Effect of red wine polyphenols and *Lactobacillus plantarum* IFPL935 on the composition and metabolism of human gut microbiota. By E. Barroso[1], M.C. Martínez-Cuesta[1], T. Requena[1], C. Peláez[1], T. van de Wiele[2]. *[1] Department of Biotechnology and Microbiology, Instituto de Investigación en Ciencias de la Alimentación, CIAL (CSIC), Nicolás Cabrera 9, 28049 Madrid, Spain, [2] LabMET, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium.*

Introduction

The human gut microbiota is a community highly diverse with densities approaching 10^{11}–10^{12} cells/g that exerts a crucial impact on the human health [1, 2]. The composition of the intestinal microbiota varies largely among individuals [3], but despite this highly divergent composition, the functional gene profiles are quite similar [4]. It seems that the huge amount of genes representing the gut microbiome provides this community with the ability to adapt and react to environmental stress and changes, thus making it functionally robust [5]. The antimicrobial effects of plant polyphenols and its impact on the human gut microbiota have been extensively reviewed [6]. Some studies have shown the antimicrobial effect of grape seed polyphenols on batch incubations with fecal samples from healthy individuals [7]. On the other hand, we have shown that *Lactobacillus plantarum* IFL935 exerted a positive impact on microbial metabolism when incubated with human colonic microbiota and a polyphenolic red wine extract [8]. Furthermore, we have found that *L. plantarum* IFL935 improved the recovery of colonic bacterial counts and metabolism after the antimicrobial impact caused by the feeding of the SHIME with a red wine polyphenolic extract [9]. But the fact that the human gut microbiota shows a large inter-individual variation makes wondering if this observed effect of *L. plantarum* IFPL935 is strictly linked to an individual response or can be extrapolated to a number of individuals.

Objective

In the present work we have studied the effect of *L. plantarum* IFPL935 on the microbiota composition and metabolism of fecal samples from ten adult volunteers incubated with red wine polyphenols. The aim of the work has been to sustain the potential probiotic effect of the strain by modulating changes in the gut microbiota beyond inter-individual variability.

Materials and Methods

Homogenated fecal samples from 10 healthy individuals were used to inoculate (8%) separately rubber-top bottles containing sterile nutritive medium [10] together with 500mg/L of a polyphenol extract of red wine (Provinols: 95% polyphenols) or Provinols plus 10^7 ufc/mL of *L. plantarum* IFPL935. Samples were incubated and analyzed by duplicate. Total microbial genomic DNA was isolated after incubating samples at 37 °C during 0, 24 and 48 h and total microbial community changes were monitored by quantitative PCR (qPCR) using specific primers for different bacterial groups [8]. Microbial production of short-chain fatty acids (SCFAs) (analyzed by GC [11]), lactic acid (Lactic acid kit; R-Biopharm) and ammonium (determined by titration [12]) in the samples were measured as indicators of fermentative and proteolytic activities. Analysis of variance (ANOVA), the least significant difference (LSD) test and the *t*-student test were carried out using the STATISTICA program for Windows, version 7.1 (StatSoft. Inc. 1984−2006, www.statsoft.com).

Results

Inter-individual microbiota variability was high and is expressed in Table 1 as the standard deviation for the mean value of all individuals at 0, 24 and 48 h of each treatment. The total number of bacteria was in the range of 10^7–10^8 copy number/mL, showing a significant decrease over the incubation time that indicates an overall antibacterial effect of the Provinols treatment. Addition of *L. plantarum* IFPL935 was related with significantly higher counts through incubation of the total population as well as the *C. coccoides-E. rectale* group, helping to maintain high values for these butyrate producers. Bifidobacteria and the lactobacilli-enterococci group were the less affected by the Provinols treatment. On the other hand, addition of *L. plantarum* IFPL935 increased the production of lactic acid and decreased the production of acetic acid, which was the dominant SCFA in the samples treated with Provinols (Figure 1).

Conclusions

Despite the initial inter-individual variability in the microbiota, addition of *L. plantarum* IFPL935 helps to modulate some of the bacterial groups that are affected by the antibacterial effect of the polyphenols in the Provinols treatment.

Table 1. Quantitative-PCR (log copy number / mL) for the microbial groups analyzed [a]

		Provinols		Provinols + *L. plantarum*	
Total Bacteria	t0	7,97 ± 0,64	A	8,03 ± 0,46	A
	t24	7,12 ± 0,60	B	7,67 ± 0,26	B *
	t48	6,89 ± 0,95	B	7,48 ± 0,45	B *
Lactobacillus-Enterococcus	t0	6,15 ± 0,81	A	7,66 ± 0,90	A
	t24	7,05 ± 0,80	B	7,66 ± 0,61	A *
	t48	7,26 ± 0,57	B	7,71 ± 0,47	A *
Bifidobacterium	t0	6,40 ± 0,47	A	6,78 ± 0,52	A
	t24	6,62 ± 0,54	A	7,17 ± 0,73	A *
	t48	6,49 ± 0,58	A	6,80 ± 0,77	A
C.coccoides - E.rectale	t0	7,69 ± 0,96	A	7,82 ± 0,98	A
	t24	5,38 ± 1,75	B	6,71 ± 0,62	B *
	t48	5,34 ± 1,80	B	6,55 ± 0,65	B *
C.leptum	t0	6,90 ± 0,57	A	6,75 ± 0,62	A
	t24	5,92 ± 0,54	B	6,07 ± 0,53	B
	t48	5,88 ± 0,71	B	6,03 ± 0,57	B
Ruminococcus	t0	6,28 ± 0,93	A	6,43 ± 1,03	A
	t24	5,55 ± 0,74	B	5,84 ± 0,91	AB
	t48	5,67 ± 1,17	B	5,80 ± 0,93	B

[a]Data are expressed as means ± standard deviation of 10 individual samples incubated in duplicate. For a given microbial group, different capital letters denote significant differences ($P < 0.05$, from LSD test) over the incubation time (for a given treatment). Asterisks denote significant differences ($P < 0.05$, from *t*- test) between treatments during incubation.

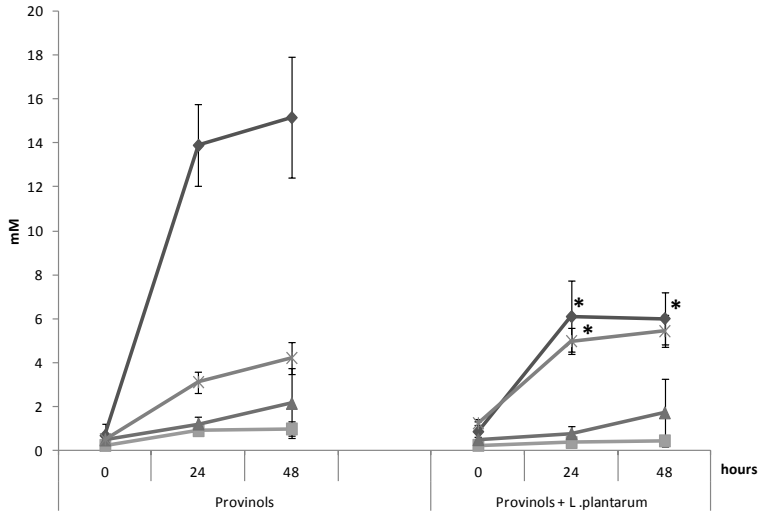

Figure 1. Mean concentrations (mM) of acetic acid (diamonds), butyric acid (triangles), propionic acid (squares) and lactic acid (crosses) over the incubation of 10 individual samples for each treatment. Vertical bars denote standard error. Asterisks indicate significant differences ($p<0.05$) between treatments during incubation.

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