TITLE: Detection of *aprX* gene encoding alkaline metalloprotease in proteolytic bacteria isolated from raw milk.

AUTHORS: ARAÚJO, L. G.; ALVES, B. K.; LOPES, J. B. A.; RIBEIRO JÚNIOR, J. C.; CESAR, K. K. F. A.; EVANGELISTA, G. D.; MACEDO, A. T.; MACEDO, G. H. R. V.; ABREU, A. G.

INSTITUTION: UNIVERSIDADE CEUMA, SÃO LUÍS, MA (JOSUÉ MONTELLO ST. 1 - RENASCENÇA II, POSTCODE: 65075-120, SÃO LUÍS - MA, BRAZIL)

ABSTRACT:

Among contaminating bacteria from milk, psychrotrophic ones stand out as the main responsible for the proteolysis because they are able to multiplying in refrigeration temperatures. aprX gene carries information for the alkaline metalloprotease production, a proteolytic enzyme related, mainly, to the *Pseudomonas* genus, the main genus of psychrotrophic bacteria in raw refrigerated milk. The aim of this was to investigate, by biomolecular methods, the presence of aprX gene in proteolytic bacteria isolated from raw milk from Maranhão microregion. There were selected 112 samples of raw milk collected from february to june- 2018 in dairy farms of Codó and Caxias - MA. Microbiological analyzes were performed at Instituto Federal do Maranhão (IFMA) Campus Caxias, and biomolecular assays at Laboratório de Inspeção de Produtos de Origem Animal (LIPOA) – Universidade Estadual de Londrina (UEL). Tests were carried out to classify proteolysis and psychrotrophic activity (7°C for 10 days). All 112 proteolytic psychrotrophic bacteria were submitted to detection of aprX genes to verify the potential of alkaline metalloprotease production by PCR, as well as submitted to molecular confirmation by searching for Pseudomonas genus using specific fragment in 16S rRNA gene, according to previously protocols. There were 12 (10.7%) positive for the aprX gene, all of them associate with the genus Pseudomonas, corroborating with other studies in the state that point to the great potential for deterioration of the products in the locality. Therefore, as in other Brazilian regions, refrigerated raw milk produced in the state of Maranhão needs to be obtained hygienically, avoiding contamination with deteriorating microorganisms that may compromise the integrity and quality of the fluid milk and derivatives intended for trading.

Keywords: *Pseudomonas; aprX;* Milk; Alkaline Metalloprotease; Psychrotrophic bacterias.

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