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Lis LSR Rocha[1]

[1]Universidade Federal de Viçosa

Lis LSR Rocha
Universidade Federal de Viçosa

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Abstract

Many efforts have been made to understand the pathogenesis of bovine mastitis to reduce losses and promote animal welfare. *Staphylococcus aureus* may cause bovine clinical mastitis, but it is mainly associated with subclinical infection, which is usually persistent and can easily reoccur. Here, we conducted a comparative genomic analysis between four strains of *S. aureus* causing subclinical infection (Sau170, 302, 1269, 1364), previously sequenced by our group, and two well-characterized strains causing clinical mastitis (N305 and RF122) to find differences that could be linked to mastitis outcome. A total of 146 virulence-associated genes were compared and no appreciable differences were found between the bacteria. However, several nonsynonymous single nucleotide polymorphisms (SNPs) were identified in genes present in the subclinical strains when compared to RF122, especially in genes encoding host immune evasion and surface proteins. The comparison of orthologous genes using OrthoMCL identified a membrane transporter in the genomes of the bacteria belonging to the subclinical group, but this finding was not confirmed by polymerase chain reaction (PCR) on a collection of field isolates of *S. aureus* associated with clinical or subclinical mastits. The secreted and surface proteins predicted by different in silico tools were compared through multidimensional scaling analysis, revealing a high degree of similarity among the six strains. However, differences were seen in the nucleotide sequences of a gene that codes for a hypothetical protein (cl3309) and a lipoprotein (cl3700). These findings were also analyzed by PCR on DNA extracted from field isolates of *S. aureus*. The lipoprotein, but not the hypothetical protein, was able to separate the clinical isolates from the subclinical ones. These results show that sequence variation among bovine *S. aureus*, and not only the presence/absence of virulence factors, is an important aspect to consider when comparing isolates causing different mastitis outcomes

Materials

Field isolates of *S. aureus* were used to validate some findings obtained by *in silico* analysis. Isolates 308, 340, 403, 1001, 1311, 1315, and 1323 originated from animals suffering from subclinical mastitis, diagnosed as having a score of two or three on the California Mastitis Test. Isolates 76, 216, 1439, 2555, 3909, 5T18-19, 9T18-16, 10T18-59, 10T18-68, 14T18-13, 22T18-52, and 22T17-54 were collected from cows presenting signs of clinical mastitis, with visible abnormalities in the milk, such as blood and clots.

Growing and storing conditions

1 The bacteria used in this study were maintained on BHI agar (Brain Heart Infusion, BHI HiMedia, Mumbai, India) at 37 °C

2 stored in the long term in BHI containing 20% glycerol.