

# MICRO BIOTECH 15

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## Antimicrobial activity of Brazilian propolis extracts for *Staphylococcus aureus* of sheep and goats mastitis origin

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Mastitis is considered the main disease causing milk yield reduction in sheep and goat and consequently a prejudice source for dairy producers[1]. This inflammation of the mammary gland is usually caused by environmental and infectious pathogens, from which the most frequently found are bacteria of the genus *Staphylococcus*, including *S. aureus* and coagulase negative staphylococci[2].

*Staphylococcus aureus* is quite resistant to antimicrobial agents due to its wide range of resistance genes [3], [4]. Considering this fact the aim of this work is to study innovative ways to control mastitis using propolis, a natural antimicrobial that has considerable activity against these resistant strains. Propolis have been studied by researchers from all over the world, especially Brazilian propolis due to its great vegetation biodiversity and their high levels of total phenolic compounds, flavonoids, tannins and anthocyanin. Propolis is a resinous mass produced by honeybees *Apis mellifera*, which manipulate resins collected in various vegetables with their salivary glands secretions producing this mass that is used to close the hive hindering the access of intruders [5], [6].

Twenty-four *S. aureus* isolates were analysed. From those, 22 were isolated from milk of goats and sheep with clinical and subclinical mastitis, from the region of Vale do São Francisco in the Brazilian Sertão and *S. aureus* ATCC 25923 plus a MRSA strain were added. Alcoholic extracts were produced from several batches of green, red and brown propolis consisting of 300 g of raw propolis in 700 mL of 70 % ethanol.

Four genes related to antimicrobial resistance were assessed: *blaZ* that determines the resistance to  $\beta$ -lactam antibiotics, and genes *icaA*, *icaD* and *bap* that influence the production of biofilm. For the tests of susceptibility to different types of propolis the microdilution method was used, in triplicate, and dilutions between 0.003672 and 15% were tested, 70 % ethanol consisted of a negative control.

The gene *blaZ* was found in 15 isolates; *icaA* gene was present in 3 isolates, *icaD* gene in 2 and *bap* gene was detected in 6 isolates. All the propolis tested exhibited antimicrobial activity, ranging from 44 to 100 % of susceptible isolates depending on different propolis batches. According to the results of this experiment the green and red propolis appear to have better antimicrobial activity than the brown variety.

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