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PCR development for molecular identification of *Staphylococcus chromogenes*

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Coagulase negative *Staphylococcus* (CNS) constitutes a group of more than 35 species and is present in skin flora of human and animals. Recently have acquired major importance in many countries being related as emergent pathogens in animal cases of mastitis, the inflammatory process of the mammary gland. In the diagnostic routine, the CNS species are treated as one group since the distinction of these species by traditional biochemical tests are expensive and time consuming. However, *S. chromogenes* is one of the most common species found in CNS of dairy herd. So, the development of identification methodologies based on molecular biology, as PCR, using conserved genes as targets for specie-specific primers, will allow a quick and accurate analysis of many samples at the same time. A monomeric thermonuclease, also known as micrococcal nuclease, codified by the *nuc* gene, is conserved in *Staphylococcus* genus and it has been used for species-specific identification. However, this gene target has not been applied yet to identify *S. chromogenes*, although its significance in mastitis. In this study we performed a sequence analysis of *nuc* gene and developed a specie-specific PCR for the identification of *S. chromogenes*. The pair of primers was designed based on the partial sequence of *nuc* gene of *S. chromogenes* available (GenBank: AB465333.1) and the PCR cycles was standardized. At the present time, 22 reference *Staphylococcus spp.* strains and 31 clinical ones, 26 *S. chromogenes*, 2 *S. aureus*, 1 *S. captis*, 1 *S. epidermidis* and 1 *S. haemolyticus* were analyzed by this PCR protocol. The amplification was verified, by electrophoresis in agarose gel 1.5% as a single band of 234 pb corresponding to the *nuc* gene, only in *S. chromogenes* strains and also a 478 pb amplicon corresponding to the *rrs* gene responsible for the *16S r*RNA in all the analyzed strains, discarding false negative results. All the strains previously identified as *S. chromogenes* have been positive for the amplification of *nuc* gene with this pair of primers, but negative for the others *Staphylococcus* species. Further tests, including other *Staphylococcus* species and a higher number of samples, will be performed in order to confirm the specificity and sensibility of the pair of primer developed in this study.