Effect of 4 different strains of *L. plantarum* on obesity and gut permeability

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**Introduction**

Obesity is considered as a global epidemic and known to cause various health problems that have increased considerably in recent decades. It is estimated that the number of people affected has tripled during the last 40 years [1]. Approximately 650 million adults and 5 million children around the world suffer from obesity [1]. Number of researches have shown strong relationship between intestinal microbiota and obesity [2,3]. Microbiota in addition to have an important physiological role in vital processes such as digestion, vitamin synthesis and metabolism; they are also responsible for intestinal permeability, induction of low-grade inflammation, increased energy efficiency from diet and the regulation of fatty tissue composition [3].

Lactic acid bacteria (LAB) have demonstrated an anti-obesity effect such as suppression of lipid absorption, inhibition of adipocyte differentiation, decrease of low density lipoproteins, down regulation of proinflammatory cytokine genes in adipose tissue, etc. [4]. In the present study, we evaluated 4 different strains of *L. plantarum* isolated from Korean white kimchi, as putative probiotics and as modulators of gut microbiota for amelioration of metabolic syndrome, including obesity in a high-fat diet (HFD) induced obesity mice model.

**Methodology**

**Bacterial strains**

Four different strains of *L. plantarum* collected from Korean white Kimchi were isolated. As a reference strain *L. rhamnosus* LGG (ATCC 53103) was used.

**Experimental groups**

All the strains were grown in MRS medium at 37°C for 18 hours and ten different groups were created for the experiment. 6 groups were used as control: NCD (non-caloric diet), HF + PBS (High fat diet + PBS), Orlistat (Anti-obesity drug), LGG (*L. rhamnosus*) and 299V (*L. plantarum 299V*). The other 4 experimental groups were *L. plantarum* X1, X2, X3 and X4.

**Experimental animals**

Mice C57BL/6J of 4 weeks of age were used (n=7/group). They were fed once a day with 1×10⁸ CFU of *L. plantarum* and *L. rhamnosus* GG for 8 weeks. The control group was fed with Saline Phosphate Buffer (PBS). Water and a high calorie diet 60 Kcal% was provided ad libitum for 12 weeks. The mice were housed at 23±1°C and 55±10% relative humidity, in a light/dark cycle of 12 hrs. Each mouse was weighted, and feed intake measured once a week. At the end of the experiment all the animals were euthanized by cervical dislocation. Brown adipose tissue (BAT), subcutaneous adipose tissue(SAT), epidermal adipose tissue (EAT), mesenteric adipose tissue (MAT), serum and feces were collected from each individual.

**Intestinal permeability evaluation**

4 kDa of dextran was conjugated with FITC and administered to mice orally with a probe at week 8 of treatment. FITC levels were quantified in plasma after 4 hours and compared with the control group. Subsequently the content and fat volume were measured using Siemens Inveon micro PET-CT and the volume of fat (mm³) was calculated automatically by Inveon software.
Blood biochemistry and gene expression analysis
Glucose, triglycerides and cholesterol levels were quantified in serum. Blood biochemistry test was performed by Technopark laboratories. The gene expression of the different enzymes associated with de novo synthesis, storage and oxidation of fatty acids was measured in the adipose tissue by the qPCR technique.

Statistical analysis
All the data collected were analyzed and interpreted using the statistical program IBM SPSS Statistics version 20 (IBM, USA). Significance (p < 0.05).

Results and Discussion
The comparison of results at the end of the experiment display that the group of *L. plantarum* supplemented X1 showed a statistically significant reduction of adipose tissue in comparison to the HF+PBS diet control groups resembling more to the value of the NCD diet group; however, it did not show a significant difference in adipose tissue measurements between the groups treated with Orlistat and LGG due to the high variation observed. These results were reflected in the blood biochemistry, reducing levels of glucose, cholesterol and triglycerides, as it is known that adipose tissue is the main storage site of triglycerides [4,5]. On the other hand, the strains *L. plantarum* X4, X2 and X3 did not show a significant reduction of adipose tissue compared to the HF+PBS groups (Figure 1.).

Figure 1. Adipose tissue weight in the different groups of obese mice induced by a high fat diet (HF) and a non-caloric diet (NCD) group. Epidermic Adipose tissue (a), mesenteric adipose tissue (b), brown adipose tissue (c), subcutaneous adipose tissue (d). NCD-PBS Non-caloric diet; HF-PBS PBS; HF-Orlistat anti-obesity drug; HF-LGG *L. rhamnosus* GG; HF299V *L. plantarum* 299v; HF-X4 *L. plantarum* X4; HF-X1 *L. plantarum* X1; HF-X2 *L. plantarum* X2; HF-X3 *L. plantarum* X3; The significant differences are described by letters A, B or C (p <0.05).
In the qPCR analysis the results demonstrated that the groups supplemented with *L. plantarum* X1 the enzymes involved in the oxidation of fatty acids were statistically significant more expressed and the enzymes involved in the synthesis and storage of fatty acids were statistically significant less expressed in comparison with the control groups and even the other *L. plantarum* treatments. These results suggest that these probiotics are able to inhibit the release of activated adenosine monophosphate (AMPK) leading to the downregulation of mitochondrial fatty acid oxidation, ketogenesis, glucose uptake, insulin secretion, regulation of lipogenesis, cholesterol and triglyceride synthesis [6,7].

The existing microbial diversity among all the groups and the observed changes in several bacterial taxa at the family and gender level confirmed the modulating effect of the *L. plantarum* groups on the composition of the intestinal microbiota (Figure 2). The groups treated with *L. plantarum* showed higher prevalence in the *Lachnospiraceae* family (phylum *Firmicutes*) and lower prevalence in the *Deferribacteraceae* family than in the control groups, although there were differences between the groups treated with *L. plantarum*, this was not significant. The low prevalence of the family *Deferribacteraceae* may be related to the reduction in the genus *Mucispirillum*. The groups treated with *Lactobacillus*, mainly LGG, also showed a relative decrease in the family *Lacticillaceae* and in the genus *Lactobacillus* (Fig. 2).

The proportion of the *Bacteroidaceae* and *Rikenellaceae* family was lower in HF-X1 but not in the other *Lactobacillus* groups compared to the HF-PBS group. However, the *Bacteroidetes* family, S24-7, was more abundant in HF-X (Fig. 2). At the genus level, *Bacteroides* was lower in HF-X mice. The relationship of *Firmicutes*, family *Ruminococcaceae* was reduced in HF-X and increased in HF-GG, compared to HF-PBS (Figure 2). The smaller numbers in the genera *Ruminococcus* and *Oscillospira* confirmed this reduction. The abundance of the genus *Allobaculum* was lower in HF-GG than in other groups.

There was no change in the proportion of *Firmicutes* and *Bacteroidetes*, typical gut microbiota of the mouse. The results suggest that the administration of *L. plantarum* results in the modulation of the composition of the intestinal microbiota. It is considered that *L. plantarum* X1 could stimulate the production of short chain fatty acids (SCFA) with the intestinal microbiota stimulates G protein-coupled receptors in adipose tissue, which leads to changes in intestinal motility and facilitates the absorption of nutrients [6,8].

![Figure 2](image-url) Composition of microbiota in stool samples. Relative abundance of OTU at family level. The readings were assigned by the uclust taxonomic classifier vs the Greengenes reference sequences through Macqiime 1.9.1.
Conclusions
The results display that there is no statistically significant difference between the groups treated with \textit{L. plantarum} X4, X2, X3 compared to the control groups; however the treatment with \textit{L. plantarum} X1 shows significant evidence of adipose tissue reduction and metabolites related to obesity. In addition, evidence of changes in intestinal microbiota was observed in the groups supplied with \textit{L. plantarum} X1 with respect to the other groups, at the same time the group with the greatest reduction in intestinal permeability was the one treated with \textit{L. plantarum} X1, which reinforces the evidence that there is a significant correlation between the modulation of the intestinal microbiome with intestinal permeability.

The results suggest that \textit{L. plantarum} X1 modulates the bacterial microbiome so it can be considered as a possible treatment for obesity and anothe gastrointestinal disorders; however, more studies are needed to confirm these results.

Bibliography