculin	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	–	±	±	±	±	–	+
Acid production from:																						
D-Mannose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	±
Lactose	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+
N-Acetylglucosamine	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Production of acetoin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	±
β-Galactosidase	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	(+)
β-Glucuronidase	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Arginine arylamidase	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	(+)
Alanine arylamidase	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Alkaline phosphatase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+

drop of 3 % H₂O₂ to the cells. DNase activity was tested on BD BBL DNase test agar. Thermonuclease activity was determined by spotting 20 µl filtered overnight culture supernatant on BD BBL DNase test agar subsequently incubated for 2 h at 60 °C and flooded with 1 M HCl. Coagulase was determined using rabbit plasma (BD BBL coagulase plasmas). Clumping factor, Protein A and group-specific antigen bound to the *S. aureus*-specific peripheral structure were tested using the rapid latex agglutination tests Slidex Staph Plus (bioMérieux) and Pastorex Staph Plus (Bio-Rad). Cytochrome oxidase was tested using BD BBL oxidase reagent droppers. Growth assays with different salt concentrations (6.5, 9 and 12 %, w/v, NaCl) and at 15, 37 and 43 °C were performed in BD Bacto brain heart infusion. Anaerobic growth was tested in thioglycolate. The lysostaphin test was performed using cells diluted in PBS to OD₆₀₀ 0.8. After addition of 25 µg lysostaphin ml^{–1} (Sigma-Aldrich), cells were incubated for 1 h at 37 °C and lysis was measured spectrophotometrically at OD₆₀₀. The diameter of cells of the novel strains was determined by scanning electron microscopy [× 10 000 magnification; DSM 982 Gemini (Zeiss Oberkochen)] by K. Kühni Boghenbor and M. H. Stoffel (Division of Veterinary Anatomy, University of Berne, Switzerland).

The novel strains can be distinguished from other species of the genus *Staphylococcus* by phenotypic and genetic criteria. On the basis of these distinct characteristics, we

propose to classify these strains within a novel *Staphylococcus* species, *Staphylococcus rostri* sp. nov.

Description of *Staphylococcus rostri* sp. nov.

Staphylococcus rostri (ros'tri. L. n. *rostrum* the snout of a swine; L. gen. n. *rostri* of a pig's snout).

Cells are Gram-stain-positive, non-motile cocci (0.5–1.1 µm). After 24 h of growth at 37 °C, colonies reach 2 mm in diameter, are slightly convex, smooth, glossy and opaque white and display α-haemolysis on sheep-blood (TSA-SB) agar. Strains have a positive Christie–Atkins–Munch–Petersen (CAMP) reaction with β-toxin-producing *S. aureus* JA32, indicating that the α-haemolysis that appears on sheep-blood agar is generated by the production of a δ-haemolysin (Frenay *et al.*, 1999). Grows aerobically and anaerobically. Grows with 9 and 12 % NaCl (w/v) and at 43 °C, but not at 45 or 15 °C. Catalase-positive, oxidase-negative and does not coagulate rabbit plasma. Does not agglutinate with Slidex or Pastorex, which identify *S. aureus*. Hydrolyses DNA at 37 °C, generating a 2–5 mm degradation halo, but does not produce thermostable nucleases. Does not produce urease, ornithine decarboxylase, β-galactosidase, β-glucuronidase, acetoin or arginine arylamidase. Positive for aerobic production of acids from D-glucose, D-fructose and sucrose, activities of pyrrolidonyl arylamidase, alanine arylamidase and alkaline phosphatase and reduction of

nitrate. No acid is produced from D-mannose, maltose, raffinose, D-ribose, D-mannitol, turanose, D-arabinose or cellobiose. Variable reactions are obtained for acid production from lactose (3 of 20 strains negative), trehalose (2 of 20 negative) and N-acetylglucosamine (3 of 20 negative). Variable reactions are also obtained for the production of arginine dihydrolase (4 of 20 negative). Hydrolyses aesculin weakly (one strain negative, 19 weakly positive). All known strains are susceptible to novobiocin and lysostaphin.

The type strain is ARI 262^T (=DSM 21968^T =CCUG 57266^T). Strain ARI 602 (=DSM 21969 =CCUG 57267) is a reference strain. These strains and 18 other strains were isolated from the snouts of pigs.

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