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.

<table>
<thead>
<tr>
<th>Probiotic strain (As indicated by the manufacturer)</th>
<th>Type of product</th>
<th>Identification on the basis of DNA analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. casei</em> (Actimel)</td>
<td>Yogurt drink</td>
<td><em>L. paracasei</em></td>
</tr>
<tr>
<td><em>L. casei</em> (Shirota)</td>
<td>Probiotic drink</td>
<td><em>L. paracasei</em></td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td>Yogurt</td>
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</tr>
</tbody>
</table>


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

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Strains</th>
<th>Relevant characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IMPC 2.1 (LMG P-22043)</td>
<td>Probiotic strain, isolated from human intestine</td>
</tr>
<tr>
<td></td>
<td>LMG P-17806</td>
<td>Probiotic strain, isolated from human intestine</td>
</tr>
<tr>
<td></td>
<td>IMPC 4.1</td>
<td>Isolated from human intestine</td>
</tr>
<tr>
<td></td>
<td>LMG 23554</td>
<td>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</td>
</tr>
<tr>
<td><strong>L. paracasei</strong></td>
<td>ATCC 334</td>
<td>Reference strain, isolated from dairy product</td>
</tr>
<tr>
<td></td>
<td>ISPA 882</td>
<td>Isolated from processed meat</td>
</tr>
<tr>
<td></td>
<td>ISPA 1040</td>
<td>Isolated from dairy product</td>
</tr>
<tr>
<td></td>
<td>ATCC 27216</td>
<td>Isolated from human saliva</td>
</tr>
<tr>
<td></td>
<td>B44f3t (ISPA 960)</td>
<td>Isolated from dairy product</td>
</tr>
<tr>
<td></td>
<td>B8S (ISPA 613)</td>
<td>Isolated from dairy product</td>
</tr>
<tr>
<td><strong>L. casei</strong></td>
<td>ATCC 393</td>
<td>Reference strain, Isolated from dairy product</td>
</tr>
</tbody>
</table>
Molecular characterization by fAFLP analysis

Strains

ISPA 882 (254)
LMG P-17806 (244)
LMG 23554 (251)
IMPC 2.1 (273)
IMPC 4.1 (265)
ATCC 27216 (241)
ISPA 960 (240)
ISPA 613 (237)
ISPA 1040 (236)
ATCC 334 (239)
ATCC 393 (231)

N. of DNA fragments

$S_D$

30 40 50 60 70 80 90 100

L. paracasei

L. casei
Evaluation of bacterial immunomodulatory properties

- BALB/c mice
- Treatment with *L. paracasei* strains
- Effect on DC maturation: CD11c, CD40, CD80, CD86
- Effect on cytokine production: TNF-α, IL-2, IL-10, IL-12

Immature dendritic cells (iDC) vs. Mature dendritic cells (mDC)
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**

![Graph showing TNF-α production by different strains of *Lactobacillus paracasei* with and without LPS stimulation.](image)
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.