Molecular typing of *Staphylococcus aureus* based on PCR-RFLP of coa gene and RAPD analysis

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Abstract

The aim of this study was molecular identification of *S. aureus* strains isolated from mastitic milk samples and establishing the genetic relationship between strains isolated from cows belonging to the same herd. In all 43 isolated strains the gap gene (930 bp) was amplified, which enabled their affiliation to the *Staphylococcus* genus to be established. PCR-RFLP with AluI endonuclease of the gap gene as well as nuc (450 bp) and coa (1130 bp) gene amplification allowed precise *S. aureus* species identification. One hundred percent of the genetic relationship between strains was established via RAPD-PCR and coa-typing.

Key words: *S. aureus*, mastitis, nuc gene, gap gene, coa gene, PCR-RFLP, RAPD

Introduction

*Staphylococcus aureus* is the primary contagious mastitis agent (Aarestrup et al. 1995, Schlegelová et al. 2003, Saei et al. 2009). In epidemiological investigations, adequate accordant identification markers should differentiate unrelated strains and indicate related isolates of bacteria as belonging to the same type (Aarestrup et al. 1995). The aim of this study was molecular identification of *S. aureus* strains isolated from mastitic cows’ milk samples, based on analysis of the gap gene, nuc gene and coa gene, and establishing the genetic relationship between *S. aureus* strains isolated from cows belonging to the same herd, using RAPD and coa typing.

Materials and Methods

Forty-three *S. aureus* strains isolated from mastitic milk samples were analyzed. Milk samples were collected four times during a 12 month period from 43 cows suffering from subclinical mastitis. Amplification of the gap gene, nuc gene and coa gene was conducted using primers and reaction conditions previously reported by Yugueros et al. (2000), Wilson et al. (1991) and Aarestrup et al. (1995) respectively. Amplified fragments of gap and coa genes were digested with AluI restriction enzyme (Fermentas) according to the manufacturer’s procedure. RAPD was conducted using primers and PCR conditions reported previously.
by Reinoso et al. (2004). Phylogenetic analysis was determined using UPGMA and Jaccard’s coefficient (GeneTools software, Syngene).

Results

All 43 S. aureus strains revealed clumping factor presence and coagulase production using rabbit plasma as well as mannitol fermentation on Mannitol Salt Agar. A 930 bp-long gap gene fragment was amplified in all 43 isolates. PCR-RFLP with AluI endonuclease of the gap gene revealed a restriction pattern specific for S. aureus (in silico analysis, Yugueros et al. 2000). A 450 bp-long fragment of coa gene, and a fragment of coa gene, approximately 1130 bp-long, were also detected in analyzed strains, thus confirming their affiliation to the S. aureus species. Digestion of the amplified coa gene fragment with AluI endonuclease revealed one restriction pattern consisting of 4 bands, about 470 bp, 300 bp, 170 bp and 90 bp in size. RAPD-PCR revealed one repetitive pattern (clonal type) for all analyzed S. aureus strains. This RAPD profile consisted of 6 amplicons ranging from 230 to 1000 bp in size. All strains showed identity on RAPD profile consisted of 6 amplicons ranging from 230 to 1000 bp in size. All strains showed identity on genome level (100% genetic correlation).

Discussion

The application value of the gap gene as a genus marker and tool for taxonomical species analysis of staphylococci was confirmed in previous studies (Yugueros et al. 2000, Karakulska and Sawicka 2008, Nawrotek et al. 2009). Coa typing enables the establishment of predominant coa genotypes of S. aureus and understanding of the epidemiology of bovine mammary gland infections, and also improves mastitis control (Aarestrup et al. 1995, Schlegelová et al. 2003). It is reported that often only a few coa genotypes dominate among isolated strains. It could be presumed that the majority of infections in particular regions could be caused by S. aureus strains with the same coa genotype (Saei et al. 2009). In this study one coa genotype was identified in all S. aureus isolates, collected over 12 months from cows from the same herd. The occurrence of identical or closely related S. aureus strains has been often reported. Saei et al. (2009) showed that while some coa genotypes isolated from bovine mastitis are predominant and common to various herds and can spread freely between cows, herds or even regions, some genotypes are unique for a particular herd. According to reported data, S. aureus strains with predominant coa genotypes have a greater capacity for potential transmission and can cause mammary gland infections and chronic mastitis (Aarestrup et al. 1995, Schlegelová et al. 2003). Results obtained in this study confirmed the significant role of predominant coa genotypes in mastitis duration. Moreover, RAPD-PCR analysis revealed that analyzed S. aureus isolates that were etiological factors of mastitis in one herd represented the same clonal type. This confirms the highly infectious nature of predominant S. aureus genotypes and their significant role in the etiology of mastitis in cows.

References