Identification of coagulase negative staphylococci isolated from bovine mastitis by 16S rDNA sequencing - Lange C.C.[1], Brito M.A.V.P.[1*], Silva F.S.[2], Alvim M.C.T.[3], Reis D.R.L.[1], Domingues R.[4]

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Mastitis is a multifactorial disease, which often has a bacterial origin, and affects cattle worldwide. The genus *Staphylococcus* contains at least 40 species and 17 subspecies and, under certain circumstances, many of these species can cause mastitis. In herds that have achieved control of infectious agents, especially *Staphylococcus aureus* and *Streptococcus agalactiae*, other *Staphylococcus* species have often been associated with mastitis in cattle. In this study a total of 63 coagulase-negative staphylococci (CNS) were subjected to sequencing of the 16S rDNA to species identification. The sequencing reactions were done on the MegaBACE 1000 DNA sequencer (GE Healthcare, NYSE, Germany). The pairs of sequence were edited and assembled using DNA Baser v3 software (Biosoft Heracle, Germany) and the resulting sequences were blasted against *Staphylococcus* spp. nucleotide database of the National Center for Biotechnology Information (NCBI). The species identified were *S. chromogenes* (25), *S. sciuri* (10), *S. haemolyticus* (7), *S. epidermidis* (6), *S. simulans* (5) and *S. hyicus* (3). All species identified in this study have been already identified in other studies about the etiology of staphylococcal mastitis. The sequenced fragments of seven isolates aligned along its entire length (100%) with two or three different species. The sequencing of a larger fragment from the same gene or other gene must be done to obtain the definitive identification of these isolates. The accurate identification to the species level of CNS is necessary to establish sources of infection, transmission mechanisms and the impact of the different species in bovine mastitis.

Key-words: *Staphylococcus chromogenes, S. sciuri, S. haemolyticus*

Embrapa project number: 01.09.01.003.05.02

Gado de Leite

Identification of coagulase negative staphylococci isolated from bovine mastitis by 16S rDNA sequencing

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INTRODUCTION

Mastitis is a multifactorial disease, which often has a bacterial origin, and affects cattle worldwide. The genus *Staphylococcus* contains at least 40 species and 17 subspecies and, under certain circumstances, many of these species can cause mastitis. In herds that have achieved control of infectious agents, especially *Staphylococcus aureus* and *Streptococcus agalactiae*, other *Staphylococcus* species have often been associated with mastitis in cattle.

The accurate identification of CNS to the species level is necessary to establish sources of infection, transmission mechanisms and the impact of the different species in bovine mastitis. The aim of this work was to identify CNS to the species level using the 16S rDNA sequencing.

MATERIAL E METHODS

In this study a total of 63 coagulase-negative staphylococci (CNS) were subjected to sequencing of the 16S rDNA to species identification. The isolates were isolated from milk of cows with subclinical mastitis from herds located in Minas Gerais and Rio de Janeiro State, between 1998 and 2002. The sequencing reactions were done on the MegaBACE 1000 DNA sequencer (GE Healthcare, NYSE, Germany). The pairs of sequence were edited and assembled using DNA Baser v3 software (Biosoft Heracle, Germany) and the resulting sequences were blasted against *Staphylococcus* spp. nucleotide database of the National Center for Biotechnology Information (NCBI).

RESULTS AND DISCUSSION

The species identified were *S. chromogenes* (25), *S. sciuri* (10), *S. haemolyticus* (7), *S. epidermidis* (6), *S. simulans* (5) and *S. hyicus* (3). All species identified in this study have been already identified in other studies about the etiology of staphylococcal mastitis (Santos et al., 2008; Taponen et al., 2008; Zadocks and Watts, 2009).

Seven isolates could not be identified with the sequencing of the 536 bp fragment of the 16S rDNA gene. The sequenced fragments of these isolates aligned along its entire length (100%) with two or three different species. The sequencing of a larger fragment from the same gene or other gene must be done to obtain the definitive identification of these isolates.

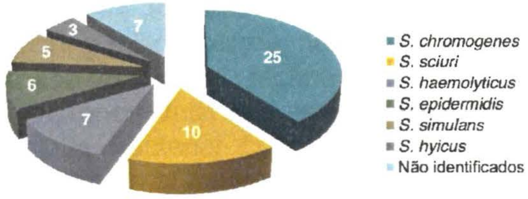

Figure 1. Identification of coagulase-negative *Staphylococcus* species isolated from bovine mastitis in the states of Minas Gerais and Rio de Janeiro

CONCLUSION

The 16S rDNA sequencing identified 56 CNS isolates of bovine mastitis at species level. The identity of seven CNS isolates remain to be cleared.

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