METHODS OF PURIFICATION OF BACTERIOCINS PRODUCED BY *Lactobacillus helveticus.*

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Introduction. The separation and purification processes depend on the medium used for production, and the microorganism used. The stability of bacteriocins decreases through the purification process (1). The techniques typically used to obtain bacteriocins are: ammonium sulfate precipitation, precipitation with trichloroacetic acid, chloroform precipitation, adsorption columns, among others. The efficiency of each technique depends on the characteristics of the peptide to be purified and the concentration of other antagonistic substances (2). The aim of this work was to purify the bacteriocin produced by *Lactobacillus helveticus.*

Methods. *Lactobacillus helveticus* was grown at 42°C for 48 h in culture medium containing: Oligomate 55 (1%) as carbon source, casein peptone (0.5%) and yeast extract (0.3%). The effect of H_2O_2 and organic acids were discarted; the presence of bacteriocin was determined as reported by Figueroa-González (2010) using as an indicator organism *Listeria innocua ATTC 33090.* Purification techniques were performed by membrane ultrafiltration reported by Joerger and Klaenhammer (1986) and cell adsorption-desorption (5). The molecular weight and purity check of the bacteriocin was determined by acrylamide SDS-PAGE.

Results. Table 1. Growth inhibition of *Listeria innocua* after purification methods employed.

Purification methods	Growth inhibition (%)
Crude extract	26.88 ±4.54
Extract bacteriocin	12.48 ±2.26
Ultrafiltration permeate	5.7 ±1.16
Ultrafiltration concentrate	8.37±0.76
Cell adsorption-desorption	11.96±0.31

Table.1 Growth inhibition of *Listeria innocua* in different purification methods

In the crude extract could be observed the growth inhibition effect due to all antimicrobial compounds produced by *Lactobacillus helveticus*. Bacteriocin extract showed the growth inhibition only by the effect of bacteriocin, since H_2O_2 and organic acids were eliminated. It was observed trough purification process with ultrafiltration that the activity of bacteriocin was lost. While that purify with cell adsorption-desorption conserves the capacity to inhibit growth, this technique proved to be most useful for preserving bacteriocin activity. Sample of bacteriocin purify by cell adsorption-desorption was used to determine the purity and molecular weight on SDS-PAGE; four bands were observed with molecular weight of 89.37 KDa, 78.433 KDa, 65,902 KDa and 40.83 KDa.

Conclusions. It was found that the most appropriate purification method for bacteriocin produced by *Lactobacillus helveticus* was through cell adsorption-desorption; by this method the bacteriocin is mainteined. It also avoids the use of reagents, which are difficult to remove or that may affect the stability of the bacteriocin as in the case of solvents or salts.

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References.

1. Moreira S., Wagner L. (1993). Tesis Doctorado Universidad complutense de Madrid Facultad de veterinaria. Departamento de Nutrición y Bromatología III.
2. Vásquez, M., Suárez, M. y Zapata, (2009). RCN
3. Figueroa-González, I. (2010) Estudio de prebióticos en el crecimiento de bacterias probióticas comerciales y en la síntesis de compuestos con actividad antimicrobiana. Tesis doctorado en Biotecnología. Universidad Autónoma Metropolitana.
4. Joerger M. C., Klaenhammer T. R. (1986). J. of B. Vol. 167, No. 2, p. 439-446.
5. Ajay Pal., Ramana K.V.,(2008).J.of F.B. 34 : 932- 948.