

**Investigation of the gen presence and formation of biofilms by *Staphylococcus* in a milk processing micro-dairy** - Santos S.S.<sup>1</sup>, Medeiros M.I.M.<sup>1</sup>, Souza V.<sup>1</sup>, Melo P.C.<sup>1</sup>, Zafalon L.F.<sup>2</sup>, Veschi J.L.A.<sup>3\*</sup>, Nader Filho A.<sup>1</sup>

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Milk is a highly nutritious food and excellent substrate for the multiplication of microorganisms. The objective of this study was to investigate the presence and formation of biofilm of *Staphylococci* before and after the cleaning process in a micro-dairy plant in Sao Paulo State. A total of 60 swab samples were collected before and after the cleaning process from the reception tank surfaces, the raw milk storage tank, the pasteurizer outlet pipe, the pasteurized milk storage tank, and the filling machine. Further samples were collected of the milk in the receiving tank, the raw milk storage tank, from the pasteurizer outlet pipe, and packaged milk, as well as the empty sealed plastic packaging used for packaging the pasteurized milk. Of the 41 *Staphylococcus* spp strains, 39.0% indicated positive in the coagulase test, while 61.0% were negative. Through genetic analysis, using the polymerase chain reaction technique, it was observed that within the 16 coagulase-positive strains, 25% presented the gene *icaA* and 100% had the gene *icaD*. Of the 25 coagulase-negative strains, 44% had the gene *icaA* and 100% had the gene *icaD*. It was seen using a scanning electron microscope the formation of biofilm for all the isolated strains. From the obtained results it was possible to see evidence to the potential risk to the health of the consumer represented by the presence of strains of *Staphylococcus* arising from the contaminated raw material or from deficiencies in hygienic cleaning, with formation of microorganisms' biofilms.

Key-words: biofilms, genes *icaAD*, milk quality

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## Investigation of the gen presence and formation of biofilms by *Staphylococcus* in a milk processing micro-dairy

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### INTRODUCTION

Milk is a highly nutritious food and excellent substrate for the multiplication of microorganisms. The objective of this study was to investigate the presence and formation of biofilm of *Staphylococcus* before and after the cleaning process in a micro-dairy plant in São Paulo State.

### METHODS

Sixty swab samples were collected before and after the cleaning process from the reception tank surfaces, the raw milk storage tank, the pasteurizer outlet pipe, the pasteurized milk storage tank, and the packaging machine. Further samples were collected of the milk in the receiving tank, the raw milk storage tank, from the pasteurizer outlet pipe, and packaged milk, as well as the empty sealed plastic packaging used for packaging the pasteurized milk. The media used for the accession of bacterial cells in vitro were coupons in stainless steel AISI 304 with 10 x 20 mm.

### RESULTS

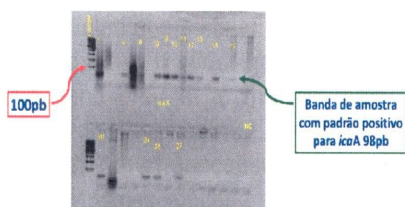


Figure 1 - Gen *icaA* positive in the columns 4, 6, 11, 12, 13, 20 e 24 (tank surfaces); 10 e 27 (pipes); a das columnas 1, 8, 9, 15, 17 e 25 (milk). NC: Negative control.

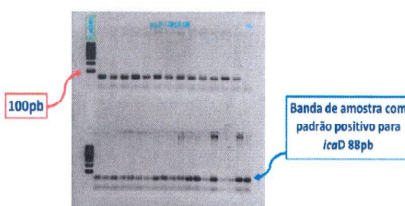


Figure 2 - *Staphylococcus* spp strains / gen *icaD* positive. NC: Negative control.



Figure 3A - Micrography of adhered cells of isolated strains on the surface of stainless steel AISI 304.

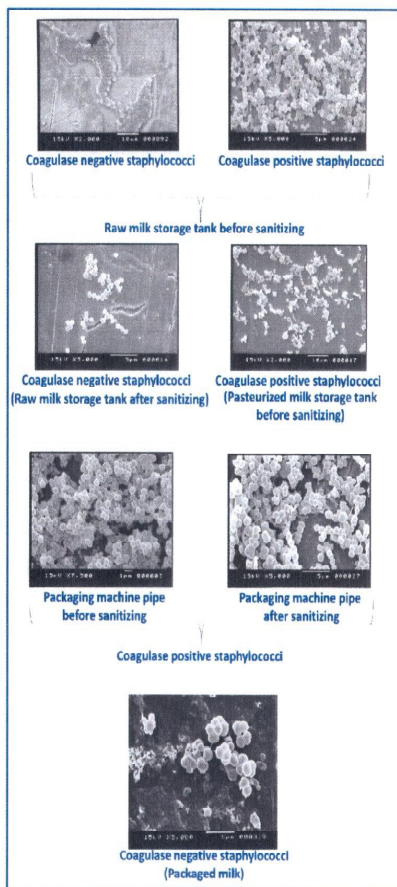


Figure 3B - Micrography of adhered cells of isolated strains on the surface of stainless steel AISI 304

### CONCLUSIONS

The presence of staphylococci strains in the contaminated material by deficiencies in hygienic cleaning with formation of biofilms represents a potential risk to the health of the consumer.

Co-financial support (CNPq/MAPA/SDA N°064/2008)

Pictures: SANTOS, S.S. 2009. Investigação da presença e da formação de biofilmes por estafilococos em micro-usina de beneficiamento de leite. 57p. Universidade Estadual Paulista Jaboticabal, SP, Brasil.