Reclassification of *Staphylococcus pulvereri* Zakrzewska-Czerwińska *et al.* 1995 as a later synonym of *Staphylococcus vitulinus* Webster *et al.* 1994

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A polyphasic taxonomic approach was applied to strains of the species *Staphylococcus vitulinus* and *Staphylococcus pulvereri* in order to clarify their taxonomic relatedness. Four reference strains, representing both species, and seven strains isolated from human clinical material were characterized by biotyping, ribotyping and SDS-PAGE analysis of whole-cell proteins, and none of the screening approaches allowed the two taxa to be distinguished. DNA–DNA hybridization experiments between four selected representative strains, including the type strains, confirmed that *Staphylococcus pulvereri* is a later synonym of *Staphylococcus vitulinus*.

Staphylococcus vitulinus and *Staphylococcus pulvereri* represent novobiocin-resistant and oxidase-positive staphylococci, together with *Staphylococcus fleurettii*, *Staphylococcus sciuri* and *Staphylococcus lentus*. Both species have been isolated from human and animal specimens. *S. vitulinus* was described by Webster *et al.* (1994) as *Staphylococcus vitulus*; the name was later corrected to *S. vitulinus* by Trüper & De'Clari (1998). The species was clearly separated by biotyping, ribotyping and DNA–DNA hybridization from *S. sciuri* and *S. lentus*. Zakrzewska-Czerwińska *et al.* (1995) described *S. pulvereri*; all strains representing this taxon were clearly separated from the novobiocin-resistant oxidase-positive staphylococci *S. sciuri* and *S. lentus* by molecular as well as chemotaxonomic methods, but unfortunately this study did not include representatives of *S. vitulinus*. Another member of this group, *S. fleurettii*, was described by Vernozy-Rozand *et al.* (2000), who included all the above-mentioned taxa in their study except *S. pulvereri*. Petráš (1998) opened the question of the synonymy of *S. vitulinus* and *S. pulvereri* on the basis of biochemical testing of *S. pulvereri* strains NT 215^T (=CCM 4481^T), NT 322 and ATCC 51699 and *S. vitulinus* SVI^T (=CCM 4511^T). He obtained aberrant results in 12

biochemical tests for one or more strains tested compared with the original descriptions. The very close phylogenetic relationship of these two staphylococci based on 16S rRNA gene sequence analysis was shown by Takahashi *et al.* (1999). Similarly, Poyart *et al.* (2001) found 99.5 % identity of the *sodA_{int}* gene sequences of *S. vitulinus* and *S. pulvereri* representatives. Chesneau *et al.* (2000) found either similar or identical ribotypes of these two species. Recently, Kwok & Chow (2003) confirmed the high 16S rRNA gene sequence similarity (99 %) and showed 98 % partial *hsp60* gene sequence similarity. Although all of these studies indicated the synonymy of these two species, their taxonomic status has not been clearly revised and clarified.

Bacterial reference strains were obtained from the Czech Collection of Microorganisms (CCM) and from the American Type Culture Collection (ATCC). The Reference Laboratory for Staphylococci, National Institute of Public Health (Prague, Czech Republic) provided seven novel *S. pulvereri*-/*S. vitulinus*-like isolates from human clinical origin: strain 98/147 from gastric contents of a newborn, strain 98/779 from an abscess, strain 01/896 from a skin smear during mycosis, strain 02/219 from pectoral puncture fluid and strains 01/317, 95/597 (=CCM 4512) and 02/418 (=CCM 7101) from urine.

Biotyping was performed by API STAPH and ID 32 STAPH

Fig. 1. Dendrogram based on cluster analysis of ribotype patterns (obtained with *EcoRI*) of *S. vitulinus*, *S. pulvereri* and related oxidase-positive taxa *S. fleurettii*, *S. lentus* and *S. sciuri*.

(bioMérieux) as well as by conventional tests as described by Mannerová *et al.* (2003). Biochemical test results assigned the novel isolates to the *S. vitulinus*/*S. pulvereri* species group, but could not assign them to one of the two species due to different and variable reactions in several tests when comparing with both original species descriptions.

Ribotyping with *EcoRI* and a probe complementary to the 16S and 23S rRNA of *Escherichia coli* as well as statistical analysis and dendrogram construction were done as described by Švec *et al.* (2001). Ribotyping grouped all analysed strains, including *S. vitulinus* and *S. pulvereri* representative strains obtained from bacterial collections, into a single cluster (Fig. 1). This group was clearly differentiated from the other novobiocin-resistant and oxidase-positive species, *S. fleurettii*, *S. lentus* and all three *S. sciuri* subspecies.

Whole-cell protein profile analysis, including preparation of whole-cell protein extracts from cells grown for 24 h on nutrient agar (Oxoid CM3) at 37 °C, SDS-PAGE, densitometric analysis and reading of protein profiles, as well as their numerical analysis, were performed in accordance with the procedure described by Pot *et al.* (1994). Protein profile analysis was performed using GelComparII software (Applied Maths). The similarity between all pairs of traces

was expressed by Pearson's product-moment correlation coefficient, converted for convenience to a percentage value. UPGMA clustering was used for the construction of the dendrogram. The level of reproducibility was investigated by the inclusion of duplicate protein extracts of several strains and a mean correlation of 0.93 was obtained. All *S. vitulinus* and *S. pulvereri* strains were grouped in one cluster with similarity values ranging from 89 to 98 % (Fig. 2). This cluster was separated from related novobiocin-resistant and oxidase-positive staphylococci.

Whole genomic DNAs for DNA–DNA hybridization were extracted from *S. vitulinus* CCM 4511^T, *S. pulvereri* CCM 4481^T and CCM 4482 and from clinical isolate CCM 7101 as described before (Mannerová *et al.*, 2003). As described by the latter authors, DNA–DNA hybridization experiments were performed using the microplate method and determination of the DNA base compositions was done using HPLC. Hybridizations were performed at 33 °C in hybridization mixture (2 × SSC, 5 × Denhardt solution, 2.5 % dextran sulfate, 50 % formamide, 100 µg denatured salmon sperm DNA ml⁻¹, 1250 ng biotinylated probe DNA ml⁻¹). The DNA–DNA relatedness values obtained ranged from 90 to 100 % and confirmed that all four strains represent one species. The DNA G + C content was 33 mol% for all four strains.

Fig. 2. Dendrogram based on SDS-PAGE analysis of *S. vitulinus*, *S. pulvereri* and related novobiocin-resistant and oxidase-positive staphylococci.

All data presented in this study as well as all results published previously show clearly the synonymy of *S. vitulinus* and *S. pulvereri*. The reclassification of *S. pulvereri* as a later synonym of *S. vitulinus* is therefore proposed in accordance with Rule 24a of the Bacteriological Code (Lapage *et al.*, 1992).

Emended description of *Staphylococcus vitulinus* Webster *et al.* 1994

Biochemical test results obtained in this study were nearly in full agreement with the species description of *S. vitulinus*, including variable reactions published by Webster *et al.* (1994). The only differences were found for acidification of mannitol (described as positive for *S. vitulinus*; we found three negative strains) and ribose (described as variable; all our strains were negative). Contradictory results were obtained for mannose acidification (described as negative for *S. vitulinus*). All our strains were negative for this test in ID 32 STAPH kit, but all were positive in API STAPH kit, and conventional testing revealed four positive strains. In addition to the species description, all tested strains were positive for glucose acidification and gelatin hydrolysis; negative for acidification of melibiose and methyl α -D-glucopyranoside, Tween 80 as well as arginine dihydrolase and DNase production.

Differentiation of *S. vitulinus* from the other novobiocin-resistant and oxidase-positive species, *S. lentus*, *S. sciuri* (including all three subspecies) and *S. fleurettii*, remains unchanged as described by Webster *et al.* (1994) and Vernozy-Rozand *et al.* (2000) except for the mannose acidification test, which gives test-kit-dependent results as discussed above. Moreover, a few controversial biochemical test results were noted for the *S. fleurettii* type strain in this study. The type strain of *S. fleurettii*, CCM 4922^T, described as turanose- and L-arabinose-positive, was repeatedly turanose- as well as L-arabinose-negative with the ID 32 STAPH kit and in conventional tests; similarly, trehalose acidification, described as positive for this strain, was negative in the ID 32 STAPH kit, positive in the API STAPH kit and delayed-positive (3 days) in the conventional test.

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