NOTE**16S rDNA sequence variations of some *Streptococcus suis* serotypes**

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***Streptococcus suis* 16S rDNA from selected serotypes has been sequenced and compared with the 16S rDNA sequences from serotypes 1 and 2 present in GenBank. After alignment the sequenced serotypes show clusters of variation. Based on these clusters, a limited phylogenetic tree showing the relationships of all of the serotypes was constructed.**

Keywords: *Streptococcus suis*, serotypes, sequencing, 16S rDNA, phylogenetic tree

Streptococcus suis is an organism that causes meningitis, arthritis, septicaemia, and sudden death in pigs (Clifton-Hadley, 1983). Furthermore, *S. suis* is a zoonotic agent that is known to cause meningitis in humans (Ahrens *et al.*, 1988). Presently 35 serotypes of *S. suis* are described (Gottschalk *et al.*, 1989, 1991; Higgins *et al.*, 1995; Perch *et al.*, 1983) with serotype 2 being the most common virulent type. However, serotypes other than 2 can be virulent (Higgins & Gottschalk, 1990), and in Denmark serotype 7 is nearly as common as serotype 2 (Aarestrup *et al.*, 1998). As a consequence, all *S. suis* strains isolated at our laboratory from received carcasses are serotyped by co-agglutination (Hommes *et al.*, 1986; Mittal *et al.*, 1983) and capillary precipitation (Higgins & Gottschalk, 1990; Perch *et al.*, 1983). Approximately 20% of the *S. suis* isolates received are non-typeable (Danish Veterinary Laboratory Annual Report, 1990–1995), so these *S. suis* isolates are only identified by biochemical methods. To ensure reliable identification of these non-typeable isolates, we are presently developing a DNA-based test which will be reported elsewhere.

During this work we realized that, as serotypes 9, 20, 22, 24, 25, 26 and 27 could not be identified, the chosen primer regions contained some serotype-specific sequence variations. Therefore, the sequencing of 16S from these serotypes became a necessity. Here we describe the presence of clusters of variation in the 16S sequence found by sequencing selected reference strains and comparing these sequences with the *S. suis* serotype 1 and 2 sequences from GenBank. Fur-

thermore, the clusters of variation were used for construction of a preliminary phylogenetic tree.

Strains

The reference strains, serotypes 9 (St. 22083), 20 (86-5192), 22 (88-1861), 24 (88-5299A), 26 (89-4109-1) and 27 (89-5259) were received from Statens Serum Institut, Copenhagen, Denmark, while serotype 25 (89-3576-3) was received from Dr Marcello Gottschalk, Université de Montréal, Canada.

The 16S rDNA sequences of *S. suis* serotype 1 (NCTC 10237, accession no. X59031), *S. suis* serotype 2 (NCTC 10234^T, AB002525), *Streptococcus acidominimus* (NCDO 2025^T, X58301), *Streptococcus bovis* (NCDO 597^T, AB002482), *Streptococcus gordonii* (ATCC 10558^T, AF003931), *Streptococcus mitis* (ATCC 49456^T, AF003929), *Streptococcus oralis* (ATCC 35037^T, AF003932), *Streptococcus parasanguinis* (ATCC 15912^T, AF003933), *Streptococcus pneumoniae* (ATCC 33400^T, AF003930) and *Streptococcus sanguinis* (ATCC 10556^T, AF003928) were obtained from GenBank.

Extraction of *S. suis* DNA

All concentrations given in parentheses are the final concentrations. A Todd-Hewitt + 5% horse serum overnight culture was centrifuged and the pellet was resuspended in 10% sucrose/50 mM Tris pH 8.0. After addition of lysozyme (1.67 mg ml⁻¹) and EDTA (0.1 M), the suspension was incubated at 20 °C for 30 min. The cell membrane was lysed by the addition of SDS (1.67%) and protein was degraded during incubation for 2 h at 60 °C with proteinase K (0.26 mg ml⁻¹). Nucleic acids were phenol/chloroform extracted and ethanol precipitated.

The GenBank accession numbers for the 16S rDNA sequences of the serotypes are: 9, AF014814; 20, AF014815; 22, AF014816; 24, AF014817; 25, AF014818; 26, AF014819; 27, AF014820.

Fig. 1. Phylogenetic tree of selected *S. suis* serotypes. Due to differences in length of the sequences, the phylogenetic tree is constructed on the basis of the sequence discrepancies in the 16S rDNA sequence from bases 106 to 1335 using the *E. coli* numbering. The axis indicates the number of substitution events. For comparison, other streptococci are included in the tree.

Amplification of 16S rDNA

16S rDNA was amplified by PCR using the primers 8FX (5'-AGAGTTTGATCCTGGCTNAG-3') and 1407R (5'-TGACGGGCGGTGTGTACAA-3'). After an initial denaturation of template DNA at 94 °C for 3 min, amplification was performed for 30 cycles of 1 min at 94 °C, 55 °C and 72 °C. The MgCl₂ concentration was 2.5 mM.

Sequencing

The produced amplicons were used as template for cycle sequencing (Murray) using the primers 8FX, 517F (5'-CCAGCAGCCGCGGTAATAC-3'), 784FX (5'-GGATTAGATACCCNGGTAGTC-3'), 535R (5'-GTATTACCGCGGCTGCTGG-3'), 804RX (5'-GACTAGCNGGGTATCTAATCC-3') and 1407R. All primers map in conservative regions of 16S (Weisburg *et al.*, 1991). Termination and electrophoresis was done using the AmpliTaqFS dye terminator kit and a 373A automatic sequencer (Applied Biosystems/Perkin Elmer).

The sequences were aligned and a consensus sequence was computed with LaserGene (DNASTAR). After alignment, most of the sequence variations were found to cluster in regions at approximately 200, 470 and 1020 bases (data not shown). These positions correspond to base 207, 477 and 1027, respectively, when using the *Escherichia coli* numbering (Brosius *et al.*, 1978). However, the region at base 200, showing only a few serotype specific variations, was very different from other streptococci when the *S. suis* sequences were aligned with the 16S rDNA sequences from *S. acidominimus*, *S. bovis*, *S. gordonii*, *S. mitis*, *S. oralis*, *S. parasanguinis*, *S. pneumoniae* and *S. sanguinis*, indicating the presence of an *S. suis*-specific region (data not shown). This region was found to be identical to the recently described V2 region (Smith *et al.*, 1997).

The sequenced serotypes could be grouped by means of their sequence variations to produce a phylogenetic

tree (Fig. 1). According to this tree the investigated *S. suis* serotypes belong to three groups. Group one consists of serotypes 2, 24, 25 and 27, group two consists of serotypes 9, 20 and 26, and group three consists of serotypes 1 and 22. However, all three groups are clearly distant from the other streptococci shown in Fig. 1, indicating that all of the analysed serotypes belong to *S. suis*.

Acknowledgements

We thank Peter Ahrens for the kind donation of the sequencing primers. A special thank to Katja Kristensen for outstanding technical assistance. Without her great skills, the duration of this work would have been prolonged. This work was supported by a grant from the Federation of Danish Pig Producers and Slaughterhouses.

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