Supplementary File

Effect of fermented whey with a probiotic bacterium on gut immune system

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Materials and methods

Whey

The microbiological and physicochemical characteristics of whey were described in Table 1 and were determined by standard procedures: fat, International Dairy Federation (IDF, 1987a); total protein (IDF, 2001); total solids (IDF, 1987b); ash (AOAC, 1995) and lactose by difference.

Lactic acid bacteria strain

The LAB strain was identified from both the fermentation pattern (API 50 CHL test) and the 16S rRNA gene sequence (Dogi et al. 2013). Stock cultures were maintained at -80°C in 15% (v/v) glycerol. *Lactobacillus rhamnosus* RC007 was grown at 37°C for 24 h without agitation in Man, Rogosa and Sharpe (MRS) broth (Britania, Buenos Aires, Argentina). Overnight fresh culture of the strain under study (MRS, 37 °C, 24 h, aerobic incubation) was centrifuged (6000 g, 15 min, 5 °C), washed twice with phosphate buffered saline (PBS), inoculated (1% v/v) in heat treated W or MRS broth and incubated overnight (37 °C, during 24 h, aerobic incubation). Bacterial growth was evaluated by taking an aliquot every two hours and plating on MRS agar. Decrease in pH was followed with a digital pH meter.

Histological studies

The small intestines from mice were fixed in formaldehyde, dehydrated using a graded series of ethanol and xylene, embedded in paraffin and sectioned at 4 μm and stained with haematoxylin and eosin. Goblet cells were counted at 40× only in villi axis. The quantification was performed on all the villi found in each section analyzed (two slides per animal/intestine, two sections per slide). The number of intraepithelial lymphocytes (IEL) per 100 epithelial cells in the complete villous was counted.

Statistical analyses

Considering that no interactions were observed between these two independent assays, results were analyzed together. Statistical analyses were performed using MINITAB 15 software (Minitab, Inc., State College, PA).

References

-A.O.A.C. 1995. Ash of milk – gravimetric method, in Official Methods of A.O.A.C. International, method 945.46 (33.2.10)

-Dogi CA, Fochesato A, Armando R, Pribull B, Souza MMS, da Silva Coelho I, Araújo de Melo, Dalcero A, Cavaglieri L. 2013. Selection of lactic acid bacteria to promote an efficient silage fermentation capable of inhibiting the activity of *Aspergillus parasiticus* and *Fusarium graminearum* and mycotoxin production. *Journal of Applied Microbiology* 114(6), 1650-1660.

-IDF (1987a). Determination of the Fat Content of Skim Milk, Whey and Buttermilk by the Rose-Gottlieb Gravimetric Method (reference method). Brussels: IDF (FIL-IDF Standard 22B)

-IDF (1987b). Milk, Cream and Evaporated milk – Dry Matter Oven Method (reference method). Brussels: IDF (FIL-IDF Standard 21B)

-IDF (2001) Milk – Determination of Nitrogen Content – Part 1: Kjeldahl Method. Brussels: IDF (FIL-IDF Standard 20-2 ISO 8968-2)

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Table 1. Composition (g/100 ml), microorganisms (log CFU/ml) and pH of cheese whey used in this study

Physicochemical evaluation		**Microbiological evaluation (log CFU/ml)**	
Total solids (% w/v)	6.05 ± 0.13	Lactobacilli	5.60 ± 0.17
Ashes (% w/v)	0.47 ± 0.02	Enterobacteria	5.23 ± 0.15
Fat (% w/v)	0.40 ± 0.01	Total anaerobes	6.36 ± 0.19
Proteins (% w/v)	0.83 ± 0.03		
Lactose (% w/v)	4.39 ± 0.15		
pH	6.2 ± 0.1		

Physicochemical and microbiological evaluation was determined in fresh whey prior to heat treatment in duplicate.

Table 2: Cell counts of *L. rhamnosus* RC007 growing in commercial MRS or cheese whey and final pH of the culture media

	Cell counts (log CFU/ml ± SD)	**pH**
MRS (14 h)	9.3 ± 0.18	4.05
Whey (14 h)	7.9 ± 0.23	4.06
Whey (16 h)	8.9 ± 0.10	4.03

Lactobacillus rhamnosus RC007 was grown in MRS or W during 24 h. Bacterial growth was evaluated by taking an aliquot every two hours and plating on MRS agar. Decrease in pH was followed with a digital pH meter.

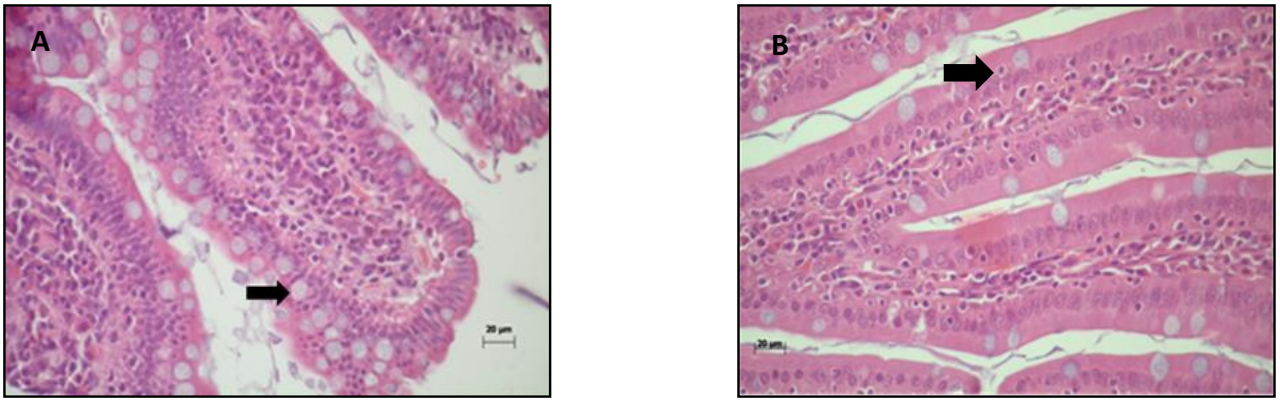

Figure 1: Microphotographs of small intestines stained with hematoxylin/eosin (H/E).

Goblet cells (A) and intraepithelial lymphocytes (B) are shown. Magnification 400X.