Identification of coalho cheese microbiot of districts of Pernambuco, Brazil

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The coalho cheese is manufactured with mass semi cooked and traditionally consumed fresh or ripened. It is produced there is more than 150 years, in several States of the Northeast Area of Brazil starting from milk of cow raw and/or pasteurized milk. Formerly it was used for coagulation of the milk the coagulum of the dry and salted stomach of wild animals or calves. Now this practice was substituted by the use of coagulum industrial (Lima, 1996). Last years, they have been increasing the interest in the studies genotipics and fenotipics wild type isolated starting from handmade cheeses produced without the addition of cultures starters (Coppola et al., 2001). Molecular techniques supply appropriate tools for the typification, taxonomies and accompaniment of the evolution of the microorganisms involved in the processing of the victuals (Germond et al., 2003; Rossetti and Giraffa, 2005). Several species-specific primers have been drawn and used for identification of acid-lactic bacteria through PCR or reactions of multiple PCR that are faster and convenient than hybridization techniques (Fortina et al., 2001).

The present work sought the identification of the goods of bacteria found in the coagulum cheese "A" and "B" produced in the small ones, averages and Micro Industries in some municipal districts of the rural area of the Pernambuco, Brazil Batches of coalho cheese produced with raw and pasteurized milk obtained starting from local producers previously registered. Batches of coalho cheese, two ripened in the autumn and winter, and the other seven in the spring and summer, were manufactured by two cheese-makers in accordance with traditional methods. These cheese samples were taken to the laboratory under refrigeration, being kept below 5°C, and were analysed on arrival. The stumps were cultivated in ldr10% (skimmed milk reconstituted to 10% of solids non greasy), for 24 at 48 hours, in greenhouse 30 °C and 37 °C (1 tube to 30 °C and other to 37 °C). After the proven growth for the coagulation of the milk, it was proceeded the plated and the purification of the microorganisms in the means Agar MRS, Agar M17 to 37°C and 30°C and Agar Miller-Hinton to 37°C for 18 at 24 hours. With the isolated bacteria, they were made the test of Gram and catalase test. With the species-specific primers, it was made the identification of the stumps through the technique of PCR (polimerase chain reaction), for a final volume of 15 ml. The amplification was accomplished for through the genic sequencing. Of ownership of the results, any difference was not verified in the growth of the isolated ones in half MRS, Agar M17 to 37°C and 30°C and Agar Miller-Hinton to 37°C.

The bacterial isolated presents in the cheese coalho were mainly *Staphylococcus* spp., *Lactobacillus* spp, *Lactococcus* spp. *Streptococcus* spp. (Guedes Neto et al, 2005, what demonstrates the contamination for other groups of microorganisms.

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References

- Coppola, S., Blaiotta, G., Ercolini, D., Moschetti, G. (2001). Molecular evaluation of microbial diversity occurring in different types of Mozzarella cheese. *Journal Applied Microbiology*, v. 90, p. 414 420.
- Germond, J. E., Lapierre, L., Delley, M., Mollet, B., Felis, G. E., Dellagio, F. (2003). Evolution of the bacterial species Lactobacillus delbrueckii: a partial genomic study with reflections on prokaryotic species concept. *Molecular Biology Evolution*, v. 20, p. 93-104.
- LIMÁ, M. H. P. (1996) *Elaboração* de queijo de coalho a partir de leite pasteurizado e inoculado com S. thermophillus e L. bulgaricus 82 f. *Dissertação* (Mestrado em Tecnologia de Alimentos), Centro de Ciências Agrárias, Universidade Federal do Ceará, Fortaleza.
- Guedes Neto, L.G., Souza, M.R., Nunes, A.C., Nicoli, J.R., Santos, W.L.M. (2005) Atividade antimicrobiana de bactérias ácido-lácticas isoladas de queijos de coalho artesanal e industrial frente a microrganismos indicadores. *Arquivo Brazileiro Medicina Veterinaria Zootecnia* v.57, supl. 2, 246 p.245-250
- Gatti, M., Rossetti, L., Fornasari, M. E., Lazzi, C., Giraffa, G., Neviani, E. (2005). Eterogeneity of Putative Surface Layer Proteins in Lactobacillus helveticus. *Appl. Environ. Microbiol.* 71: 7582-7588
- Fortina, M. G., Ricci, G., Mora, D., Parini, C., Manachini, P. L. (2001). Specificidentification of Lactobacillus helveticus by PCR with pepC, pepN and htrA targeted primers. *FEMS Microbiology Letters*, v. 198, p. 85 – 89.