sentatives of these isolates and the five *B. mycoides* strains listed in the DSM Catalogue of Strains 1993. Sequence analysis showed the five isolates, and three of the strains, to have between 99.6 and 100% similarity to the type strain of *B. mycoides* (DSM 2048^T). Two strains considered to be *B. mycoides* on the basis of phenotype, namely DSM 303 and DSM 307, showed 99.4 and 98.8% sequence similarity respectively to the type strain of *B. mycoides*.

Comparison of 16S rDNA sequences of *B. mycoides*, *B. cereus*, *B. thuringiensis* and *B. anthracis* between positions 180 and 199 (*E. coli* numbering) indicated that the region could be useful as an oligonucleotide probe target for the differentiation of *B. mycoides* from the other *Bacillus* species. An oligonucleotide probe of 20 nucleotides was designed, 3'-end labelled with the DIG System (Boehringer Mannheim) and tested against a dot blot of PCR products of all isolates, available *B. mycoides* strains and controls of other closely related *Bacillus* species.

Positive signals were obtained with 33 of the 34 isolates, and with all tested *B. mycoides* strains with the exception of strains DSM 303 and DSM 307. No signals were obtained for the other *Bacillus* species tested. The *B. mycoides* strains DSM 303 and DSM 307 had been assigned to *B. mycoides* on the basis of phenotypic characteristics. Comparison of the sequence of the probe region for these two strains showed strain DSM 303 to differ at one position, while strain DSM 307 differed at four positions.

In order to determine the specificity of the probe in distinguishing *B. mycoides* strains from other closely related species, DNA-DNA hybridization experiments were carried out using the thermal reassociation method. Two of the isolates that gave a positive signal with the oligonucleotide probe had ~70% DNA-DNA similarity to the type strain of *B. mycoides*, but 50–60% to the type strains of *B. cereus* and *B. thuringiensis*, indicating their membership of the species *B. mycoides*. *B. mycoides* strains DSM 303 and DSM 307 which did not give a signal with the probe had ~65% and ~44% DNA-DNA homology to the type strain of *B. mycoides*; such values could speak in favour of their exclusion from the species *B. mycoides*.

This study demonstrates the application of 16S rDNA oligonucleotide probes to the differentiation of species which have highly similar 16S rDNA sequences and are phenotypically difficult to differentiate. A 16S rDNA-based oligonucleotide probe has been designed which differentiates *B. mycoides* strains from the closely related *B. cereus*, *B. thuringiensis* and *B. anthracis*. The specificity of the probe is in accord with results of DNA-DNA hybridization studies. Based on the findings of this study, the species description of *B. mycoides* should be amended to include both rhizoid and non-rhizoid colony morphology and motile and non-motile cells.

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Length polymorphisms detected by PCR for rRNA gene spacers and ERIC2 sequence flanked regions indicate a clonal relatedness among groups of *Staphylococcus aureus* strains

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A clonal relatedness among definite major groups of *S. aureus* strains is already indicated by multilocus enzyme analysis and *Sma*I-macrorestriction patterns[1,2,4]. The aim of our study was to check whether length polymorphisms of rRNA gene spacers and ERIC sequence[3] flanked regions are concordant with this grouping and applicable to epidemiological questions.

Forty-six *S. aureus* strains consisting of 40 strains defined by definite phage- and *Sma*I-macrorestriction patterns and 6 representatives of epidemic MRSA clones[5] have been checked for rRNA gene spacer length polymorphism. DNA extraction and amplification of DNA sequences by PCR have been described previously[5]. The spacer between the 16S rRNA gene and the 23S rRNA gene was amplified by use of primer sequences located in the conserved areas of both flanking genes; I: 5'-TTGTACACACCGCCCGTCA-3'; II: 5'-GGTACCTTAGATCTTTCAGTTC-3'; cycling scheme: 30 cycles 94 °C, 1 min; 55 °C, 1 min; 72 °C, 1 min; last cycle with 4 min at 72 °C. The primer for the ERIC2 sequence[3] was 5'-AAGTAAGTGACTGGGTGAGCG-3'; cycling scheme: 29 cycles: 94 °C, 30 s; 25 °C, 30 s; 72 °C, 30 s; last cycle: 94 °C, 30 s; 25 °C, 30 s; 72 °C, 4 min. *Sma*I-macrorestriction patterns and analysis of fragment similarities (according to molecular masses) were performed as described[4]. rRNA gene spacer size patterns (rRGS) were less discriminative than *Sma*I-macrorestriction patterns. Strains grouped according to macrorestriction patterns and phage-patterns as probably clonally related exhibit common rRGS patterns (fig. 1). This is of special interest for 6 different epidemic MRSA, where 4 strains exhibit similar *Sma*I-macrorestriction and identical rRGS patterns.

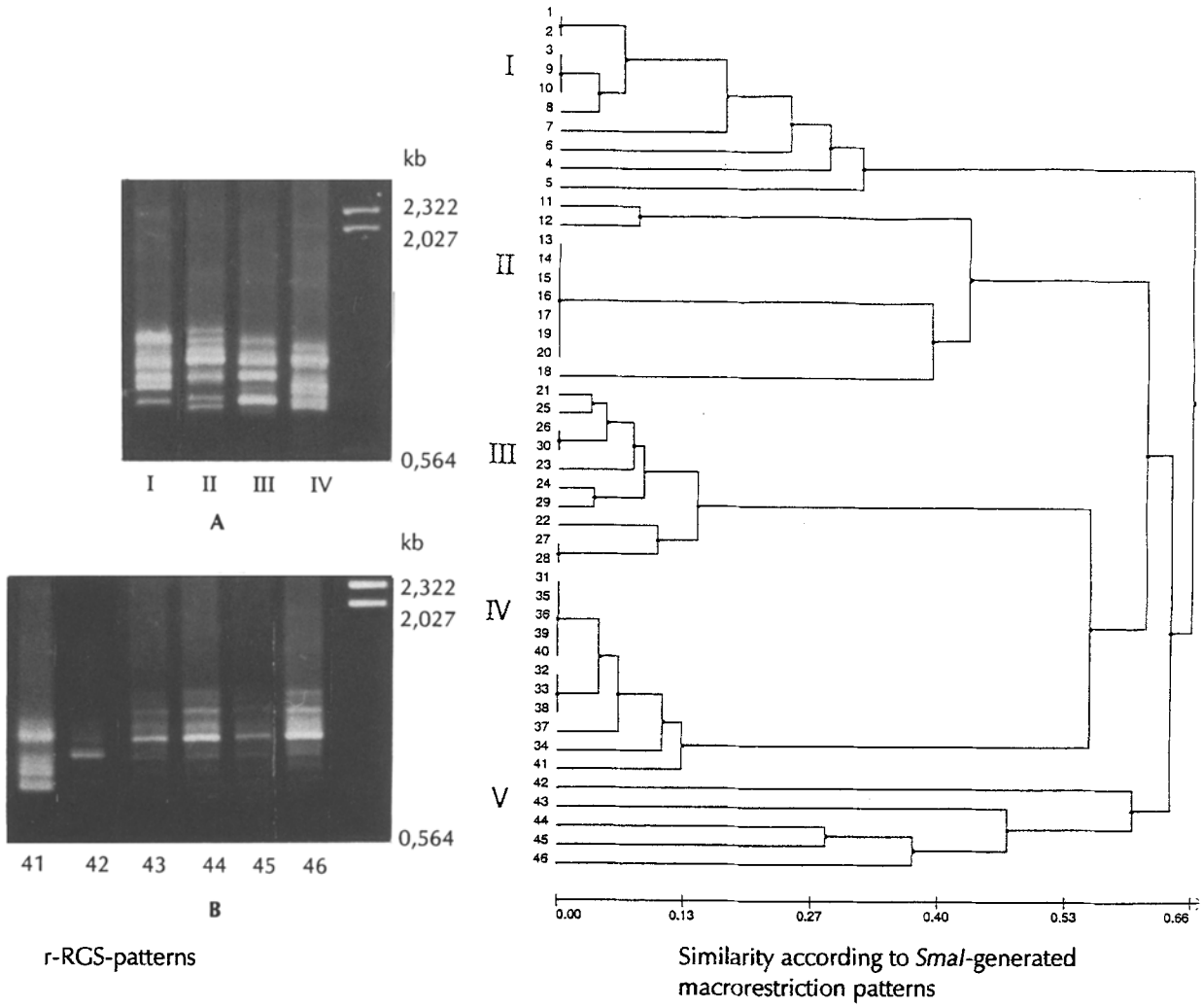

Figure 1. rRNA gene spacer length patterns typical for strains exhibiting related *Sma*I macrorestriction patterns (A I to IV; B V, MRSA strains 41 to 46). (I pattern 29, II group II phage pattern, III phage pattern 94, 96; IV phage pattern 95).

Two strains are clearly different. In both, the *Sma*I-macrorestriction pattern and the rRGS pattern of MRSA 41 correspond to the patterns of clonally related phage-group 95 strains[2] which until now have been sensitive to methicillin. These findings suggest an ancestral relatedness. The same group of strains was obtained by PCR for ERIC2 flanked regions.

The PCR for the length polymorphism of the rRNA gene spacer and ERIC2 flanked regions is therefore a useful approach to the rapid assessment of probably clonal relations among *S. aureus* strains of clinical and epidemiological significance. It is less useful for identification of single strains.

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