**The impact of *Lactobacillus plantarum* IFPL935 on the composition and activity of a complex microbiota developed in a simulator of the Human Intestinal Microbial Ecosystem.** By E. Barroso[1], M.C. Martínez-Cuesta[1], T. van de Wiele[2], T. Requena[1], and C. Peláez[1], *[1] Department of Biotechnology and Microbiology, Instituto de Investigación en Ciencias de la Alimentación, CIAL (CSIC-UAM), Nicolás Cabrera 9, 28049 Madrid, Spain, [2] LabMET, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium*

***Introduction***

The human intestinal microbiota is a highly complex and dynamic ecosystem that harbors over a thousand different strains. In the colon, bacterial numbers can reach 100 trillion bacteria and such large numbers have numerous important functions for human host health, including the maintenance of intestinal homeostasis(1). Some specific lactic acid bacteria (LAB) are considered as probiotics which, when administered in adequate amounts, confer a health benefit on the host. In previous studies, we have reported that *Lactobacillus plantarum* IFPL935 was able to grow in the presence of a flavan-3-ol extract from grape seeds(2) and furthermore to initiate the metabolism of these polyphenols(3) to other compounds that in turn could have an impact on the intestinal microbiota. Interestingly, *L. plantarum* IFPL935 also tended to increase the butyric acid production, which is known to play an important role for maintenance of gut health, when added to *in vitro* batch-culture systems inoculated with a colon region specific microbiota(4).

***Objective***

This study aims to investigate the role of the potential probiotic *L. plantarum* IFPL935 strain on the composition and/or activity of a human intestinal microbiota in a long-term feeding assay with a commercial polyphenolic red wine extract.

*Materials and Methods*

*Twin-SHIME*. This study was performed in two parallel units of the dynamic Simulator of the Human Intestinal Microbial Ecosystem (SHIME)(5). At first, ascending colon (AC), descending colon (DC) and transverse colon (TC) reactors from the Twin-SHIME systems were inoculated with the same faecal sample. After a 3-weeks period of stabilization of the colonic microbiota, a 2-weeks experiment was run by feeding both parallel systems daily

with 200 mg of a commercial red wine extract. In addition, one of the Twin-SHIME systems was simultaneously fed with *L. plantarum* IFPL935 ($10^{10}$ cfu, daily). Finally, a wash-out period (1- week) was run in both systems.

*Quantitative PCR (qPCR)*. Microbial community changes were monitored by Quantitative PCR (qPCR) analyses on total bacteria and different groups and genera of bacteria. Enumeration of the butyryl-CoA:acetate CoA transferase gene (BCoAT) was used for estimating the number of butyrate-producing bacteria in samples containing a complex microbiota[6].

*Fermentative and proteolytic activities*. Short- (SCFA) and branched-chain fatty acids (BCFA) were extracted from the samples and analyzed by gas chromatography (GC) as previously described[7]. Lactic acid was measured spectrophotometrically using the D-/L-lactic kit (R-Biopharm) according to the manufacturer´s instructions. Ammonium was released from samples as ammonia which was determined by titration as previously described [8].

***Results***

*Microbial community analyses*. Feeding the gut microbiota with the phenolic extract showed a slight decrease of total bacterial counts in the AC reactor (1 log number) after 24 h of treatment. All bacterial groups analyzed were affected. Among them, *Bacteroides*, *Bifidobacterium* and the butyrate producers *Clostridium cocoides/Eubacterium rectale* were the most affected groups. In most cases, bacterial numbers were readily recovered after the first week of treatment. Besides, addition of *L. plantarum*IFPL935 to one of the Twin-SHIME systems resulted in relative stable numbers of total bacteria, *Lactobacillus* and *Enterobacteriaceae* throughout the experiment. Related to the butyrate producers, BCoAT gene copy numbers showed significantly higher values in the TC and DC reactors than in the AC ones at the end of the treatment, and particularly, in the DC reactor corresponding to the SHIME unit supplemented with *L. plantarum* IFPL935. Cluster analysis of *Lactobacillus* DGGE profiles grouped the samples based on the different treatments (Fig. 1).

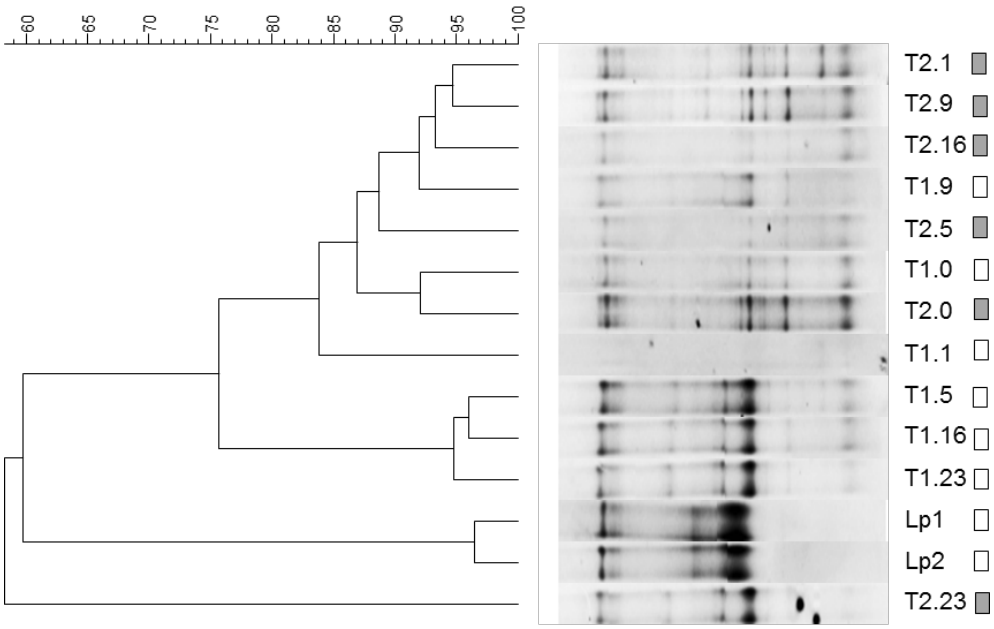


Fig.1. Clustering tree of the *Lactobacillus* DGGE-profiles in the transverse colon (TC) reactor of the Twin-SHIME during the treatment and wash out periods (days 0, 1, 5, 9 and 16, 19 and 23) with the red wine phenolic extract (grey symbols) and the extract supplemented with *L. plantarum* IFPL935 (white symbols). Lp, DNA from *L. plantarum* IFPL935.

*Fermentative and proteolytic activities*. Daily intake of the polyphenolic extract caused a sharp decrease in both fermentation and proteolysis markers measured during the first days of treatment in all the colon reactors. Regarding proteolysis, no differences between treatments were observed when *L. plantarum* IFPL935 was added. Focusing in the fermentation profile, butyric acid production decreased to no detectable levels during the first two days of treatment in all the vessels excepting the DC and TC vessels where *L. plantarum* IFPL935 were added. (Fig. 2). In this regard, addition of *L. plantarum* IFPL935 also showed an increase of lactic acid in the AC compartment at the starting of the period studied followed by a further decrease associated to the recovery of the butyrate levels (cross-feeding). In general, the microbial metabolic activity present at the start of the feeding was recovered in all vessels for both treatments after 5 days of the experiments, except for the production of butyric acid in the DC vessel without *L. plantarum* IFPL935, which only returned to initial values at the end of the wash-out period (Fig. 2).

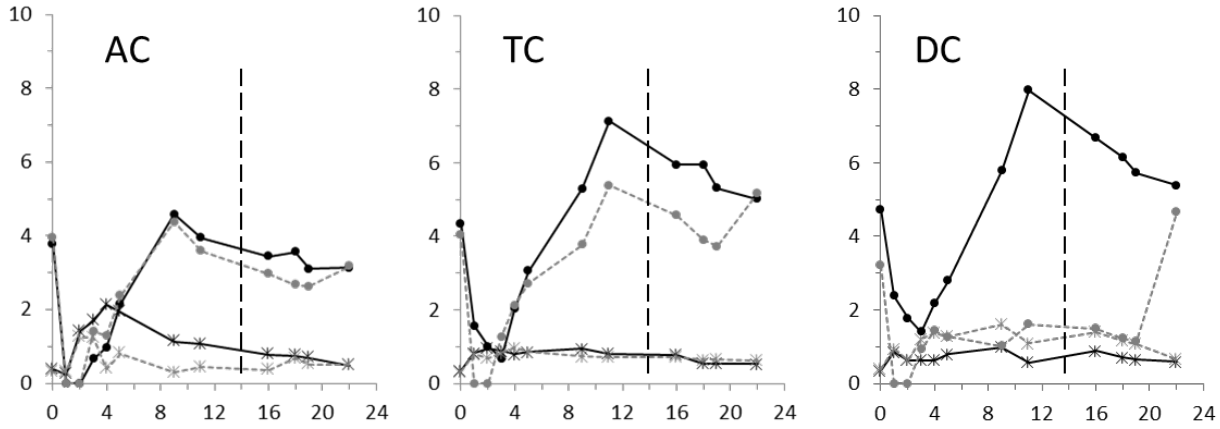


Fig.2. Concentration (mM) of butyric acid (dots) and lactic acid (asterisks) in the colon reactors of the Twin-SHIME during the treatment with the red wine phenolic extract (grey symbols) and the extract supplemented with *L. plantarum* IFPL935 (black symbols). The vertical dotted line indicates the wash-out period.

***Conclusions***

Feeding of the gut microbial ecosystem with a polyphenolic red wine extract exerted an overall antimicrobial effect on the gut microbiota in the AC reactor being *Bacteroides*, *C. coccoides*/*E. rectale* and *Bifidobacterium* the most affected groups. Addition of *L. plantarum* IFPL935 gave rise to a more steady numbers of total bacteria, *Lactobacillus* and *Enterobacteriaceae* and led to an increase in the formation of lactic acid at the start of the polyphenol treatment in the AC vessel as well as butyric acid in the TC and DC reactors after 5 days. Thus, *L. plantarum* IFPL935 may possibly influence SCFAs production, and in particular butyrate, which have numerous documented effects promoting bowel large function.

*References*

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