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Short communication

Identification of specific genes in *Staphylococcus aureus* strains associated with bovine mastitis

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ABSTRACT

Staphylococcus aureus is a common cause of bovine mastitis that is responsible for the main economic loss to the dairy industry. For identification of putative, bovine-specific molecular marker a genome comparison between bovine *S. aureus* strain RF122 and 52 previously sequenced *S. aureus* isolates associated with human infections using genome viewer, annotation tool Artemis Comparison Tool (ACT), KEGG and NCBI BLAST databases was carried out. This led to the identification of 16 unique RF122 gene sequences that may be used as molecular marker to distinguish bovine from human strains. The distribution of these genes was analyzed in a collection of bovine mastitis strains from the Netherlands and human clinical isolates by PCR and Southern blotting. Only four genes within the pathogenicity island SaPIbov3 (*sab1890*, *sab1891*, *sab1892*, *sab1893*) were present in the majority of isolates from cattle but were absent from human clinical *S. aureus* isolates. These results suggest that there is no gene/ORF uniformly shared by all bovine *S. aureus* strains that could be uniformly used as a diagnostic marker gene.

1. Introduction

Staphylococcus aureus is a common cause of bovine mastitis. Preventive strategies such as optimization of milking practices, culling, postmilking teat disinfection and antibiotic therapy are being applied, however, the prevalence of subclinical and chronic mastitis is still high (Zecconi et al., 2003; Volling and Kromker, 2008). Vaccines developed to control this disease showed limited protection due in part to the lack of common antigens among the mastitis isolates (Foster, 1991; Giraudo et al., 1997; Calzolari et al., 1997; Leitner et al., 2003). The molecular mechanisms by which *S. aureus* causes bovine mastitis remain poorly understood as multiple virulence factors are expressed specifically for an individual strain under variable conditions (Tuchscherr et al., 2007; Otto, 2010). Several epidemiological studies evidence a host-specialization among clones associated with human and bovine infection and suggests that some clonal groups have achieved a widespread distribution and are responsible for the majority of infections (Smith et al., 2005; Leonard et al., 2006; Sung et al., 2008; Smyth et al., 2009). Recently, whole genome sequencing and comparative genomic hybridization has been applied to identify molecular genetic features that distinguish bovine-associated *S. aureus* optimized for mastitis pathogenesis in cattle from those that infect humans (Herron-Olson et al., 2007; Ben Zakour et al., 2008). In particular, mobile genetic elements such as pathogenicity islands and bacteriophages contributes to the variation in genome content between strains of different origin (Ben Zakour et al., 2008; Sung et al., 2008). For example, multiple putative horizontal

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gene transfer DNA sequences from different causative agents of bovine mastitis were found in the hospital epidemic methicillin-resistant MRSA252 strain and not present in other human isolates (Brody et al., 2008). All these studies strongly suggest host-specialization of *S. aureus* clones and indicate that certain clones are particularly successful at causing bovine mastitis (Haveri et al., 2005; Herron-Olson et al., 2007). However, the genomic variability among *S. aureus* clones is high and the molecular basis of host-specialization remains to be determined. The aim of the present work was to identify genetic features specific for bovine isolates that may serve as diagnostic marker for *S. aureus* causing bovine mastitis. Thus, the genome sequence of the bovine *S. aureus* isolate RF122 was compared with the genome of 52 human *S. aureus* isolates available in the database. Overall, 16 genomic features have been identified which are specifically associated with the bovine isolate RF122. The distribution of these genes was further analyzed using a strain collection of bovine isolates and representative human strains including epidemic dominant lineages.

2. Material and methods

2.1. Bacterial strains

In the present study, we report on the characterization of a collection of bovine *S. aureus* isolates from The Netherlands sampled in 2005–2006 (Table 1). Human strains were selected from the German reference center for staphylococci (Robert-Koch Institute, branch Wernigerode) (Table 1). *S. aureus* strain RF122, of which the full-genome nucleotide sequence has recently been published, was also included in the study (Herron-Olson et al., 2007). Isolates were identified to the species level by biochemical characterization using the API-20-Staph system (bioMerieux, Marcy l'Etoile, France) and 16S rRNA gene sequencing. In addition, the strains were tested with the Staphytect Plus test (Oxoid, Wesel, Germany).

2.2. Comparative analyses of different S. aureus genomes

For identification of putative, bovine-specific targets a genome comparison between bovine *S. aureus* strain RF122 and previously sequenced 52 *S. aureus* isolates (N315, Mu50, Mu3, MW2, MRSA252, MSSA476, COL, USA300, NCTC8325, JH9, JH1, Newman, TW20, ST398, 55/2053, M876, 65-1322, 68-397, E1410, MN8, TCH60, TCH130, TCH70, JKD6008, JKD6009, A017934/97, H19, C101, C427, D139, M899, WBG10049, WW2703/97, Btn1260, C160, A5937, A5948, A6224, A6300, A8115, A9299, A9635, A9719, A9763, A9781, A9765, A10102, A8117, 930918-3, D30, 132, CF-Marseille) associated with humans (mostly from infections) was carried out by using Artemis Comparison Tool (ACT) and KEGG and BLAST databases. The distribution of selected genes within a collection of bovine mastitis isolates from The Netherlands and human clinical isolates was examined by use of Southern blot hybridization and PCR. Isolation of chromosomal DNA, restriction enzyme cleavage, and Southern blotting were done as described previously (Cramton et al., 1999). Specific gene probes were amplified using the primers and conditions described in Table S1, with *S. aureus* RF122 chromosomal DNA as the template.

2.3. PFGE

The genetic relationships of 51 bovine *S. aureus* isolates from our collection were classified by pulsed-field gel electrophoresis (PFGE). Chromosomal DNA was digested with the endonuclease SmaI (New England Biolabs, Frankfurt, Germany) at 37 °C for 4 h. Then, electrophoresis was carried out in a CHEF DR-III apparatus (Bio-Rad, München, Germany) for 18 h at 14 °C at 6 V/cm with pulses from 5 to 40 s. A standard pattern (Lamda Ladder PFG Marker, New England Biolabs) was included in the gels to allow comparison of the digitally normalized PFGE profiles.

2.4. MLST

Internal PCR fragments of seven housekeeping genes were amplified using previously described primers (Enright et al., 2000). Clonal analysis of the STs was performed using eBURST, a Web-implemented clustering algorithm (http://www.mlst.net), which divides MLST data sets into groups of related isolates and predicts the founding genotype of each clonal complex. STs with at least five of seven identical alleles were defined as a clonal group.

3. Results and discussion

3.1. Genotyping of bovine S. aureus isolates by pulsed-field gel electrophoresis (PFGE) and MLST

Given that bovine *S. aureus* strains are epidemiologically distinctive from human strains we were looking for putative, bovine-specific genes/ORFs which could serve as a diagnostic marker for bovine isolates or do have the potential as a target for vaccine development. First, the heterogenicity of bovine *S. aureus* isolates from a collection of 51 isolates was assessed by pulsed-field gel electrophoresis (PFGE) to select isolates with different genetic background for analysis of bovine-associated marker genes (see below). The analysis revealed 24 different pulsefield types (data not shown). Based on their PFGE profiles, 28 bovine *S. aureus* strains with different and four partly with the same PFGE pattern were selected for further analysis using MLST. The analyzed strains represent 15 different STs, including three STs which have been not described so far. The new STs resulted from new allele combinations, as well as new alleles (Fig. 1). Of the 28 bovine isolates analyzed here, 46% were from the dominant bovine lineages ST151 ($n = 1$), ST 97 ($n = 7$), and ST71 ($n = 5$). Previous epidemiological studies using PFGE, MLST and multi-strain microarray reported that relatively few widely distributed clones of *S. aureus* are responsible for the majority of cases of bovine intramammary infections (Kapur et al., 1995; Zadoks et al., 2000; Smith et al., 2005; Sung et al., 2008). In the UK, USA and Chile, bovine mastitis is mostly caused by isolates of lineage ST151, ST771 and