IS THERE ANY HEALTH RISK DUE TO THE PRESENCE OF ENTEROCOCCI IN COTIJA CHEESE®?

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Introduction. *Enterococcus* spp. play a key role in the intestinal microbial balance in mammal species. Besides, they are involved in spontaneous fermentation of traditional food worldwide (1). However, their presence in food is controversial, because they have been related to clinical infections (2). Cotija cheese® is a farmhouse made product, from which we have isolated lactic acid bacteria. From those, some *Enterococcus* strains showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.* The aim of this research was to search for genes involved with pathogenicity, to evaluate the production of antibacterial compounds and to assess the antibacterial activity against microorganisms important in food safety, in populations of *Enterococcus* spp. isolated from Cotija cheese®.

Methods. Populations of enterococci isolated from 12 Cotija chesses® and six strains, isolated previously, were tested to detect, through PCR, the following genes *asa, esp, cylA* (virulence factors); *entA* (enterocin) and *atlA* (peptidoglycan hydrolases, PGH). SDS-PAGE and zymograms against *Micrococcus lysodeikticus* (3) and *Listeria innocua* were performed for protein analysis and lytic activity. Biochemical tests were performed to assess their hemolytic activity, adherence ability to Caco-2 and HT-29 cell lines, biofilm formation on polyestyrene microplates (Abs 620 nm) and MRS-culture supernatant diffusion assays against bacteria of food interest.

Results. Gene detection results by PCR are summarized in Table 1. All *asa*$^+$ samples had the ability to adhere to Caco-2 and HT-29 cell lines (Fig.1A). Although only two populations and an isolated strain are *esp*$^+$, other samples were able to form biofilms, but only few of them formed a well-structured one (D.O. >0.22). *cyl A* gene was not detected; thus, β-hemolytic activity was not observed. Despite of the low incidence of *atlA* gen in the samples, all of them showed a 75-80 kDa band with bacteriolytic activity against *M. lysodeikticus* (Fig.1B). The enterocin A was detected in more than 50% of the samples and a 5 kDa band with activity against *L. innocua* was observed. Antibacterial activity in MRS-culture supernatant was observed against *S. typhimurium, S. aureus, Y. enterocolitica, L. monocytogenes, L. innocua* (Fig. 1C)*, S. pyogenes, B. cereus and E. coli*, through agar diffusion assays.

Table 1. Screening virulence factors, enterocin A and PGH genes

	Virulence Factor genes			Enterocin A gen	PGH gen
	asa	*esp*	*cylA*	*entA*	*atlA*
Populations	7	2	0	6	2
Isolated strains	3	1	0	4	1

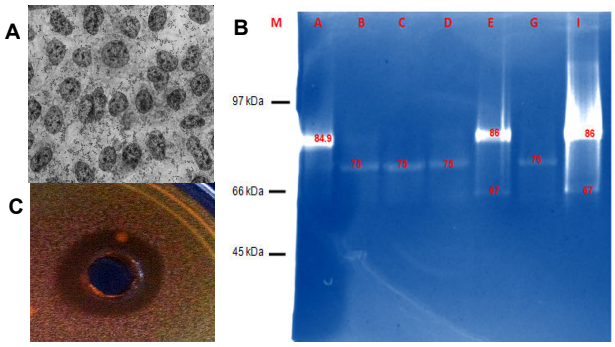

Fig 1. A) Adhesion of *E. faecalis* A to Caco-2 cells. B) Zymogram against *M. lysodeitikus*. M: high molecular weight marker; A, D, I: *E. faecalis*; B, C, G: *E. faecium*. C) Inhibition of *L. innocua* due to enterocin A production.

Conclusions. Although some genes encoding virulence factors were found in the enterococci studied, they have not been linked so far as the direct cause of clinical infections (4). Instead, the presence and expression of genes involved with probiotic behavior were detected, as well as enterocin and PGH activity. These results indicate their potential use in fermented foods.

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References.

1. Giraffa, G. 2003. *Int J Food Microbiol* 88: 215-222.
2. Shankar N., Coburn P. y Pillar C. 2004. *Int JMedical Microbiol* 293:609-618.
3. Leclerc, D. and Asselin, A. 1989. *Can J Microbiol* 35, 749–753.
4. Eaton, T.J. y Gasson, M. J. 2001. *Appl Environ Microb* 67: 1628-1635.