

***In vitro* immunomodulatory activity of two probiotic strains isolated from human breast milk: *Lactobacillus salivarius* CECT5713 and *Lactobacillus fermentum* CECT5716**

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Literature from human and animal studies shows that lactobacilli can modulate both natural and acquired immunity, and that these immunomodulatory effects are strain-specific. On the other hand, commensal bacteria, including some species of lactobacilli commonly present in human breast milk, appear to colonise the neonatal gut and contribute to protection against infant infections.

In this study, we evaluated the potential of two lactobacillus strains isolated from human milk to modulate the activation status and phenotypic pattern of peripheral blood mononuclear cell (PBMC) subsets *in vitro*. *Lactobacillus salivarius* CECT5713 (Ls) and *Lactobacillus fermentum* CECT5716 (Lf)⁽¹⁾ at 10⁵, 10⁶ and 10⁷ bacteria/ml (provided by Puleva Biotech, Granada, Spain) were co-cultured with PBMC (10⁶/ml) from healthy donors (N = 8) for 24 h. PBMC were also activated using a polyclonal T cell stimulator, concanavalin A (ConA), as a positive control. Phenotypes of Natural killer cells (NK cells, CD56⁺), total T-cells (CD3⁺), cytotoxic T-cells (CD8⁺) and CD4⁺ T-cells and their activation status (CD69 and CD25 expression) were determined by flow cytometry. Regulatory T-cells (T reg) were also quantified by intracellular Foxp3 evaluation.

Regarding innate immunity, activation status of peripheral CD8⁺ NKT cells was not substantially affected by either ConA or bacteria (2–3%). However, the CD8⁺ NK subset was highly activated by addition of both lactobacilli strains: CD69 expression in these cells was 24% for unstimulated cells and 73, 90 and 92% for cells exposed to ConA, Lf and Ls, respectively. With respect to acquired immunity, approximately 10% of CD8⁺ T-cells became activated after co-cultivation with Lf or Ls (Table). The expression of CD25 by CD4⁺ T-cells was increased 2-fold by Lf and Ls relative to unstimulated cells. Moreover, expansion of T reg cells (CD4⁺CD25⁺Foxp3⁺) was induced after exposure to both Lf and Ls. This work demonstrates that *L. salivarius* CECT5713 and *L. fermentum* CECT5716 enhanced both natural and acquired immune responses, as evidenced by the activation of peripheral mature CD8⁺ NK and CD4⁺ cell subsets and the expansion of T reg cells.

Cell subset	Control	ConA	<i>L. fermentum</i> CECT5716	<i>L. salivarius</i> CECT5716
T cells (CD3 ⁺)				
%CD69 ⁺	1.97 ± 0.76	50.77 ± 3.06*	7.19 ± 1.25* [⊙]	8.24 ± 0.90* [⊙]
%CD25 ⁺	5.10 ± 0.56	45.88 ± 5.38*	8.52 ± 1.00* [⊙]	9.10 ± 1.28* [⊙]
T CD8 ⁺ cells				
%CD69 ⁺	3.43 ± 1.18	38.62 ± 3.32*	9.05 ± 1.16* [⊙]	8.56 ± 0.49* [⊙]
% CD25 ⁺	2.88 ± 0.03	22.57 ± 4.15*	2.34 ± 0.21 [⊙]	3.81 ± 2.69 [⊙]
T CD4 ⁺ cells				
%CD69 ⁺	2.87 ± 0.45	56.43 ± 8.24*	5.84 ± 1.35* [⊙]	6.40 ± 1.11* [⊙]
%CD25 ⁺	9.68 ± 1.40	48.69 ± 8.23*	11.09 ± 1.14 [⊙]	12.07 ± 1.21* [⊙]

Data are expressed as means ± SEM values of eight healthy donors. Significant differences between control, ConA and bacterial species were tested by one-way ANOVA. Differences: *P < 0.05 v. control; [⊙]P < 0.05 v. ConA.

1. Martin R, Olivares M, Marin ML *et al.* (2005) *J Hum Lact* **21**, 8–17.