EXPRESSION OF RECOMBINANT BOVINE LACTOFERRIN IN KM71-H STRAIN OF *Pichia Pastoris*

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Introduction. Lactoferrin, a no hematic iron-binding glycoprotein belonging to the family of transferrins, with a molecular weight of approximately 80 kDa (2); exerts antimicrobial activity against a broad spectrum of microorganisms such as bacteria, viruses, fungi and parasites, also has the ability to modulate the immune response (3).
The objective of this work was determine the methodology to obtain a recombinant protein that in the future can serve as a nutraceutical protein with antimicrobial and immune capacity.

Methods. Yeast transformation was achieved by electroporation with expression vector containing synthetic lactoferrin pJ901 and pJ902. Transformed yeast clones were selected with G418 and Zeo respectively (5). To assess lactoferrin expression, clones were induced with 0.5% methanol in intervals of 24 h, during 72 h in liquid culture medium (4). Protein extracts were recovered by cell lysis and were analyzed by SDS-PAGE and subsequently by Western blot using anti-bLF HRP. Best expressed clones were purificated by chromatography. The antimicrobial effect of recombinant bovine lactoferrin was tested in *S. aureus* and *S. epidermidis* (1).

Results. It was demonstrated that recombinant bovine lactoferrin. The total level of proteins expression is achieved obtain 1,521 mg / mL. The synthetic bovine Lactoferrin inhibited bacterial growth of *S. aureus* and *S. epidermidis.*

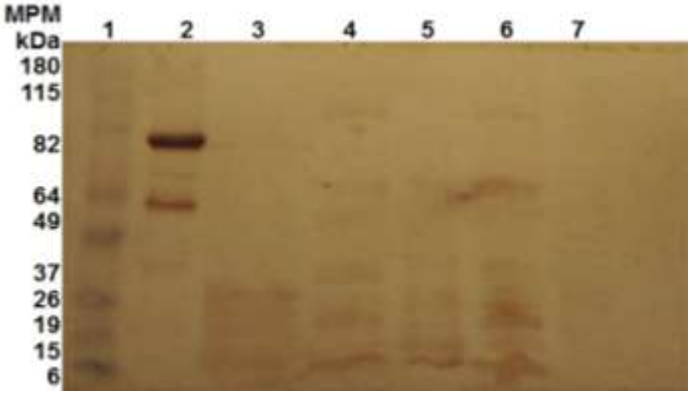

Fig 1. Wester blotting of Concentrated protein extracts C2 pJ901 and C2 pJ902. Lane 1. MPM BenchMark™ Pre-Stained Protein Ladder. Lane 2. comercial bLf 5 mg/mL. Lane 3. Soluble fractions. C2 pJ901. Lane 4. Soluble proteins C2 pJ902. Lane5. Insoluble fractions. C2 pJ901. Lane 6. Insoluble protein C2 pJ902. Lane 7. KM71-H strain P. pastoris without plasmid.

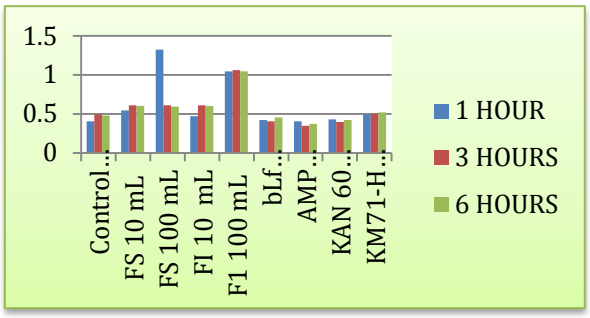

Fig 2. Antimicrobial assay against *S. aureus*. FS: soluble fractions. FI: insoluble fractions. AMP: Ampicile. Kan:Kanamicin

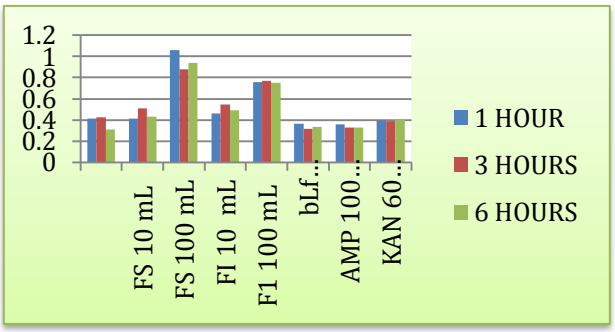

Fig 2. Antimicrobial assay against *S. epidermidis*.

The antimicrobial effect against *S. aureus* and *S. epidermidis* was observed, where it was determinate the minimum concentration of recombinant bovine lactoferrin to cause an inhibitory effect on certain bacteria.

Conclusions. It is possible the introduction of synthetic genes pJ901: synthetic 98782 lactoferrin, and pJ902: synthetic lactoferrin 98783, plasmid DNA from Mach 1 *E. coli* strain to P. KM71-H strain of *P. pastoris*. The synthetic bovine lactoferrin expressed has bactericidal and / or bacteriostatic activity in *S. aureus* and *S. epidermidis.* There are needed at least 21.6 g of synthetic bovine lactoferrin to exert an inhibitory effect.

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