

Cytokine analysis to differentiate immunomodulatory properties of *Lactobacillus paracasei* strains and for the identification of potentially unsafe strains

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Lactobacillus paracasei is generally recognized as a safe species and *L. paracasei* strains are frequently present in fermented food products and also used as probiotic strains.

Probiotic strain (As indicated by the manufacturer)	Type of product	Identification on the basis of DNA analysis
<i>L. casei</i> (Actimel)	Yogurt drink	<i>L. paracasei</i>
<i>L. casei</i> (Shirota)	Probiotic drink	<i>L. paracasei</i>
<i>L. casei</i>	Yogurt	<i>L. paracasei</i>
<i>L. casei</i>	Yogurt	<i>L. paracasei</i>

Holzapfel W.H. *et al.*, *Am J Clin Nutr* 2001; 73:365S

Nevertheless, it is noteworthy that same *L. paracasei* strains have been associated with cases of infective endocarditis.

Oakey H.J. *et al.*, *J App Bacteriol* 1995; 78:142

Daniel C. *et al.*, *App Environ Microbiol* 2006; 72:5799

Vankerckhoven V. *et al.*, *J Med Microbiol* 2007; 56:1017

Aim of the study

Comparison of genetically characterized *Lactobacillus paracasei* strains to reveal their immunomodulatory properties and the potential relationship between the immune response and their different behaviors.

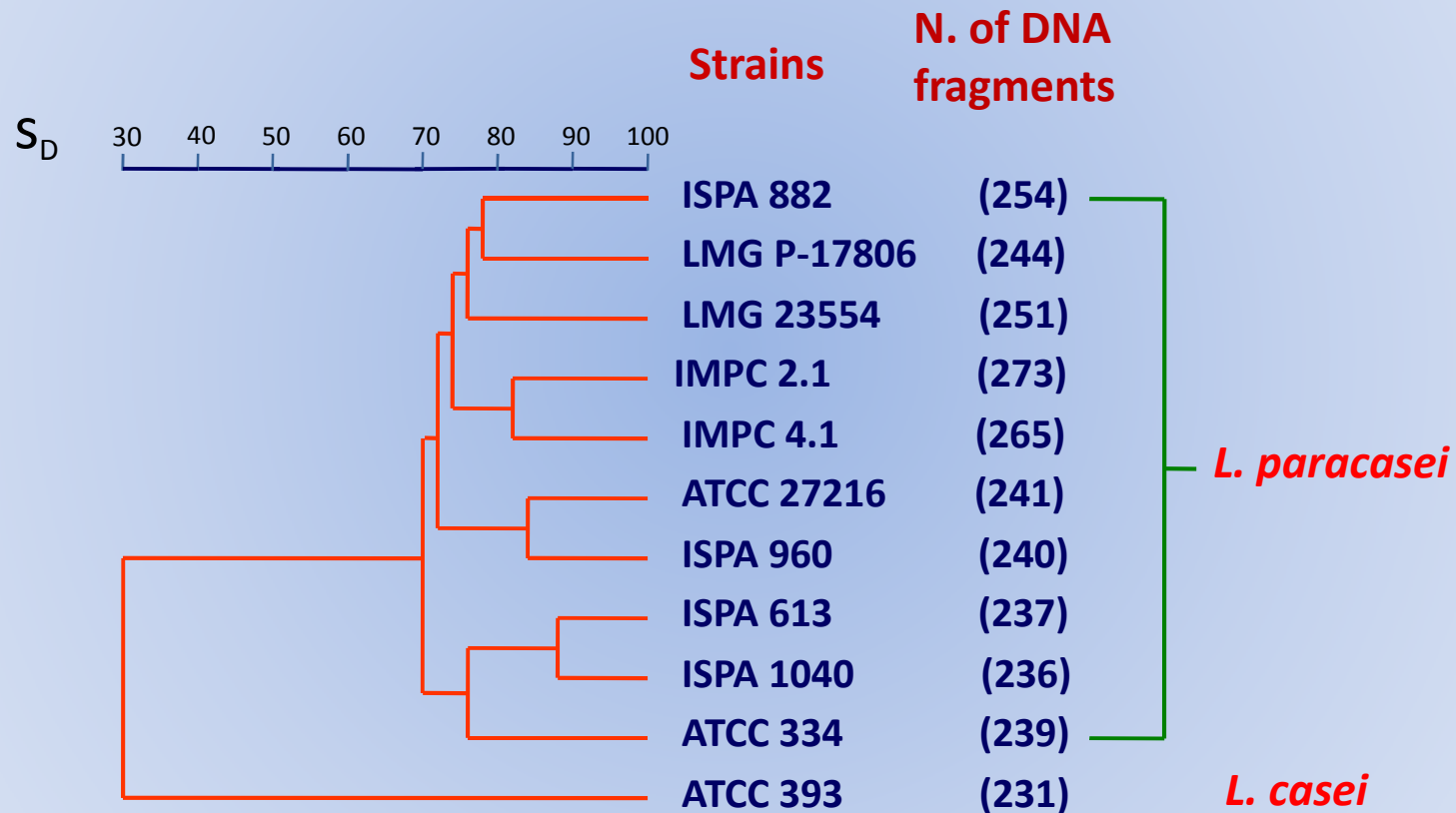
To ascertain if immunological tests can be useful to predict peculiar probiotic aptitudes and the safety of a new proposed probiotic strain.

Therefore, bacterial strains were characterized by using the fAFLP technique and their ability to modulate the immune response of mouse dendritic cells (DCs) was evaluated.

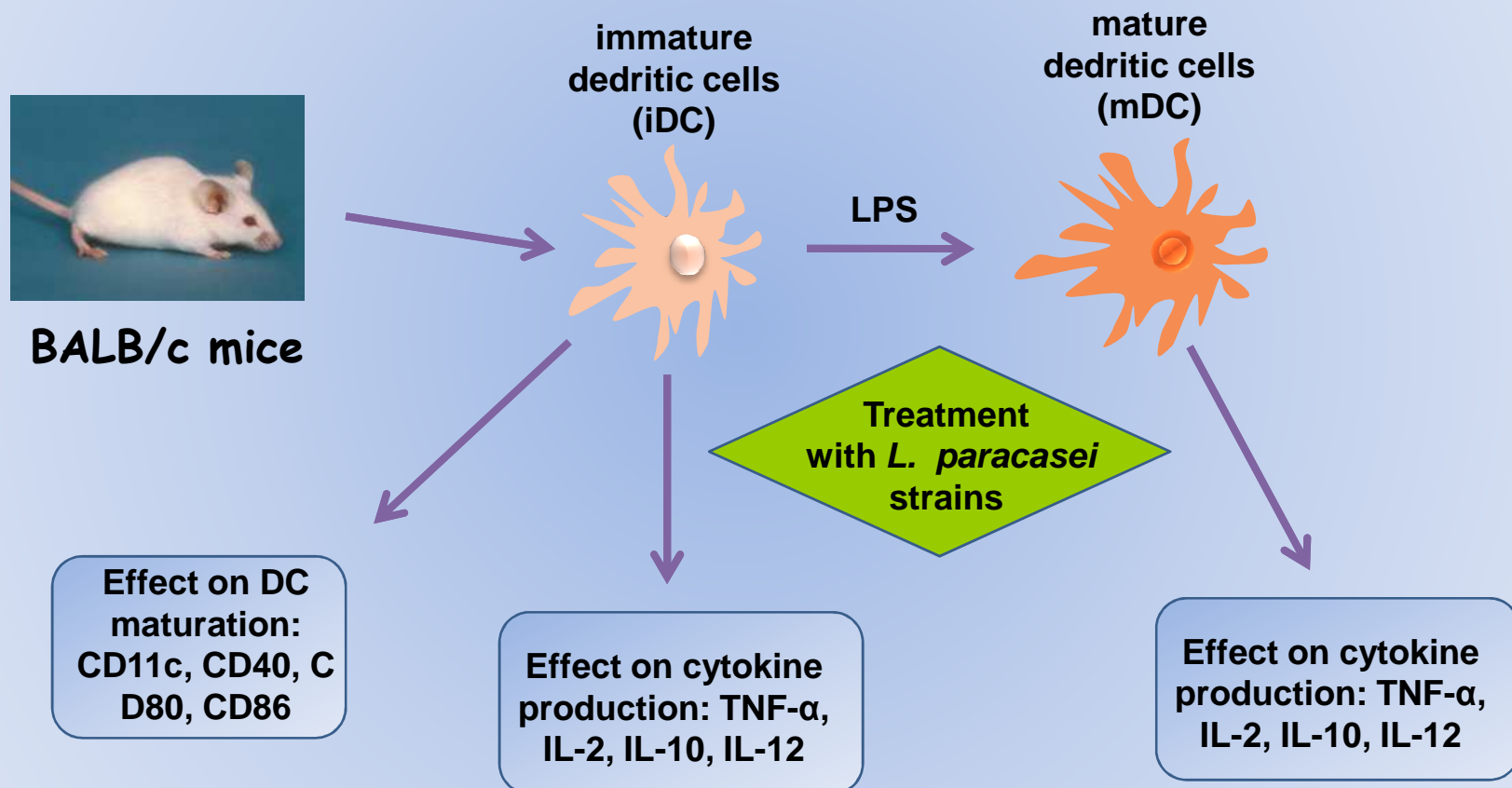
Bacterial strains used in this study

Species	Strains	Relevant characteristics
<i>L. paracasei</i>	IMPC 2.1 (LMG P-22043)	Probiotic strain, isolated from human intestine
	LMG P-17806	Probiotic strain, isolated from human intestine
	IMPC 4.1	Isolated from human intestine
	LMG 23554	Unsafe strain, Isolated from human blood of a patient with infective endocarditis. The strain is also capable of exacerbating colitis in mice and of translocation to extra-intestinal organs
	ATCC 334	Reference strain, isolated from dairy product
	ISPA 882	Isolated from processed meat
	ISPA 1040	Isolated from dairy product
	ATCC 27216	Isolated from human saliva
	B44f3t (ISPA 960)	Isolated from dairy product
B8S (ISPA 613)	Isolated from dairy product	
<i>L. casei</i>	ATCC 393	Reference strain, Isolated from dairy product

Molecular characterization by fAFLP analysis

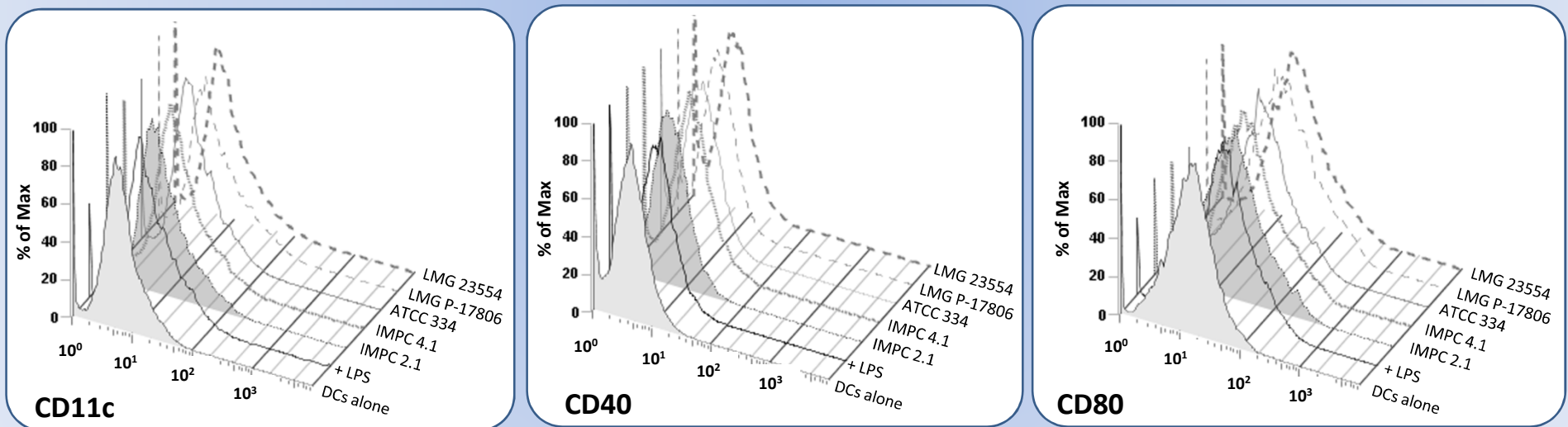


Evaluation of bacterial immunomodulatory properties

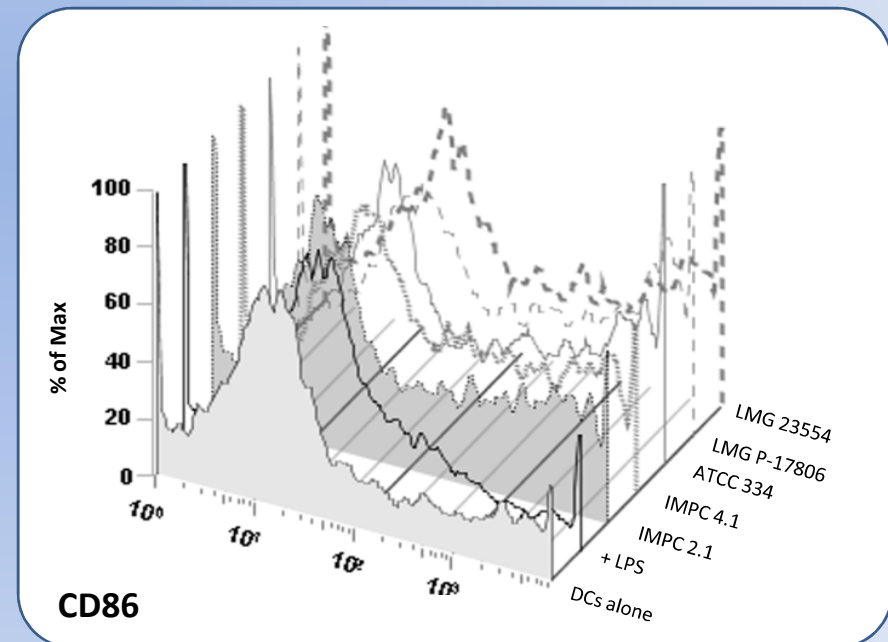


Effects of *L. paracasei* strains on the maturation of mice bone-marrow DCs

All strains increased the surface expressions of CD11c and CD80, but not of CD40, in iDCs at levels comparable with LPS stimulation.

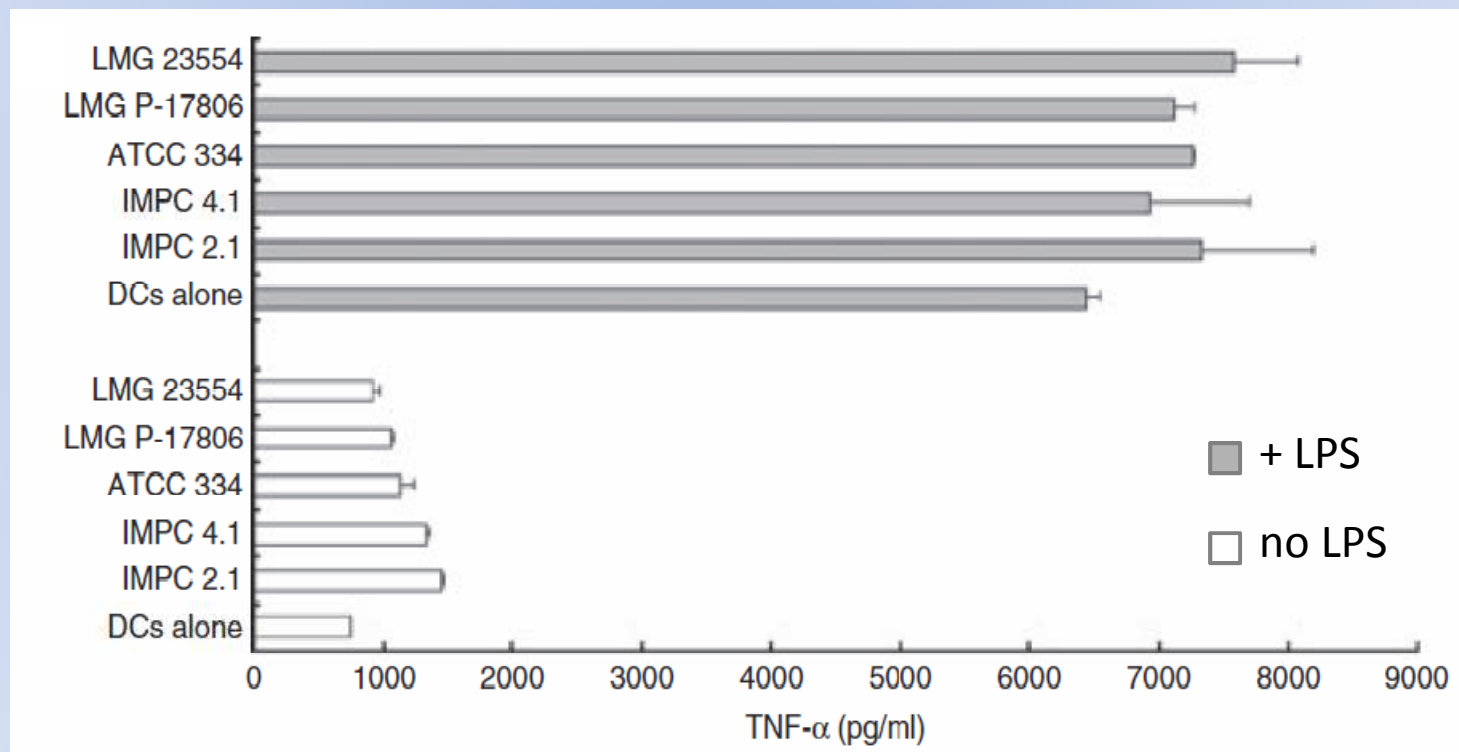


Surface expression of CD86, following incubation with bacterial strains, was even higher than the expression caused by LPS stimulation. Up-regulation of CD86 is consistent with other studies in which probiotic *Lactobacillus* species caused a similar enhancement.

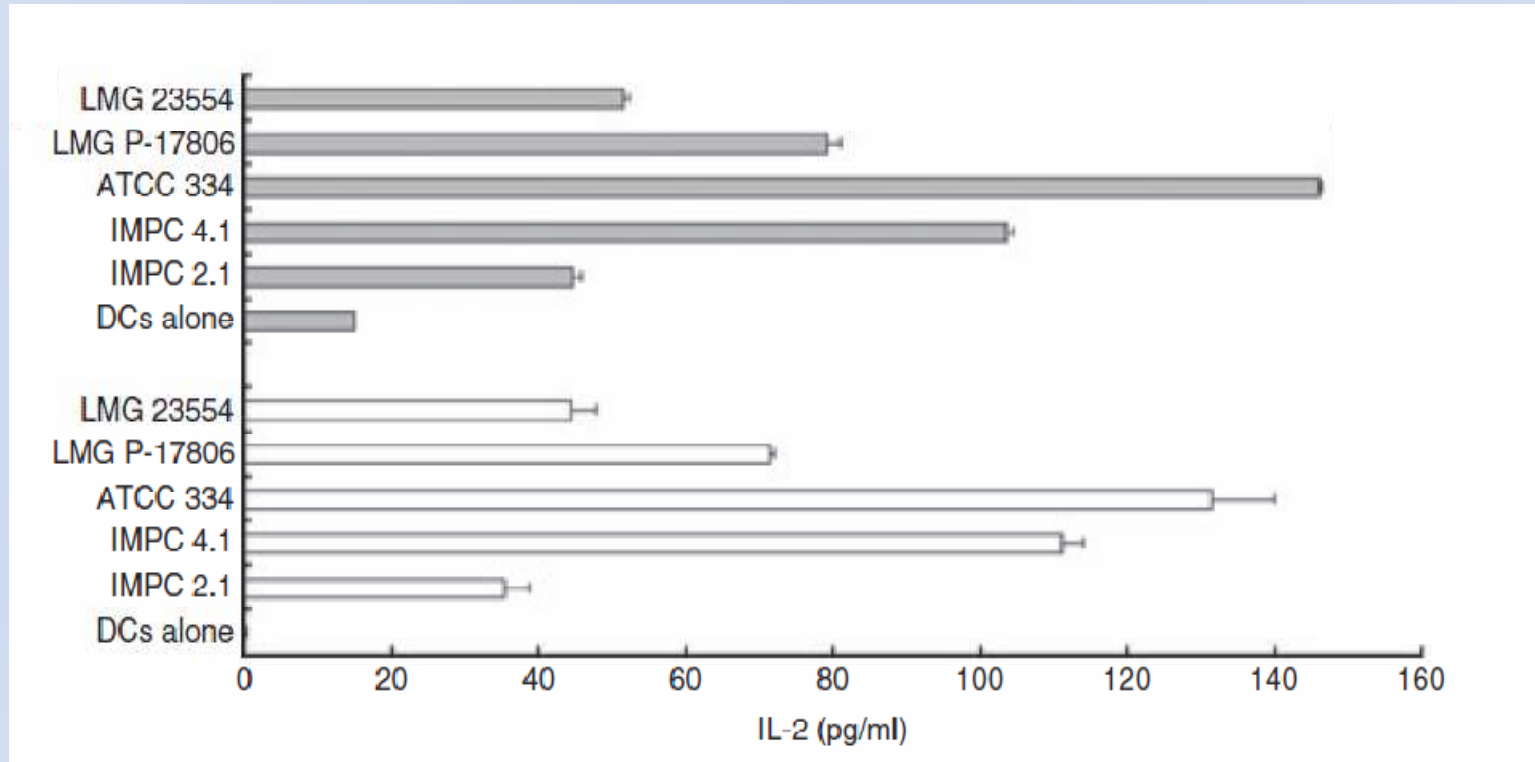


Effect of *Lactobacillus paracasei* strains on cytokine production by dendritic cells (DCs)

TNF- α



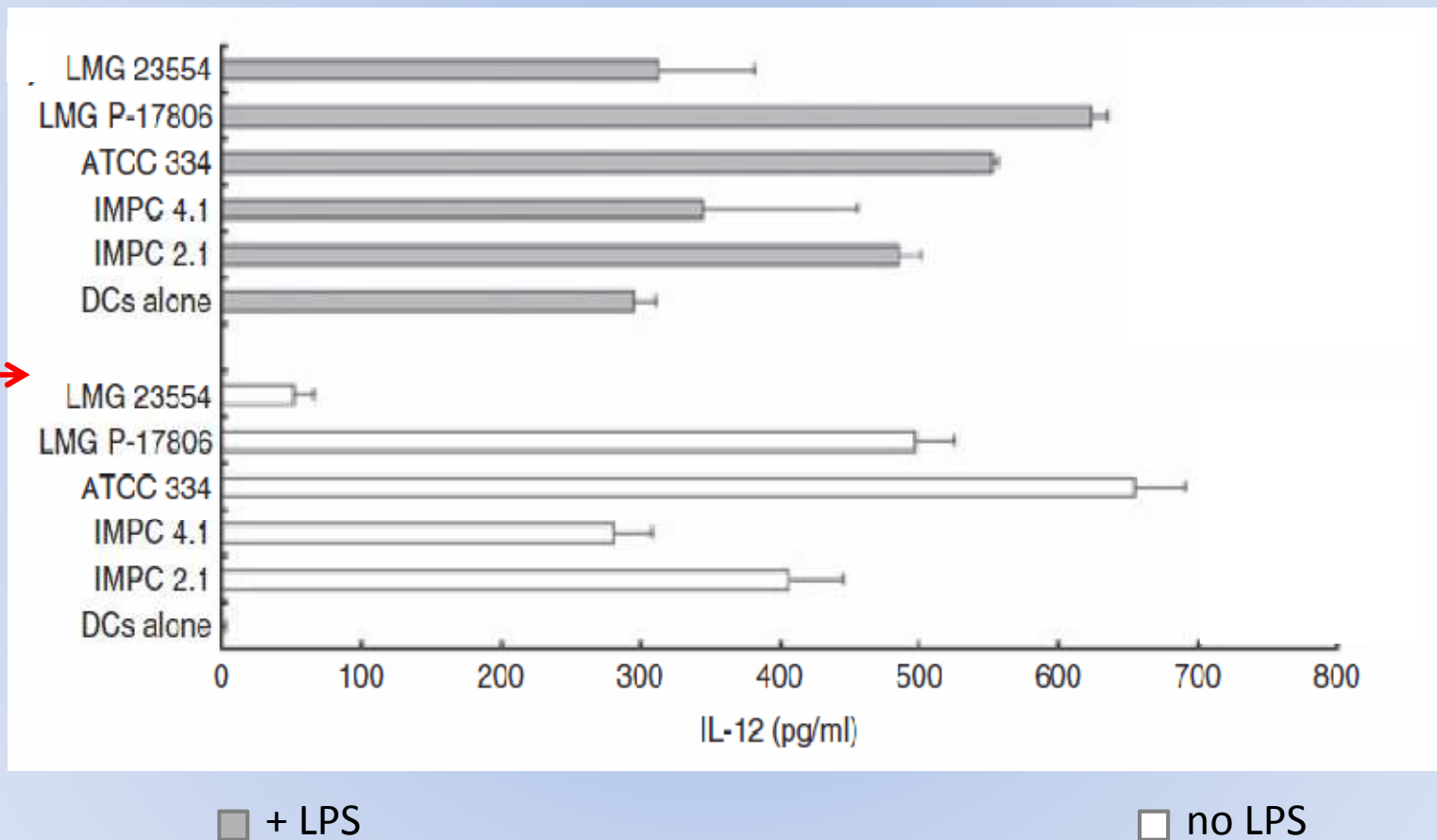
IL-2



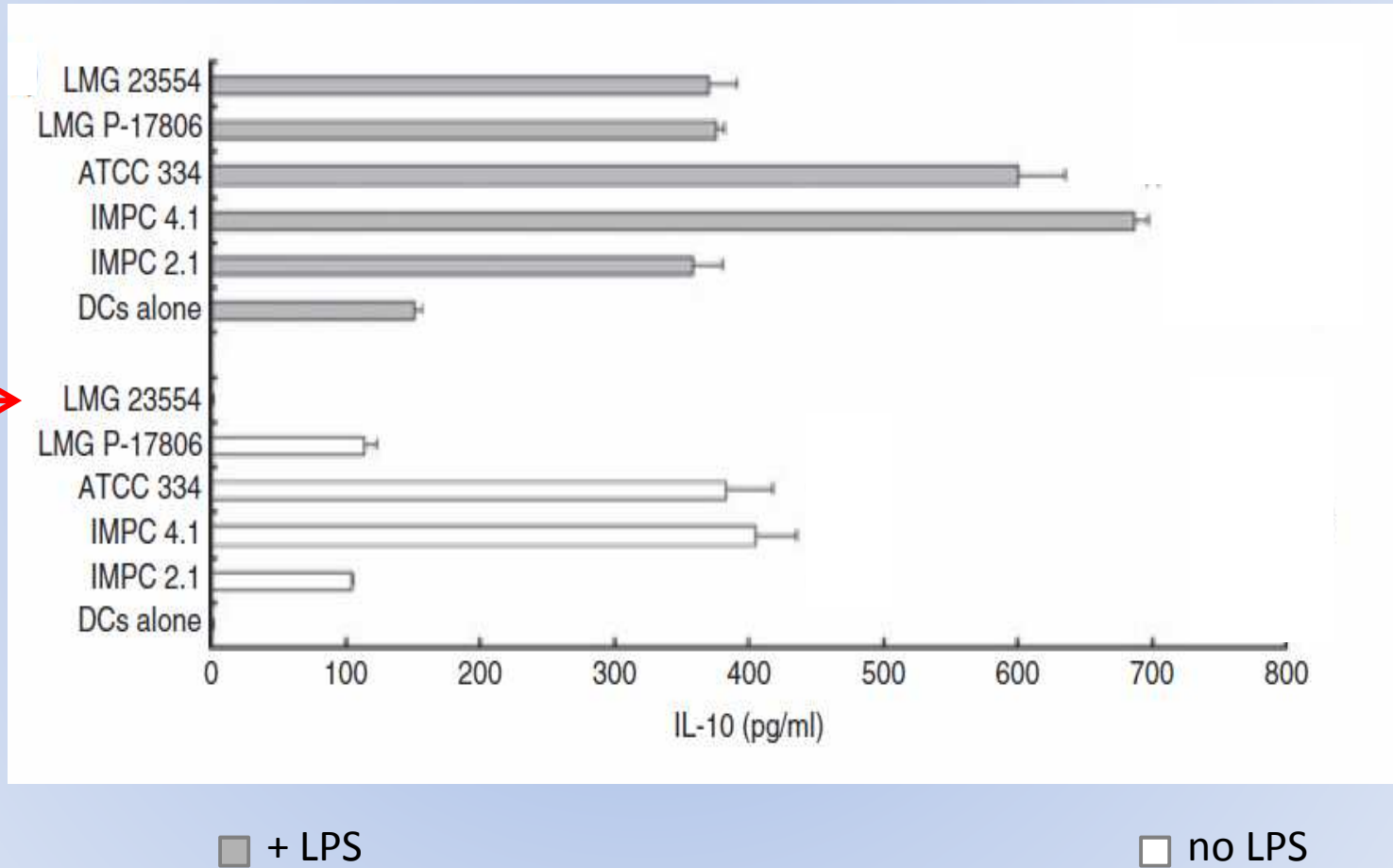
■ + LPS

□ no LPS

IL-12



IL-10



Conclusions

- ❖ Our results confirm that each single strain of a bacterial species appears to influence the immune system in a peculiar manner: strain IMPC 4.1 showed an interesting anti-inflammatory ability (high IL-10/IL-12); probiotic strains IMPC 2.1 and LMG P-17806 were characterized by a similar and intermediate ability to induce cytokine secretion in contrast to the very low ability of strain LMG 23554 to stimulate production of IL-10 and IL-12.
- ❖ The evaluation of the different types and/or levels of cytokines whose secretion is induced by each strain could be relevant to define its pro- or anti-inflammatory properties and its more appropriate clinical use.

- ❖ Our results suggest that cytokine (IL-10, IL-12) pattern analysis of DCs stimulated with bacterial cells can be considered as an useful *in vitro* method to predict potentially unsafe features of new proposed probiotic strains before embarking on time-consuming clinical studies.

In fact, in this regard, the unsafe strain LMG 23554 was clearly differentiated from the other *L. paracasei* strains and its low ability to stimulate IL-10 and IL-12 production could explain the ability of the strain to cross the intestinal mucosal barrier and / or persist in the extra-intestinal organs or circulation.