Rapid identification of *Staphylococcus epidermidis*

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During the collection of airborne bacteria in a museum in England some bacterial strains were isolated which due to their fatty acid profiles were clearly identified as members of the genus *Staphylococcus*. As fatty acid compositions of coagulase-negative staphylococci are very similar, differing only in quantities but not in qualities, further identification at the species level without a fatty acid database was not achieved. Investigation of the isolates using the Staph ID 32 API system resulted in an identification of the isolates as *Staphylococcus epidermidis* (probabilities of 79.7–95.5%). For further genotypic characterization of these isolates, some *Staphylococcus epidermidis* strains from different sources and the type strains of *Staphylococcus aureus*, *Staphylococcus capitis*, *Staphylococcus epidermidis*, *Staphylococcus gallinarum*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus warneri* and *Staphylococcus xylosus* were subjected to repetitive-sequence PCR, including enterobacterial repetitive intergenic consensus (ERIC) PCR, BOX-PCR and repetitive extragenic palindromic unit sequence (REP) PCR. ERIC- and BOX-PCR yielded a species-specific banding pattern for all *Staphylococcus epidermidis* strains. Furthermore, all staphylococcal reference strains investigated exhibited distinct banding patterns, clearly distinguishable from that of *Staphylococcus epidermidis*. No species-specific banding patterns could be observed after REP-PCR. As species identification of coagulase-negative staphylococci by fatty acid analyses and biochemical tests is known to be difficult ERIC- and BOX-PCR seem to be excellent tools for the identification of *Staphylococcus epidermidis* isolates.

Keywords: *Staphylococcus epidermidis*, ERIC-PCR, REP-PCR

INTRODUCTION

Staphylococci are ubiquitous in the environment. Natural populations are associated with skin, skin glands and mucous membranes of warm-blooded animals. They have been isolated from animal products such as meat, milk and cheese, and other sources such as soil, sand, seawater, fresh water, dust and air (Kloos *et al.*, 1991). Some staphylococcal species are known to be frequently encountered in severe infections. Historically, only the coagulase-positive species *Staphylococcus aureus* was considered to be pathogenic. In the last two decades coagulase-

negative staphylococci have emerged as significant pathogens, especially in medical-device-related infections and in immunocompromised patients. In the 1980s many surveys indicated a higher frequency of *Staphylococcus epidermidis*, *Staphylococcus saprophyticus* and *Staphylococcus haemolyticus* strains associated with human infections compared to other coagulase-negative staphylococcal species (Kloos & Wolfshohl, 1982; Marisk & Brake, 1982; Sewell *et al.*, 1982; Jordan *et al.*, 1980). Recently, it was reported that strains belonging to the species *Staphylococcus hominis*, *Staphylococcus capitis*, *Staphylococcus lugdunensis*, *Staphylococcus warneri* and *Staphylococcus saccharolyticus* can occasionally cause bacteremia and infective endocarditis (Kloos & Bannerman, 1994; Schnitzler *et al.*, 1997; Weinstein *et al.*, 1998). *Staphylococcus epidermidis* is the coagulase-negative species most prevalent in diseases, frequently

Abbreviations: ERIC, enterobacterial repetitive intergenic consensus; RAPD, randomly amplified polymorphic DNA; REP, repetitive extragenic palindromic unit sequence; rep-PCR, repetitive-sequence PCR.

associated with bacteraemia, urinary-tract infections and post-catheterization (Martineau *et al.*, 1996).

Due to the pathogenic potential of certain staphylococcal species, effective methods are needed for the identification of clinically significant strains. In common routine diagnostics, biochemical tests and fatty acid analyses are performed. These methods enable the identification of *Staphylococcus aureus* isolates, but often fail in the identification of coagulase-negative staphylococci (Martineau *et al.*, 1996; Stoakes *et al.*, 1994). Since some coagulase-negative species can be distinguished only by a limited number of stable biochemical tests, the precise assignment of a staphylococcal strain to a species is difficult to obtain. What is more, atypical characteristics such as phosphatase-negative reactions in certain *Staphylococcus epidermidis* strains often result in their misidentification as *Staphylococcus hominis* (Ieven *et al.*, 1995). Fatty acid compositions of coagulase-negative staphylococcal species showing quantitative but not qualitative differences in certain acids are applied for their identification (Kotilainen *et al.*, 1990). However, because of considerable overlaps in mean fatty acid compositions, standard deviations and minimum and maximum values, members of the species *Staphylococcus hominis* are often assigned to *Staphylococcus epidermidis* (Stoakes *et al.*, 1994). Other methods for species identification such as DNA-DNA hybridization, 16S rDNA analyses, ribotyping and serotyping are too laborious for routine diagnostics.

ERIC (enterobacterial repetitive intergenic consensus)- and REP (repetitive extragenic palindromic unit sequence)-PCR analyses have provided promising results in strain identification of Gram-negative bacteria such as *Citrobacter diversus* (now *Citrobacter koseri*) and *Rhizobium meliloti* (De Bruijn, 1992; Woods *et al.*, 1992). There is some evidence that these sequence elements also exist in the genomes of Gram-positive bacteria. Recently, PCR with only one primer corresponding to the ERIC sequence was used for differentiation of *Staphylococcus aureus* strains (Van Belkum *et al.*, 1992) and primers derived from the REP sequence were used in PCR for typing of *Streptococcus pneumoniae* isolates (Versalovic *et al.*, 1993).

In repetitive-sequence (rep) PCR, the primers used are designed from interspersed repetitive sequence elements like the ERIC, REP and BOX (derived from the BOX A element) sequences. PCR with these primers generate genomic fingerprints of sufficient complexity for similarity studies within different strains of a species. ERIC and REP sequences were originally identified in the genome of *Escherichia coli* and *Salmonella typhimurium* (Lupski & Weinstock, 1992) while the BOX repetitive DNA element was first found in the genome of *Streptococcus pneumoniae* (Martin *et al.*, 1992).

During a study on airborne bacteria collected in the Sainsbury Center of Visual Arts, in Norwich, England,

some bacterial strains were isolated which could be clearly assigned to the genus *Staphylococcus* on the basis of fatty acid profiles. Further investigation of the isolates with the Staph ID 32 API system resulted in an identification of the isolates as *Staphylococcus epidermidis* with probability values of 79.5–95.5%. It is known that the Staph ID 32 API system is not very reliable, especially when low probability values are obtained (Renneberg *et al.*, 1995). For confirmation of these identifications we subjected these isolates to rep-PCR. Here we show that ERIC- and BOX-PCR generate species-specific banding patterns.

METHODS

Bacterial strains. For collection of airborne bacteria, the Biotest Hycon air sampler RCS Plus was used. Bacteria were isolated on a *Legionella* selective medium agar [*Legionella*-CYE-agar base with *Legionella*-BCYE α -supplement (Oxoid) and *Legionella*-MWY-selective supplement (Oxoid)] or on Casein Mineral Medium (Altenburger *et al.*, 1996) and subcultivated on Tryptic Soy Agar (Oxoid) at 37 °C. The staphylococcal isolates were designated 3.8-1, 3.8-2, 3.8-7, 3.8-11, 36 and 5.8-3. Isolate 36 was isolated by Ursula Ulrych of our group. Isolate 5.8-3 was lost during the subcultivation process. The reference strains used were *Staphylococcus aureus* DSM 20231^T, *Staphylococcus capitis* subsp. *capitis* DSM 20326^T, *Staphylococcus epidermidis* DSM 20044^T, *Staphylococcus gallinarum* DSM 20610^T, *Staphylococcus haemolyticus* DSM 20263^T, *Staphylococcus hominis* DSM 20328^T, *Staphylococcus warneri* DSM 20316^T and *Staphylococcus xylosus* DSM 20266^T, which were provided by Peter Kämpfer (University of Giessen, Germany). *Staphylococcus epidermidis* strains 99, 115, 116, 122, 136, 138 and 140 were provided by Dr Angelika Lehner (Veterinary University, Vienna). *Staphylococcus epidermidis* strains ATCC 12228, MCCM 01992, MCCM 02571, MCCM 02721, MCCM 02726, MCCM 02714 and *Staphylococcus hominis* strain MCCM 02720 were supplied by Dr Reinier Mutters (University of Marburg, Germany). *Staphylococcus epidermidis* strain 047 was provided by Dr Friedrich Götz (University of Tübingen, Germany).

Phenotypic characteristics. Cell morphology was determined by using phase-contrast microscopy. Gram staining was performed as described by Murray *et al.* (1994). Catalase activity was studied by dropping 3% H₂O₂ on well grown colonies on agar plates and observing the bubbles. Oxidase production was tested using Bactident Oxidase strips (Merck) according to the instructions of the manufacturer. Staph ID32 API system strips were inoculated according to the directions of the manufacturer (bioMérieux).

Fatty acid composition. Fatty acid analysis was performed as described by Groth *et al.* (1996) using a Hewlett Packard HP 6890 Series GC System with a HP-5 capillary (30 m × 0.32 mm; film thickness 0.25 µm).

Preparation of DNA and PCR amplification. Subcultures of the isolates and the reference strains were grown on Tryptic Soy Agar and incubated at 37 °C for 2 d. Approximately two loops of biomass were scraped off the agar plates, suspended in 100 µl sterile water and lysed by repeated cycles of freezing in liquid nitrogen and thawing at 65 °C, and finally centrifuged. Samples of *Staphylococcus aureus* were treated with proteinase K (250 µg ml⁻¹). Between 1 and 3 µl of the supernatant were used as template DNA for PCR amplification.

RAPD (randomly amplified polymorphic DNA) PCRs were performed by using the RAPD primers 2, 3, 4, 5 and 6 of the RAPD Analysis Primer Set (Pharmacia Biotech) according to the instructions of the manufacturer. ERIC-, BOX- and REP-PCR were done as described by Louws *et al.* (1994). Primers for ERIC-PCR (ERIC1R: 5'-ATGTAAGCTCCTGGGGATTAC-3', ERIC2: 5'-AAGTAAGTGACTGGGGTGAGCG-3'), REP-PCR (REP1R-I: 5'-IIICGICGICATCIGGC-3', REP2-I: 5'-ICGICTTATCIGGCCTAC-3') and BOX-PCR (BOXA1R: 5'-CTACGGCAAGGCGACGCTGACG-3') were synthesized at the Service Department of the Vienna Biocenter (MIG-BASE). A primer concentration of 50 pmol was used. PCR amplification reactions were done using Ready to Go beads (25 µl; Pharmacia Biotech) and a Primus thermal cycler (MWG-Biotech), and an initial denaturation step (95 °C for 7 min) followed by 30 cycles of denaturation (94 °C for 1 min), annealing (44, 52 or 53 °C for 1 min with REP, ERIC or BOX primers, respectively), extension (65 °C for 8 min) and a final extension step (65 °C for 16 min). Between 4 and 8 µl PCR product was separated by gel electrophoresis at room temperature on 1.5% agarose gels, stained with ethidium bromide and photographed on a UV transilluminator. Fingerprints were compared visually.

RESULTS

Phenotypic characteristics

The six airborne isolates, 3.8-1, 3.8-2, 3.8-7, 3.8-11, 36 and 5.8-3, were Gram-positive, catalase-positive and oxidase-negative spherical cocci that occurred singly, in pairs, in short chains and in irregular clusters. No spores were observed. Colonies were small, entire, circular and white pigmented.

Fatty acid composition

All isolates investigated exhibited nearly identical fatty acid profiles. They displayed large amounts of the fatty acids anteiso- $C_{15:0}$, $C_{18:0}$ and $C_{20:0}$ and smaller but significant amounts of the fatty acids iso- $C_{15:0}$, $C_{16:0}$, iso- $C_{17:0}$, anteiso- $C_{17:0}$ and two unidentified fatty acids. The obtained fatty acid profiles clearly identified these isolates as members of the genus *Staphylococcus* (Kotilainen *et al.*, 1990).

Staph ID 32 system

The Staph ID 32 system identified the isolates 3.8-1, 3.8-2, 3.8-7, 3.8-11 and 36 as *Staphylococcus epidermidis* strains with probability values of 92.8, 79.7, 79.7, 79.7 and 95.5%, respectively. No Staph ID 32 test could be performed for isolate 5.8-3 as this strain was lost during subcultivation.

RAPD-PCR

Banding patterns obtained after RAPD-PCR with the primers 2, 3, 4, 5 and 6 demonstrated that the isolates 3.8-1, 3.8-2, 3.8-7, 3.8-11 and 36 represented different strains of *Staphylococcus epidermidis* (data not shown). The isolates 3.8-1 and 3.8-11, on one hand, and strain 36 and *Staphylococcus epidermidis* DSM 20044^T, on the other, displayed nearly identical banding patterns

differing only in one band using primers 5 and 4 (data not shown).

ERIC-PCR

ERIC-PCR banding patterns (Fig. 1) displayed the highest similarity between the isolates 3.8-1, 3.8-2, 3.8-7, 3.8-11 and 36 to *Staphylococcus epidermidis* DSM 20044^T. Isolates 3.8-1, 3.8-2 and 3.8-11 exhibited identical patterns, whereas in the sample from strain 3.8-7 one band was missing. Very similar genomic fingerprints were obtained for *Staphylococcus epidermidis* DSM 20044^T and isolate 36. These strains only differed in the absence or presence of bands in the high molecular mass range. However, bands of high molecular masses (above 1200 bp) were less reproducible. For example, the ERIC-PCR pattern of *Staphylococcus epidermidis* DSM 20044^T in figs 1 and 2 differed in three bands of high molecular masses. However, all investigated *Staphylococcus epidermidis* strains (Figs 1 and 2) possessed five bands in common with sizes of approximately 300, 450, 550, 600 and 800 bp. Isolate 5.8-3 and the two strains of *Staphylococcus hominis*, DSM 20328^T and MCCM 02720, exhibited identical banding patterns after ERIC-PCR (Figs 1 and 2). The type strains of the other investigated staphylococcal species had distinct banding patterns (Fig. 1) useful for differentiation from each other. Although *Staphylococcus epidermidis* DSM 20044^T and *Staphylococcus warneri* DSM 20316^T possessed two identical bands they were clearly distinguishable from each other (Fig. 1).

BOX-PCR

Genomic fingerprints generated after BOX-PCR (Figs 3 and 4) were identical for all *Staphylococcus epidermidis* strains including the isolates 3.8-1, 3.8-2, 3.8-7, 3.8-11 and 36. They exhibited three predominant bands with sizes of 350, 1000 and >1100 bp. The investigated reference strains exhibited distinct BOX fingerprints (Fig. 3). Banding patterns of isolate 5.8-3 shared the highest similarity to banding patterns of *Staphylococcus hominis* DSM 20328^T and *Staphylococcus capitis* DSM 20326^T (Fig. 3).

REP-PCR

Obtained banding patterns showed low complexity. Patterns of the isolates 3.8-1, 3.8-2, 3.8-11 and 3.8-7 consisted of only two or three bands. Strain 36 and *Staphylococcus epidermidis* DSM 20044^T exhibited only one band. REP-PCR yielded identical banding patterns for the isolates 3.8-1, 3.8-2 and 3.8-11, on one hand, and for the *Staphylococcus epidermidis* DSM 20044^T and strain 36, on the other. Strain 3.8-7 exhibited a nearly identical pattern to the first group (data not shown). These two groups possessed no bands in common. The banding pattern of strain 5.8-3 consisted of only one band, which could also be found in the pattern of *Staphylococcus hominis* DSM 20328^T

Fig. 1. ERIC-PCR-generated DNA fingerprints of the staphylococcal isolates 3.8-1, 3.8-2, 3.8-7, 3.8-11, 36 and 5.8-3, and the type strains of *Staphylococcus aureus*, *Staphylococcus capitis*, *Staphylococcus epidermidis*, *Staphylococcus xylosus*, *Staphylococcus warneri*, *Staphylococcus gallinarum*, *Staphylococcus haemolyticus* and *Staphylococcus hominis*.

Fig. 2. ERIC-PCR-generated DNA fingerprints of fifteen *Staphylococcus epidermidis* strains from different sources and two *Staphylococcus hominis* strains.

Fig. 3. BOX-PCR-generated DNA fingerprints of the staphylococcal isolates 3.8-1, 3.8-2, 3.8-7, 3.8-11, 36 and 5.8-3, and of the type strains of *Staphylococcus aureus*, *Staphylococcus capitis*, *Staphylococcus epidermidis*, *Staphylococcus xylosus*, *Staphylococcus warneri*, *Staphylococcus gallinarum*, *Staphylococcus haemolyticus* and *Staphylococcus hominis*.

Fig. 4. BOX-PCR-generated DNA fingerprints of fifteen *Staphylococcus epidermidis* strains from different sources and two *Staphylococcus hominis* strains.

which additionally possessed a second band. The reference strains *Staphylococcus xylosus* DSM 20266^T, *Staphylococcus gallinarum* DSM 20610^T and *Staphylococcus hominis* DSM 20328^T, showed distinct banding patterns, but they also consisted of only one or two bands. Furthermore, no amplification products were obtained after REP-PCR for *Staphylococcus aureus* DSM 20231^T, *Staphylococcus capitis* DSM 20326^T, *Staphylococcus warneri* DSM 20316^T and *Staphylococcus haemolyticus* DSM 20263^T.

DISCUSSION

In the study presented here, several staphylococcal strains including the type strains of *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Staphylococcus warneri*, *Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus gallinarum*, *Staphylococcus aureus* and *Staphylococcus xylosus*, fourteen strains of the species *Staphylococcus epidermidis* from different strain collections, one strain of *Staphylococcus hominis* and six isolates of the genus *Staphylococcus* were subjected to rep-PCR.

These reference strains were chosen according to their relatedness to *Staphylococcus epidermidis* as demonstrated from DNA–DNA reassociation experiments (Kloos *et al.*, 1991). The species *Staphylococcus capitis*, *Staphylococcus warneri*, *Staphylococcus hominis* and *Staphylococcus haemolyticus* are closely related to *Staphylococcus epidermidis*, whereas the species *Staphylococcus aureus*, *Staphylococcus xylosus* and *Staphylococcus gallinarum* belong to three different clusters, each distantly related to the *Staphylococcus epidermidis* group.

REP-PCR

After REP-PCR several strains including *Staphylococcus aureus* displayed no band whereas in other strains one to two bands were observed. The lack of

any band after REP-PCR demonstrates that in the genome of *Staphylococcus aureus* sequences complementary to the REP primer are not present. This is in agreement with hybridization experiments shown by Versalovic *et al.* (1991). Although we were able to detect a few bands in some staphylococcal strains, the observed banding patterns were neither strain nor species specific. This observation indicated that REP-PCR is not suitable for identification of staphylococcal strains.

ERIC-PCR

So far, no studies have been reported where ERIC-PCR was applied to identify staphylococcal species. Although ERIC sequences have not been demonstrated to be present in Gram-positive bacteria, PCR studies using the primers ERIC-1R or ERIC-2 under low-stringency conditions indicated the presence of ERIC-like sequences in *Staphylococcus aureus* (Van Belkum *et al.*, 1992). According to our data, ERIC-like sequences exist in the genomes of staphylococci. This finding is confirmed by application of the stringent experimental conditions described for ERIC-PCR (Louws *et al.*, 1994).

Banding patterns generated after ERIC-PCR displayed a high degree of similarity between *Staphylococcus epidermidis* DSM 20044^T, the airborne *Staphylococcus epidermidis* isolates and the other strains of *Staphylococcus epidermidis* obtained from different strain collections. Although *Staphylococcus epidermidis* DSM 20044^T and strain 36 displayed some variability in the presence of bands in the high molecular mass range (above 1200 bp), the presence of the remaining predominant bands was highly reproducible. Some similarities were observed in the banding patterns of *Staphylococcus epidermidis* and *Staphylococcus warneri*, however they were clearly distinguishable from each other. The observation that all type strains of the other staphylococci species investigated

showed unique patterns after ERIC-PCR indicated that these species also generated a species-specific banding pattern. This finding is confirmed by the high degree of similarity found in the banding patterns of *Staphylococcus hominis* DSM 20328^T and the methicillin-resistant strain *Staphylococcus hominis* MCCM 02720. Furthermore, isolate 5.8-3 displayed a fingerprint which indicates that it probably belongs to *Staphylococcus hominis*.

BOX-PCR

BOX-PCR was originally developed for strain identification of *Streptococcus pneumoniae* isolates. Southern blot hybridization experiments have demonstrated that the presence of the BOX sequence is restricted to *Streptococcus pneumoniae* (Hermans *et al.*, 1995). The BOX probe did not hybridize with chromosomal DNA from *Staphylococcus aureus*, *Staphylococcus warneri* or *Staphylococcus epidermidis* and other Gram-positive bacteria.

The application of BOX-PCR to our collection of staphylococci always yielded a banding pattern. This result demonstrates the presence of several copies of this sequence in the genomes of members of this genus. Genotypic fingerprints obtained after BOX-PCR were identical for all *Staphylococcus epidermidis* strains and the airborne isolates of this species. Furthermore, banding patterns of *Staphylococcus epidermidis* strains were distinguishable from those of all other reference strains used. This observation indicated that BOX-PCR provides a species-specific banding pattern for *Staphylococcus epidermidis*, which enables the rapid identification of *Staphylococcus epidermidis* isolates. Although BOX fingerprints of the investigated species had at least one band in common, the banding pattern appeared to be species specific. A high degree of similarity was observed for *Staphylococcus capitis* DSM 20326^T, *Staphylococcus haemolyticus* DSM 20263^T and *Staphylococcus hominis* DSM 20328^T where their banding patterns differed only in two bands. Since it is a well known problem that PCR is not reproducible between laboratories, this fact may cause problems in the identification of isolates related to these three species. Due to the high similarity in the BOX-PCR patterns of isolate 5.8-3, *Staphylococcus hominis* and *Staphylococcus capitis*, no clear differentiation was possible.

Conclusions

According to our data, within the staphylococcal genomes examined here, sequences are present that are complementary to the sequences of the primers used for ERIC- and BOX-PCR. However, recent studies have shown that the stringent conditions used by Louws *et al.* (1994) are not indicative for the presence of ERIC- and BOX-sequences in a target DNA (Gillings & Holley, 1999). These authors have demonstrated that ERIC- and BOX-PCR only indicate the

presence of these sequences in a genome if the annealing temperature is significantly increased. Thus ERIC- and BOX-PCR performed under less stringent conditions can be considered as variants of the RAPD method. Sequences complementary to REP primers were only found in several *Staphylococcus* species. PCR amplification using primers derived from these repetitive sequences (ERIC, REP and BOX sequences) did not yield strain-specific banding patterns within selected species of the genus *Staphylococcus* and are therefore not suitable for epidemiological studies. For example, isolate 36 and *Staphylococcus epidermidis* DSM 20044^T, which were clearly distinguishable by RAPD-PCR (results not shown), displayed identical ERIC-, REP- and BOX-PCR banding patterns.

ERIC- and BOX-PCR provided species-specific banding patterns for *Staphylococcus epidermidis* strains. Based on this observation, the genomic fingerprints of the airborne isolates 3.8-1, 3.8-2, 3.8-7, 3.8-11 and 36 clearly identify these strains as *Staphylococcus epidermidis*. Furthermore, it is possible that there is also a species-specific banding pattern for *Staphylococcus hominis* strains. Thus, these approaches, especially ERIC-PCR, allow the clear differentiation of *Staphylococcus epidermidis* and *Staphylococcus hominis* strains which has always remained a problem in biochemical-test and computer-assisted fatty acid identification systems. Our results demonstrate that ERIC- and BOX-PCR are excellent tools for rapid identification of *Staphylococcus epidermidis* strains at the species level. For species differentiation ERIC-PCR appears to be more suitable than BOX-PCR, however, the combination of these two PCR methods will provide more reliable results in classifying and identifying staphylococcal isolates.

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REFERENCES

- Altenburger, P., Kämpfer, P., Makristathis, A., Lubitz, W. & Busse, H.-J. (1996). Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* **47**, 39–52.
- De Bruijn, F. J. (1992). Use of repetitive sequences and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria. *Appl Environ Microbiol* **58**, 2180–2187.
- Gillings, M. & Holley, M. (1999). Repetitive element PCR is a variant of the randomly amplified polymorphic DNA method. In *Abstracts of the 9th International Congress of Bacteriology & Applied Microbiology*. Sydney: International Union of Microbiological Societies.
- Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.

- Hermans, P. W. M., Sluijter, M., Hoogenboezem, T., Heersma, H., Van Belkum, A. & de Groot, R. (1995). Comparative study of five different DNA fingerprint techniques for molecular typing of *Streptococcus pneumoniae* strains. *J Clin Microbiol* **33**, 1606–1612.
- Ieven, M., Verhoeven, J., Pattyn, S. R. & Goossens, H. (1995). Rapid and economical method for species identification of clinically significant coagulase-negative staphylococci. *J Clin Microbiol* **33**, 1060–1063.
- Jordan, P. A., Iranvani, A., Richard, G. A. & Baer, H. (1980). Urinary tract infection caused by *Staphylococcus saprophyticus*. *J Infect Dis* **142**, 510–515.
- Kloos, W. E. & Bannerman, T. L. (1994). Update on clinical significance of coagulase-negative staphylococci. *Clin Microbiol Rev* **7**, 117–140.
- Kloos, W. E. & Wolfshohl, J. F. (1982). Identification of *Staphylococcus* species with the API STAPH-Ident system. *J Clin Microbiol* **16**, 509–516.
- Kloos, W. E., Schleifer, K.-H. & Götz, R. (1991). The genus *Staphylococcus*. In *The Prokaryotes: a Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Application*, 2nd edn, pp. 1369–1420. Edited by Balows, A., H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.
- Kotilainen, P., Huovinen, P. & Eerola, E. (1990). Application of gas-liquid chromatographic analysis of cellular fatty acids for species identification and typing of coagulase-negative staphylococci. *J Clin Microbiol* **29**, 315–322.
- Louws, F. J., Fulbright, D. W., Stephens, C. T. & de Bruijn, F. J. (1994). Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas* pathovars and strains generated with repetitive sequences and PCR. *Appl Environ Microbiol* **60**, 2286–2295.
- Lupski, J. R. & Weinstock, G. M. (1992). Short, interspersed repetitive DNA sequences in prokaryotic genomes. *J Bacteriol* **174**, 4525–4529.
- Marisk, F. J. & Brake, S. (1982). Species identification and susceptibility to 17 antibiotics of coagulase-negative staphylococci isolated from clinical specimens. *J Clin Microbiol* **15**, 640–645.
- Martin, B., Humbert, O., Camara, M. & 10 other authors (1992). A highly conserved repeated DNA element located in the chromosome of *Streptococcus pneumoniae*. *Nucleic Acids Res* **20**, 3479–3483.
- Martineau, F., Picard, F. J., Roy, P. H., Ouellette, M. & Bergeron, M. G. (1996). Species-species and ubiquitous DNA-based assays for rapid identification of *Staphylococcus epidermidis*. *J Clin Microbiol* **34**, 2888–2893.
- Murray, R. G. E., Doetsch, R. N. & Robinow, C. F. (1994). Determinative and cytological light microscopy. In *Methods for General and Molecular Bacteriology*, pp. 22–41. Edited by G. Philipp, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Renneberg, J., Rieneck, K. & Gutschik, E. (1995). Evaluation of Staph ID 32 System and Staph-Zym System for identification of coagulase-negative staphylococci. *J Clin Microbiol* **33**, 1150–1153.
- Schnitzler, N., Meilicke, R., Conrads, G., Frank, D. & Haase, G. (1997). *Staphylococcus lugdunensis*: report of a case of peritonitis and easy-to-perform screening strategy. *J Clin Microbiol* **36**, 812–813.
- Sewell, C. M., Clarridge, J. E., Young, E. J. & Guthrie, R. K. (1982). Clinical significance of coagulase-negative staphylococci. *J Clin Microbiol* **16**, 236–239.
- Stoakes, L., John, M. A., Lannigan, R., Schieven, B. C., Ramos, M., Harley, D. & Hussain, Z. (1994). Gas-liquid chromatography of cellular fatty acids for identification of staphylococci. *J Clin Microbiol* **32**, 1908–1910.
- Van Belkum, A., Bax, R., Peerbooms, P., Goessens, W. H. F., Van Leeuwen, N. & Quint, W. G. V. (1992). 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.
- Versalovic, J., Koeuth, T. & Lupski, J. R. (1991). Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* **19**, 6823–6831.
- Versalovic, J., Kapur, V., Mason, E. O., Shah, U., Koeuth, T., Lupski, J. R. & Musser, J. M. (1993). Penicillin resistant *Streptococcus pneumoniae* strains recovered in Houston: identification and molecular characterization of multiple clones. *J Infect Dis* **167**, 850–856.
- Weinstein, M. P., Mirrett, S., van Pelt, L., McKinnon, M., Zimmer, B. L., Kloos, W. & Reller, L. B. (1998). Clinical importance of identifying coagulase-negative staphylococci isolated from blood cultures: evaluation of MicroScan Rapid and dried overnight gram-positive panels versus a conventional reference method. *J Clin Microbiol* **36**, 2089–2092.
- Woods C. R., Versalovic, J., Jr, Koeuth, T. & Lupski, J. R. (1992). Analysis of relationships among isolates of *Citrobacter diversus* by using DNA fingerprints generated by repetitive sequence-based primers in the polymerase chain reaction. *J Clin Microbiol* **30**, 2921–2929.