

### IMMUNOMODULATORY PROPERTIES OF A PROBIOTIC COMBINATION: EFFECTS ON IMMUNE CELL POPULATIONS AND MACROPHAGE POLARIZATION IN MICE

Hélène Authier <sup>(1)</sup> - Stéphanie Hernandez <sup>(2)</sup> - Bénédicte Bertrand <sup>(1)</sup> - Elsa Jacouton <sup>(3)</sup> - Sophie Holowacz <sup>(3)</sup> - Agnès Coste <sup>(1)</sup>

Université de Toulouse, INSERM, CNRS, EFS, UPS, RESTORE Research Center, Toulouse, France <sup>(1)</sup> - Genibio, Research Department, Lorp-Sentaraille, France <sup>(2)</sup> - PiLeJe, Applied Research, Paris, France <sup>(3)</sup>

#### Objective:

To assess the immunomodulatory properties of a probiotic combination on innate and adaptive immune responses in mice.

#### Methods:

C57BL/6 mice were orally treated with the combination of *L. acidophilus* LA201 and *L. paracasei* LA802 (Lactibiane® Immuno, PiLeJe Laboratoire; 10<sup>9</sup> CFU/mouse) or phosphate-buffered saline (n=10/group) once daily for 14 days and then *intraperitoneally* injected or not with LPS (0.5mg/kg). Twelve hours after LPS injection, modulation of immune cell populations and activation of macrophages were assessed by flow cytometry and RT-qPCR, respectively. *Ex-vivo* microbicidal functions of macrophages (phagocytosis and killing) were evaluated against *Streptococcus pneumoniae*.

#### Results:

Interestingly, the probiotic combination decreased inflammatory response to LPS and induced changes in peritoneal immune cell populations by increasing the percentages of neutrophils, dendritic cells, natural killer cells, CD4+ and CD8+-activated T cells, Th1 cells and decreasing those of eosinophils and Th2 cells. Interestingly, peritoneal macrophage subpopulations were also modified. Although SPM were decreased in favor of LPM under physiological conditions, they were strongly induced by LPS treatment in line with the decrease of precursor blood monocytes.

Moreover, probiotics reduced the expression of pro-inflammatory genes (*Tnf-α*, *Il-1β*, *Il-6*) by peritoneal macrophages, thus promoting a less inflammatory phenotype without any change of macrophages' response to LPS. Phagocytosis and bactericidal activity against *S. pneumoniae* was increased in macrophages isolated from mice supplemented with the probiotic combination *versus* phosphate-buffered saline.

#### Conclusions:

Our study promotes the probiotic combination tested as a good candidate to stimulate immune responses involved in defense mechanisms against invading pathogens.

### AGGREGATION PROPERTIES OF PROBIOTIC STRAINS UNDER AEROBIC AND ANAEROBIC CONDITIONS

Daniela Machado <sup>(1)</sup> - José Carlos Andrade <sup>(2)</sup> - Joana Cristina Barbosa <sup>(1)</sup> - Francisca Costa <sup>(1)</sup> - Mariana Fonseca <sup>(1)</sup> - Diana Almeida <sup>(1)</sup> - Ana Cristina Freitas <sup>(1)</sup> - Ana Maria Gomes <sup>(1)</sup>

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal <sup>(1)</sup> - CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Paredes, Portugal <sup>(2)</sup>

#### Objective:

Aggregation between microorganisms from the same species (auto-aggregation) and from different species (co-aggregation) is considered a desirable property of probiotics, since it has been related with successful gut colonisation by probiotic strains and clearance of intestinal pathogens, respectively. Our study aimed to evaluate the auto- and co-aggregation, with the pathogens Methicillin-resistant *Staphylococcus aureus* [MRSA] and *Escherichia coli* O157:H7, of a novel probiotic candidate *Akkermansia muciniphila* DSM 22959 and the commercial probiotics *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus rhamnosus* GG, under two atmospheric conditions (aerobiosis and anaerobiosis).

#### Methods:

Auto- and co-aggregation abilities of *A. muciniphila* DSM 22959, *B. animalis* subsp. *lactis* BB-12 and *L. rhamnosus* GG were determined at different time-points (2, 4, 20 and 24-h) under aerobic and anaerobic conditions, via spectrophotometric method, according to the protocols of Collado et al. (DOI: 10.1007/s00217-007-0632-x) and Jena et al. (DOI: 10.1111/1348-0421.12054).

#### Results:

All tested probiotic strains were able to auto- and co-aggregate with pathogens at all time-points and under both atmospheres and, in general, these aggregation properties increased with increasing incubation period.

#### Conclusions:

This work provides novel insights regarding aggregation properties of novel probiotic candidate *A. muciniphila* DSM 22959 and commercial probiotics (*B. animalis* subsp. *lactis* BB-12 and *L. rhamnosus* GG) under two atmospheric conditions. Furthermore, the proven aggregation properties of *A. muciniphila* DSM 22959 support its use as probiotic.