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Short communication

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 *Alu*I 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 *Alu*I restriction enzyme (Fermentas) according to the manufacturer's procedure. RAPD was conducted using primers and PCR conditions reported previously

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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 *Alu*I 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 *nuc* 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 *Alu*I 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 the 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

Aarestrup FM, Dangler CA, Sordillo LM (**1995**) Prevalence of coagulase gene polymorphism in *Staphylococcus aureus* isolates causing bovine mastitis. Can J Vet Res 59: 124-128.

Karakulska J, Sawicka I (**2008**) Identification of *Staphylococcus spp.* strains isolated from milk samples of mastitic cows by PCR-RFLP analysis of *gap* gene. Adv Agric Sci 12: 91-98.

Nawrotek P, Karakulska J, Czernomysy-Furowicz D, Borkowski J, Furowicz AJ (**2009**) Molecular typing of *Staphylococcus aureus* strains isolated from milk of mastitic cows. Med Weter 65: 127-130.

Reinoso E, Bettera S, Frigerio C, DiRenzo M, Calzolari A, Bogni C (**2004**) RAPD-PCR analysis of *Staphylococcus aureus* strains isolated from bovine and human hosts. Microbiol Res 159: 245-255.

Saei HD, Ahmadi M, Mardani K, Batavani RA (**2009**) Molecular typing of *Staphylococcus aureus* isolated from bovine mastitis based on polymorphism of the coagulase gene in the north west of Iran. Vet Microbiol 137: 202-206.

Schlegelová J, Dendis M, Benedík J, Babák V, Ryšánek D (**2003**) *Staphylococcus aureus* isolates from dairy cows and humans on a farm differ in coagulase genotype. Vet Microbiol 92: 327-334.

Wilson IG, Cooper JE, Gilmour A (**1991**) Detection of enterotoxigenic *Staphylococcus aureus* in dried skimmed milk: use of the polymerase chain reaction for amplification and detection of staphylococcal enterotoxin genes *entB* and *entC1* and the thermonuclease gene *nuc*. Appl Environ Microbiol 57: 1793-1798.

Yugueros J, Temprano A, Berzal B, Sanchez M, Hernanz C, Luengo JM, Naharro G (**2000**) Glyceraldehyde-3-phosphate dehydrogenase-encoding gene as a useful taxonomic tool for *Staphylococcus spp*. J Clin Microbiol 38: 4351-4355.