Differentiation of *Streptococcus suis* between Serotypes 1 and 14, and Serotypes 2 and 1/2, Using PCR-RFLP

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Abstract

Background: *Streptococcus suis* is an important zoonotic pathogen causing streptococcosis in pigs and severe invasive disease in humans, including meningitis and septicemia. Among the 29 recognized serotypes, serotype 2 is considered the most virulent. Two-step multiplex PCR has been used for routine serotyping. However, this method cannot distinguish between serotypes 1 and 14 or between serotypes 2 and 1/2. Capillary precipitation is therefore required for definitive differentiation, but this technique is costly and requires antisera produced by laboratory animals. A single nucleotide polymorphism (SNP) at position 483 (G→C/T) in the *cpsK* gene has been reported to differentiate these serotypes, providing a potential molecular alternative.

Objectives: To differentiate *S. suis* serotypes 1 and 14, and serotypes 2 and 1/2, using a PCR-restriction fragment length polymorphism (PCR-RFLP) assay targeting the *cpsK* gene at position 483.

Methods: A total of 76 *S. suis* isolates obtained from diseased pigs, together with reference strains representing the four serotypes, were included. Isolates were initially classified as serotype 1 or 14 (n = 6) and serotype 2 or 1/2 (n = 70). A DNA fragment covering position 483 of the *cpsK* gene was amplified and subjected to digestion with the restriction enzyme *Bst*NI. Serotypes were confirmed by Sanger sequencing of the same locus and verified by capillary precipitation using serotype-specific antisera (serotypes 1, 2, and 14).

Results: PCR-RFLP results were fully concordant with both Sanger sequencing and capillary precipitation. Among 6 isolates initially classified as serotype 1 or 14, while 2 isolates were identified as serotype 1 and 4 as serotype 14. Among the 70 isolates classified as serotype 2 or 1/2, 64 isolates were confirmed as serotype 2 and 6 isolates as serotype 1/2. In addition, Sanger sequences show that all serotypes 1 and 1/2 contain allele T at the SNP while all serotypes 2 and 14 contain allele G at the same loci.

Conclusion: The PCR-RFLP assay targeting the *cpsK* SNP at position 483 (G→C/T) accurately differentiates *S. suis* serotypes 1 and 14, as well as serotypes 2 and 1/2. This method provides a reliable, cost-effective alternative to capillary precipitation and reduces the need for laboratory animals.

Keywords: *Streptococcus suis*, Serotyping, PCR-RFLP, Pig, SNP

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