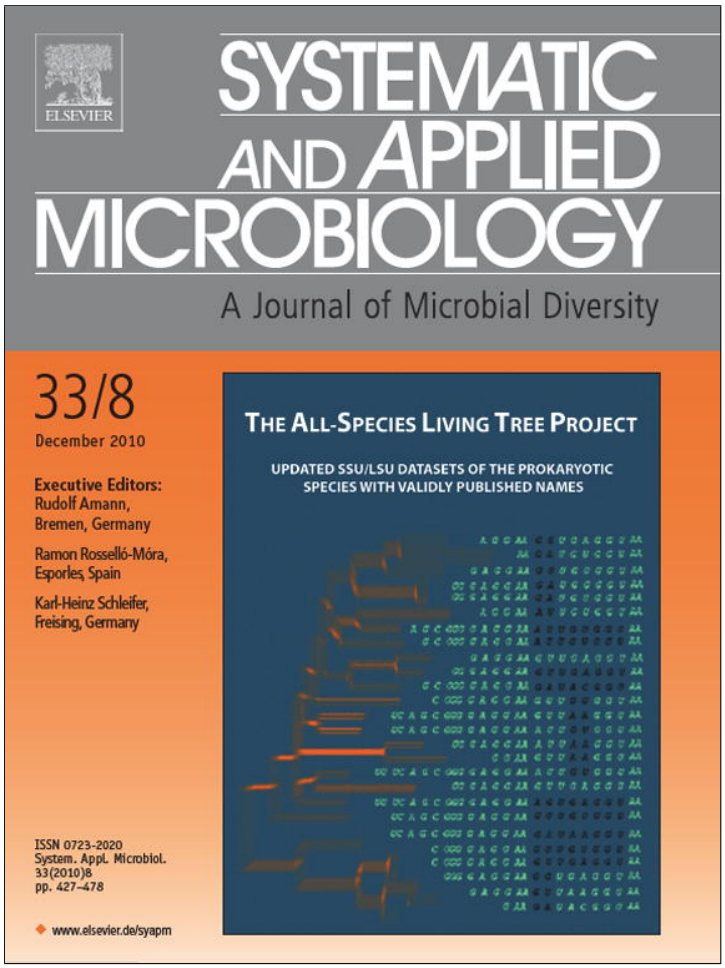

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Identification of *Staphylococcus* spp. using $(GTG)_5$-PCR fingerprinting

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ABSTRACT

A group of 212 type and reference strains deposited in the Czech Collection of Microorganisms (Brno, Czech Republic) and covering 41 *Staphylococcus* species comprising 21 subspecies was characterised using rep-PCR fingerprinting with the $(GTG)_5$ primer in order to evaluate this method for identification of staphylococci. All strains were typeable using the $(GTG)_5$ primer and generated PCR products ranging from 200 to 4500 bp. Numerical analysis of the obtained fingerprints revealed (sub)species-specific clustering corresponding with the taxonomic position of analysed strains. Taxonomic position of selected strains representing the (sub)species that were distributed over multiple rep-PCR clusters was verified and confirmed by the partial *rpoB* gene sequencing. *Staphylococcus caprae*, *Staphylococcus equorum*, *Staphylococcus sciuri*, *Staphylococcus piscifermentans*, *Staphylococcus xylosus*, and *Staphylococcus saprophyticus* revealed heterogeneous fingerprints and each (sub)species was distributed over several clusters. However, representatives of the remaining *Staphylococcus* spp. were clearly separated in single (sub)species-specific clusters. These results showed rep-PCR with the $(GTG)_5$ primer as a fast and reliable method applicable for differentiation and straightforward identification of majority of *Staphylococcus* spp.

Introduction

Staphylococci are major natural inhabitants of skin, skin glands and mucous membranes of humans and many animals. They have been isolated from a wide variety of environmental sources and food, and they are recognised as most important agents causing a wide range of human and veterinary infections [23]. Not only the well-known methicillin-resistant *Staphylococcus aureus* (MRSA) strains, but also the highly resistant coagulase-negative staphylococci are important causative agents of nosocomial diseases, particularly bloodstream infections. Reliable identification of staphylococcal species using traditional biochemical testing is becoming difficult nowadays due to the increasing number of known staphylococcal (sub)species often revealing considerable phenotypic similarity of each other. Plenty of phenotypic and genotypic methods have been described for characterisation of staphylococci [25]; however, many of these techniques require specialised equipment unattainable to routine laboratories. Repetitive element sequence-based polymerase chain reaction (rep-PCR) fingerprinting represents an easy-to-perform technique utilising primers directed to repetitive sequences interspersed in bacterial genomes [33]. A wide variety of the repetitive sequences representing several families have been found among bacteria [11,34]. A few studies showed that rep-PCR primers are not amplifying only specific repetitive DNA regions, but they are probably hybridizing with similar arbitrary sequences due to the low annealing temperatures used in rep-PCR protocols [5,24,36]. Although these results are challenging the definition of the basic rep-PCR principle [33] they have no impact on the practical applicability of these methods for characterisation of bacterial strains.

The oligonucleotide primers complementary to BOX, ERIC, REP and $(GTG)_5$ sequences are most frequently used in rep-PCR assays applied in bacterial taxonomic studies. Automatic performance of the rep-PCR typing of bacteria was developed and is possible using the DiversiLab™ system [8]. These methods have been shown as valuable tools for typing and identification of different bacterial taxa e.g. lactobacilli [6], enterococci [26], geobacilli [16], streptomycetes [14] or acetic acid bacteria [3]. Application of rep-PCR methods for identification of staphylococci was demonstrated only in a few studies [13,35]. However, these works dealing with particular groups of staphylococci cover only a limited number of hitherto described staphylococcal species. According to our best knowledge, an extensive evaluation of a rep-PCR fingerprinting method for the identification of *Staphylococcus* spp. has not been reported in the literature.

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The aim of the present study was to apply rep-PCR fingerprinting using the $(GTG)_5$ primer for the characterisation of a group of strains containing multiple representatives of *Staphylococcus* spp. originating from different sources to determine the value of this method for identification of staphylococci.

Materials and methods

Bacterial strains

A group of 212 reference and type strains representing 41 *Staphylococcus* species (comprising 21 subspecies) was included in the present study (Fig. 1). All strains were obtained from the Czech Collection of Microorganisms (Brno, Czech Republic). A list of individual strains including the information about their origin is given in Table S1. Further data are available via the online catalogue of the Czech Collection of Microorganisms (http://www.sci.muni.cz/ccm/). An additional group of *Staphylococcus intermedius* and *Staphylococcus pseudintermedius* strains originating from veterinary and human clinical materials (Fig. S4) was obtained from the Reference Laboratory for Staphylococci, National Institute of Public Health, Prague, Czech Republic (35 strains) and from the BCCM/LMG Bacteria Collection, Ghent, Belgium (strains LMG 9079 and LMG 19136).

Repetitive sequence-based PCR (rep-PCR) fingerprinting

The rep-PCR fingerprinting was performed as described previously [27]. Briefly, the total genomic DNA was isolated by alkaline extraction procedure as follows: a 1 μl loopful of bacterial cells was homogenised in 20 μl of lysis solution (0.25% SDS, 0.05 M NaOH) and heated at 95 °C for 15 min. Obtained cell lysate was diluted by adding of 180 μl of sterile deionized water, centrifuged at 13,000 rpm for 5 min and maintained at −20 °C. Isolated DNA was amplified using the $(GTG)_5$ primer: 5′-GTGGTGGTGGTGGTG-3′. Totally, 1 μl of a cell lysate and 24 μl of a PCR mixture containing 1 μM of $(GTG)_5$ primer, 200 μM of each dNTP (Jena Bioscience), 2.5 μl of 10× ThermoPol Reaction Buffer (100 mM KCl, 100 mM $(NH_4)_2SO_4$, 200 mM Tris–HCl, 20 mM $MgSO_4$, 0.1% Triton X-100, pH 8.8) and 2 U of *Taq* DNA polymerase (New England BioLabs) were included into PCR reactions performed in a Tpersonal thermocycler (Biometra). Initial denaturation (94 °C, 7 min) was followed by 30 cycles of denaturation (94 °C, 1 min), primer annealing (40 °C, 1 min), and extension (65 °C, 8 min). The last cycle was followed by the final single extension step (65 °C, 16 min). Obtained PCR products were separated for 16 h at 1.55 V cm^{-1} in 1.5% (w/v) agarose gels (20 × 25 cm) containing ethidium bromide (0.5 μg mL^{-1}). Molecular weight marker consisting of a mixture of 100 bp and 500 bp PCR Molecular Rulers (Bio-Rad) was positioned in every sixth lane to allow later normalisation of gel images. The resulting fingerprints were visualised under the UV light 302 nm, digitised using a CCD camera and processed by the Bionumerics v. 6.0 software (Applied Maths). The dendrogram was constructed using Pearson's correlation coefficients with the unweighted pair-group method using arithmetic averages (UPGMA) clustering method. Optimization value of 2% was automatically calculated by the Bionumerics software and allowed for the densitometric curves.

RNA polymerase β-subunit (rpoB) gene sequencing

Partial *rpoB* gene sequences (nucleotides 1444–1858 corresponding to *S. aureus rpoB* gene positions of the GenBank accession number X64172) were determined for the selected strains of (sub)species that were distributed over multiple clusters based on rep-PCR pattern analysis. The anticipated gene fragments for the *rpoB* were amplified with primers rpoB 1418f: 5′-CAATTCATGGACCAAGC-3′ and rpoB 3554r: 5′-CCGTCCCAAGTCATGAAAC-3′ as described previously [17]. The obtained amplicons were purified with a QIAquick PCR Purification Kit (Qiagen) and sequenced with rpoB 1418f and rpoB 1876r: 5′-GAGTCATCITTYTCTAAGAATGG-3′ primers by Eurofins MWG Operon Sequencing Department (Germany). The GenBank/EMBL/DDBJ accession numbers for *rpoB* gene sequences determined in this study are HM146311–HM146327 and HQ259711–HQ259717; *rpoB* gene sequences of type strains of analysed staphylococcal taxa were retrieved from the GenBank database. The sequences were analysed using BioNumerics software v. 6.0 (Applied Maths).

Results

All analysed *Staphylococcus* spp. strains were typeable using the $(GTG)_5$ primer and generated PCR products ranging from 200 to 4500 bp. Two independent DNA isolations and $(GTG)_5$-PCR runs were performed on a subgroup of 20 reference strains selected randomly from the analysed group to verify reproducibility of the method (Fig. S1). All couples of strains revealed visually close fingerprint profiles. Generally, differences in the band and background signal intensities between fingerprint patterns originating from the same strain were evident. Certain fingerprint patterns obtained from a single strain showed variability in the presence of a few bands (e.g. *Staphylococcus capitis* subsp. *capitis* CCM 2764^T or *Staphylococcus xylosus* CCM 2738^T), however these minor differences did not influence the final cluster analysis and the grouping remained stable because the basic band patterns typical for individual staphylococcal taxa were not significantly affected.

Cluster analysis of the $(GTG)_5$-PCR fingerprints obtained from the tested *Staphylococcus* spp. clustered most of the analysed strains into well-separated groups representing individual species or subspecies (Fig. 1). Fingerprint profiles revealed by individual taxa were generally visually similar, and distinctive (sub)species-specific basic band patterns were evident. The majority of the species formed single cluster except the following cases. Two *Staphylococcus caprae* reference strains CCM 4546 and CCM 7784 were separated from the type strain *S. caprae* CCM 3573^T. Both *Staphylococcus equorum* and *Staphylococcus sciuri* subspecies were split into two clusters and one extra strain separated from the two clusters. *Staphylococcus piscifermentans* and *S. xylosus* representatives were split into two groups. *Staphylococcus saprophyticus* subsp. *saprophyticus* CCM 2354 was separated from eleven remaining *S. saprophyticus* strains grouped in a single cluster. The most heterogeneous band patterns were revealed by *S. sciuri* strains. Certain subspecies were clearly differentiated from each other using the $(GTG)_5$-PCR fingerprinting (*S. capitis* subsp. *capitis* and subsp. *ureolyticus*; *Staphylococcus cohnii* subsp. *cohnii* and subsp. *urealyticum*). Differentiation of *Staphylococcus carnosus* subsp. *carnosus* from subsp. *utilis* was not so obvious because both subspecies formed two subclusters within a single cluster. Similarly, *S. aureus* subsp. *anaerobius* strains formed a subcluster within *S. aureus* subsp. *aureus* cluster. Analysed strains of the remaining subspecies of *S. hominis*, *S. schleiferi* and *S. succinus* did not form separate subspecies-specific clusters.

Seventeen representative strains revealing diverging $(GTG)_5$-PCR fingerprint profiles separated from the remaining band patterns generated by type strains or the remaining members of the same species were re-identified using the partial *rpoB* gene sequencing to verify their taxonomic position (Figs. S2 and S3). The identification results obtained by the *rpoB* gene sequencing corresponded with the original assignment of all strains except the

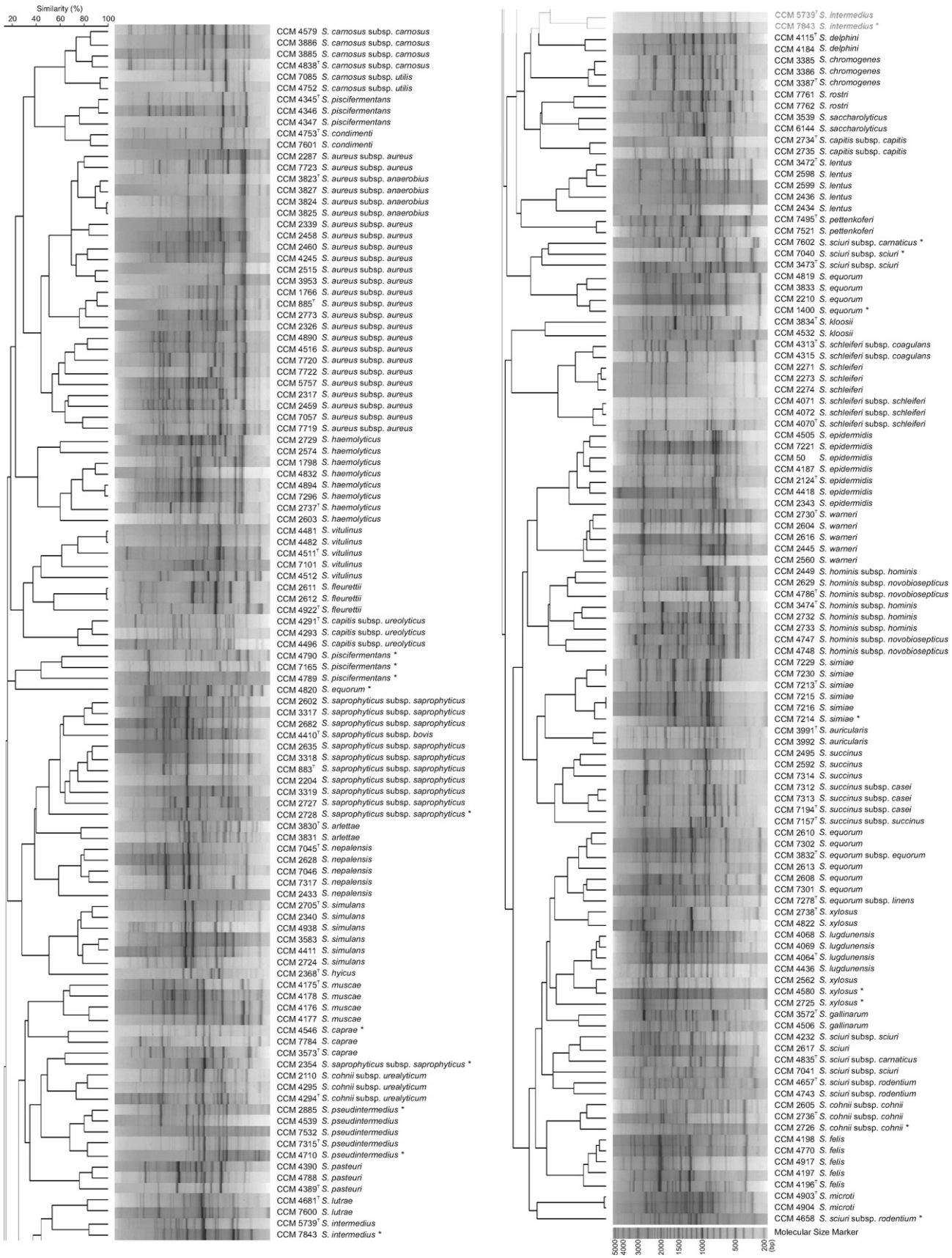

Fig. 1. Dendrogram based on the cluster analysis of the $(GTG)_5$-PCR fingerprint profiles revealed by *Staphylococcus* spp. The dendrogram was constructed with the Pearson's product moment correlation coefficients using the UPGMA clustering method. The strains marked with asterisks (*) were characterised using the rpoB gene sequence analysis.

strains CCM 2885 and CCM 4710 originally deposited as *S. intermedius* in the CCM collection. These two strains were grouped with *S. pseudintermedius* type and reference strains CCM 7315^T and CCM 7532 on the basis of $(GTG)_5$-PCR cluster analysis. The *rpoB* gene sequences from the strains CCM 2885 and CCM 4710 were clearly differentiated from the *rpoB* gene sequence of *S. intermedius* type strain (accession number AF325869) and they both showed 99.75% similarity with the *rpoB* gene sequence of *S. pseudintermedius* (accession number EU888126) which confirmed both strains as representatives of *S. pseudintermedius* species. The reclassification stimulated us to study additional 37 strains identified tentatively as *S. intermedius* which were obtained from the National Institute of Public Health and from the BCCM/LMG Bacteria Collection. They were all but one clearly clustered with *S. pseudintermedius* reference strains. Only one strain NRL/St 03/087 (=CCM 7843) showed $(GTG)_5$-PCR fingerprint corresponding to *S. intermedius* CCM 5739^T (Fig. S4). The *rpoB* gene sequence of *S. intermedius* NRL/St 03/087 (=CCM 7843) (accession number HQ259717) was identical (100% similarity) with the *rpoB* sequence of the type strain *S. intermedius* CIP 8160^T (accession number AF325869). The similarity level of the *rpoB* sequences of six representative *S. pseudintermedius* strains (HQ259711–HQ259716) towards the type strain *S. pseudintermedius* CCM 7315^T (accession number EU888126) were 99.94% (one residue difference) and clearly confirmed identification outcomes obtained by the $(GTG)_5$-PCR fingerprinting.

Discussion

The presented results demonstrate that $(GTG)_5$-PCR fingerprinting is an efficient tool for taxonomic delineation and identification of majority of *Staphylococcus* spp. Most of the staphylococcal species formed (sub)species-specific clusters corresponding with the taxonomic position of analysed strains in the present study. Only *S. caprae*, *S. equorum*, *S. piscifermentans*, *S. saprophyticus*, *S. sciuri* and *S. xylosus* species revealed heterogeneous fingerprints distributed over multiple clusters. Two *S. caprae* strains CCM 4546 and CCM 7784 originating from human clinical material were separated from the type strain *S. caprae* CCM 3573^T which originates from goat milk. This finding implies that some correlation between the source of isolation and the resulting $(GTG)_5$-PCR fingerprints may exist, but such a correlation was not obvious among the remaining species revealing heterogeneous fingerprints. However, separation of certain taxa into intraspecies $(GTG)_5$-PCR subclusters has been already described in the literature as in the case of *Enterococcus faecium* [26] or *Streptococcus mutans* [27].

Notable results were obtained with erroneously identified *S. intermedius* strains which were reclassified as *S. pseudintermedius* in the present study. In total, three strains (CCM 2885, CCM 4539, and CCM 4710) deposited originally as *S. intermedius* in the CCM collection were reclassified as *S. pseudintermedius*. Subsequently, a group of 36 additional strains originating from the Reference Laboratory for Staphylococci and from the BCCM/LMG Bacteria Collection and classified tentatively as *S. intermedius* were revealed to be representatives of *S. pseudintermedius*. These two phylogenetically close species are difficult to differentiate using classical phenotypical methods but the presented results showed that they can be reliably identified using the $(GTG)_5$-PCR fingerprinting. Similar results we published by Sasaki et al. [21] who analysed a series of 117 phenotypically identified *S. intermedius* strains. Identification of these strains using *sodA*, *hsp60* and *nuc* gene sequencing showed that the large majority (83 strains) was represented by *S. pseudintermedius* but only 12 strains were true *S. intermedius* in their study.

In contrast to the present work the application of various rep-PCR fingerprinting methods for the characterisation of staphylococci has been mostly utilised in studies dealing with differentiation of staphylococcal isolates to the strain level. Rantsiou et al. [19] and Iacumin et al. [10] applied rep-PCR fingerprinting for typing of *S. xylosus* strains isolated from fermented sausages. Epidemiological typing of *S. aureus* strains was performed using the primers RW3A [4,18,32], ERIC [30], REP [37] and IS*256* [5]. The DiversiLab^{TM} system has been used for typing of *S. aureus* [7,20,22,28] and *Staphylococcus epidermidis* [29]. In contrast, application of rep-PCR fingerprinting for identification of staphylococci has been described sporadically in the literature. Wieser and Busse [35] demonstrated the REP primer as unsuitable for identification of *S. epidermidis* while the BOX and ERIC primers revealing *S. epidermidis* species-specific fingerprints have been shown as reliable tools for identification of this species. Another study dealing with the application of rep-PCR with the $(GTG)_5$ primer for identification of *Staphylococcus* spp. was published by Koreňová et al. [13] who described the application of this method for the identification of staphylococci associated with ewe's milk and meat processing enterprises. However, these two studies covered only a limited number of staphylococcal species.

In this study, particular differences in the DNA band patterns and background intensities were found between fingerprints originating from the repeatedly tested strains. Isolation of the whole genomic DNA using an alkaline extraction procedure is the most difficult to standardise step in the rep-PCR protocol performed in this work. This fast procedure significantly speeds up and reduces laboriousness and expenses, however it could provide variable DNA concentration in the obtained cell lysates due to different cell quantity picked up using a bacteriological loop and physiological characteristics of the bacterial culture [1]. Various template quality and DNA concentrations included in the rep-PCR reactions can theoretically result in the fingerprint profiles revealing different band intensities; bands exhibiting poor/weak intensity could be even missing in a reaction with low template DNA concentration. Taking these facts in consideration, visual inspection of the fingerprint profiles and careful evaluation of the cluster analysis results is important for obtaining reliable taxonomic outcomes, as recommended also in a study published by Burr and Pepper [2]. The slightly different band patterns obtained in our study repeatedly from the same strain did not affect clustering of individual strains into (sub)species-specific clusters; however they limit applicability of the rep-PCR fingerprinting method performed in this work for the strain-typing purposes. These results correspond with previously published studies dealing with reproducibility of rep-PCRs. Minor differences in the band intensity of individual $(GTG)_5$-PCR fingerprints were noticed between repeatedly tested *Lactobacillus* [6], *Enterococcus* [26] and *Acinetobacter baumannii* [9] strains, and a good reproducibility of rep-PCRs with RW3A and ERIC primers was demonstrated by Kang and Dunne [12]. However, DNA used in these studies was isolated using a classical phenol-chlorophorm isolation procedure or using a commercial kit. In contrast, rep-PCR performed with the DNAs isolated by a simple lysis of cells showed less satisfactory results than that with purified DNA. Reproducibility of rep-PCR fingerprinting with the ERIC primer has been questioned by Meacham et al. [15] who found quantitative differences in the presence and intensity of bands between fingerprints revealed by the repeatedly analysed strain. Similarly, low intralaboratory and interlaboratory reproducibility of rep-PCR techniques was shown by Deplano et al. [5] in a multicenter study dealing with typing of *S. aureus*. They obtained markedly different rep-PCR fingerprints from the same strain using the same methodology in different laboratories; however the clustering of the analysed strains into related patterns was reproducible in their study. It is generally known that the application of rep-PCR methods for epidemiological studies, especially for setting up libraries in longitudinal studies dealing with high numbers of strains is strongly limited by high sensitivity of PCR fingerprinting to minor variations in experimental

conditions [31]. However, application of these methods for identification purposes is not so significantly influenced by such variations, because the final clustering is not usually affected by minor differences in fingerprint profiles as shown in our work.

In summary, the $(GTG)_5$-PCR fingerprinting of a group of 212 staphylococci covering numerous representatives of *Staphylococcus* spp. revealed (sub)species-specific clustering corresponding predominantly with the taxonomic position of analysed strains and were grouped into a single cluster in the present study. Out of 41 analysed species only *S. caprae*, *S. equorum*, *S. piscifermentans*, *S. saprophyticus*, *S. sciuri* and *S. xylosus* species revealed heterogeneous fingerprints and were distributed over multiple clusters. Obtained results imply $(GTG)_5$-PCR fingerprinting as a valuable tool for delineation of staphylococci originating from different sources. The rapid performance and low laboriousness of the $(GTG)_5$-PCR fingerprinting favour this method for the fast initial screening of numerous groups of strains enabling straightforward identification of the majority of *Staphylococcus* spp.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.syapm.2010.09.004.

References

[1] Abolmaaty, A., El-Shemy, M.G., Khallaf, M.F., Levin, R.E. (1998) Effect of lysing methods and their variables on the yield of *Escherichia coli* O157: H7 DNA and its PCR amplification. J. Microbiol. Methods 34, 133–141.
[2] Burr, M.D., Pepper, I.L. (1997) Variability in presence-absence scoring of AP PCR fingerprints affects computer matching of bacterial isolates. J. Microbiol. Methods 29, 63–68.
[3] De Vuyst, L., Camu, N., De Winter, T., Vandemeulebroecke, K., Van de Perre, V., Vancanneyt, M., De Vos, P., Cleenwerck, I. (2008) Validation of the $(GTG)_5$-rep-PCR fingerprinting technique for rapid classification and identification of acetic acid bacteria, with a focus on isolates from Ghanaian fermented cocoa beans. Int. J. Food Microbiol. 125, 79–90.
[4] Del Vecchio, V., Petroziello, J., Gress, M., McCleskey, F., Melcher, G., Crouch, H., Lupski, J. (1995) Molecular genotyping of methicillin-resistant *Staphylococcus aureus* via fluorophore-enhanced repetitive-sequence PCR. J. Clin. Microbiol. 33, 2141–2144.
[5] Deplano, A., Schuermans, A., Van Eldere, J., Witte, W., Meugnier, H., Etienne, J., Grundmann, H., Jonas, D., Noordhoek, G.T., Dijkstra, J., van Belkum, A., van Leeuwen, W., Tassios, P.T., Legakis, N.J., van der Zee, A., Bergmans, A., Blanc, D.S., Tenover, F.C., Cookson, B.C., O'Neil, G., Struelens, M.J., The European Study Group on Epidemiological Markers of the ESCMID (2000) Multicenter evaluation of epidemiological typing of methicillin-resistant *Staphylococcus aureus* strains by repetitive-element PCR analysis. J. Clin. Microbiol. 38, 3527–3533.
[6] Gevers, D., Huys, G., Swings, J. (2001) Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. FEMS Microbiol. Lett. 205, 31–36.
[7] Grisold, A.J., Zarfel, G., Hoenigl, M., Krziwanek, K., Feierl, G., Masoud, L., Leitner, E., Wagner-Eibel, U., Badura, A., Marth, E. (2010) Occurrence and genotyping using automated repetitive-sequence-based PCR of methicillin-resistant *Staphylococcus aureus* ST398 in Southeast Austria. Diagn. Microbiol. Infect. Dis. 66, 217–221.
[8] Healy, M., Huong, J., Bittner, T., Lising, M., Frye, S., Raza, S., Schrock, R., Manry, J., Renwick, A., Nieto, R., Woods, C., Versalovic, J., Lupski, J.R. (2005) Microbial DNA typing by automated repetitive-sequence-based PCR. J. Clin. Microbiol. 43, 199–207.
[9] Huys, G., Cnockaert, M., Nemec, A., Dijkshoorn, L., Brisse, S., Vaneechoutte, M., Swings, J. (2005) Repetitive-DNA-element PCR fingerprinting and antibiotic resistance of pan-European multi-resistant *Acinetobacter baumannii* clone III strains. J. Med. Microbiol. 54, 851–856.
[10] Iacumin, L., Comi, G., Cantoni, C., Cocolin, L. (2006) Molecular and technological characterization of *Staphylococcus xylosus* isolated from naturally fermented Italian sausages by RAPD, rep-PCR and Sau-PCR analysis. Meat Sci. 74, 281–288.
[11] Ishii, S., Sadowsky, M.J. (2009) Applications of the rep-PCR DNA fingerprinting technique to study microbial diversity, ecology and evolution. Environ. Microbiol. 11, 733–740.
[12] Kang, H.P., Dunne, W.M. (2003) Stability of repetitive-sequence PCR patterns with respect to culture age and subculture frequency. J. Clin. Microbiol. 41, 2694–2696.
[13] Koreňová, J., Lopašovská, J., Kuchta, T. (2009) Biofilm forming bacterial contaminants in small and medium-sized ewes' milk and meat processing enterprises in Slovakia. J. Food Nutr. Res. 48, 115–120.
[14] Lanoot, B., Vancanneyt, M., Dawyndt, P., Cnockaert, M., Zhang, J.L., Huang, Y., Liu, Z.H., Swings, J. (2004) BOX-PCR fingerprinting as a powerful tool to reveal synonymous names in the genus *Streptomyces*. Emended descriptions are proposed for the species *Streptomyces cinereorectus*, *S. fradiae*, *S. tricolor*, *S. colombiensis*, *S. filamentosus*, *S. vinaceus* and *S. phaeopurpureus*. Syst. Appl. Microbiol. 27, 84–92.
[15] Meacham, K.J., Zhang, L., Foxman, B., Bauer, R.J., Marrs, C.F. (2003) Evaluation of genotyping large numbers of *Escherichia coli* isolates by enterobacterial repetitive intergenic consensus-PCR. J. Clin. Microbiol. 41, 5224–5226.
[16] Meintanis, C., Chalkou, K.I., Kormas, K.A., Lymperopoulou, D.S., Katsifas, E.A., Hatzinikolaou, D.G., Karagouni, A.D. (2008) Application of *rpoB* sequence similarity analysis, REP-PCR and BOX-PCR for the differentiation of species within the genus Geobacillus. Lett. Appl. Microbiol. 46, 395–401.
[17] Mellmann, A., Becker, K., von Eiff, C., Keckevoet, U., Schumann, P., Harmsen, D. (2006) Sequencing and staphylococci identification. Emerg. Inf. Dis. 12, 333–336.
[18] Nascimento, J. dos S., Giambiagi-deMarval, M., de Oliveira, S.S., Ceotto, H., dos Santos, K.R.N., Bastos, M. do C. de F. (2005) Genomic fingerprinting of bacteriocin-producer strains of *Staphylococcus aureus*. Res. Microbiol. 156, 837–842.
[19] Rantsiou, K., Iacumin, L., Cantoni, C., Comi, G., Cocolin, L. (2005) Ecology and characterization by molecular methods of *Staphylococcus* species isolated from fresh sausages. Int. J. Food. Microbiol. 97, 277–284.
[20] Ross, T.L., Merz, W.G., Farkosh, A., Carroll, K.C. (2005) Comparison of an automated repetitive sequence-based PCR microbial typing system to pulsed-field gel electrophoresis for analysis of outbreaks of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. 43, 5642–5647.
[21] Sasaki, T., Kikuchi, K., Tanaka, Y., Takahashi, N., Kamata, S., Hiramatsu, K. (2007) Reclassification of phenotypically identified *Staphylococcus intermedius* strains. J. Clin. Microbiol. 45, 2770–2778.
[22] Shutt, C.K., Pounder, J.I., Page, S.R., Schaecher, B.J., Woods, G.L. (2005) Clinical evaluation of the DiversiLab microbial typing system using repetitive-sequence-based PCR for characterization of *Staphylococcus aureus* strains. J. Clin. Microbiol. 43, 1187–1192.
[23] Schleifer, K.H., Bell, J.A. (2009) Genus I. Staphylococcus Rosenbach 1884, 18AL (Nom. Cons. Opin. 17 Jud. Comm. 1958, 153.), in: De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.H., Whitman, W.B. (Eds.), Bergey's Manual of Systematic Bacteriology, The Firmicutes, vol. 3, Springer, New York, pp. 392–421.
[24] Snelling, A., Gerner-Smidt, P., Hawkey, P., Heritage, J., Parnell, P., Porter, C., Bodenham, A., Inglis, T. (1996) Validation of use of whole-cell repetitive extragenic palindromic sequence-based PCR (REP-PCR) for typing strains belonging to the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex and application of the method to the investigation of a hospital outbreak. J. Clin. Microbiol. 34, 1193–1202.
[25] Štěpán, J., Pantůček, R., Doškař, J. (2004) Molecular diagnostics of clinically important staphylococci. Folia Microbiol. 49, 353–386.
[26] Švec, P., Vancanneyt, M., Seman, M., Snauwaert, C., Lefebvre, K., Sedláček, I., Swings, J. (2005) Evaluation of $(GTG)_5$-PCR for identification of *Enterococcus* spp. FEMS Microbiol. Lett. 247, 59–63.
[27] Švec, P., Nováková, D., Žáčková, L., Kukletová, M., Sedláček, I. (2008) Evaluation of $(GTG)_5$-PCR for rapid identification of *Streptococcus mutans*. Anton. Leeuw. Int. J. G. 94, 573–579.
[28] te Witt, R., Kanhai, V., van Leeuwen, W.B. (2009) Comparison of the DiversiLab™ system, pulsed-field gel electrophoresis and multi-locus sequence typing for the characterization of epidemic reference MRSA strains. J. Microbiol. Methods 77, 130–133.
[29] Trevino, M., Martinez-Lamas, L., Romero-Jung, P.A., Giraldez, J.M., Alvarez-Escudero, J., Regueiro, B.J. (2009) Endemic linezolid-resistant *Staphylococcus epidermidis* in a critical care unit. Eur. J. Clin. Microbiol. Infect. Dis. 28, 527–533.
[30] van Belkum, A., Bax, R., Peerbooms, P., Goessens, W.H.F., Vanleeuwen, N., Quint, W.G.V. (1993) Comparison of phage typing and DNA fingerprinting by polymerase chain reaction for discrimination of methicillin-resistant *Staphylococcus aureus* strains. J. Clin. Microbiol. 31, 798–803.
[31] van Belkum, A., Tassios, P.T., Dijkshoorn, L., Haeggman, S., Cookson, B., Fry, N.K., Fussing, V., Green, J., Feil, E., Gerner-Smidt, P., Brisse, S., Struelens, M. (2007) Guidelines for the validation and application of typing methods for use in bacterial epidemiology. Clin. Microbiol. Infect. 13, 1–46.
[32] van der Zee, A., Verbakel, H., van Zon, J.C., Frenay, I., van Belkum, A., Peeters, M., Buiting, A., Bergmans, A. (1999) Molecular genotyping of *Staphylococcus aureus* strains: comparison of repetitive element sequence-based PCR with various typing methods and isolation of a novel epidemicity marker. J. Clin. Microbiol. 37, 342–349.
[33] Versalovic, J., Schneider, M., de Bruijn, F.J., Lupski, J.R. (1994) Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Methods Mol. Cell. Biol. 5, 25–40.
[34] Versalovic, J., Lupski, J.R. (1999) Interspersed repetitive sequences in bacterial genomes. In: De Bruijn, F.J., Lupski, J.R., Weinstock, G.M. (Eds.), Bacterial Genomes: Physical Structure and Analysis, 2nd ed., Kluwer Academic Publishers, Norwell, MA, pp. 38–48.

[35] Wieser, M., Busse, H.J. (2000) Rapid identification of *Staphylococcus epidermidis*. Int. J. Syst. Evol. Microbiol. 50, 1087–1093.
[36] Wilson, L.A., Sharp, P.M. (2006) Enterobacterial repetitive intergenic consensus (ERIC) sequences in *Escherichia coli*: evolution and implications for ERIC-PCR. Mol. Biol. Evol. 23, 1156–1168.
[37] Zhong, Z., Chai, T., Duan, H., Miao, Z., Li, X., Yao, M., Yuan, W., Wang, W., Li, Q., Zucker, B.-A., Schlenker, G. (2009) REP-PCR tracking of the origin and spread of airborne *Staphylococcus aureus* in and around chicken house. Indoor Air 19, 511–516.