

Abstracts *Lactobacillus plantarum*

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New evidence on oral *L. plantarum* P17630 product in women with history of recurrent vulvovaginal candidiasis (RVVC): a randomized double-blind placebo-controlled study.

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OBJECTIVE:

To evaluate activity and tolerability of an oral product containing the specific probiotic strain *Lactobacillus plantarum* P17630 on vaginal colonization of lactic acid bacteria.

PATIENTS AND METHODS:

L. plantarum P17630 (5x10⁹ CFU/capsule) or placebo were formulated in capsules and administered orally to a total of 93 women with a history of recurrent vulvovaginal candidiasis over a period of 3 treatment cycles (15 days/cycle) separated by 15-day wash-out intervals. Vulvovaginal examinations and vaginal mycological culture, including Nugent score and Lactobacillary grade (LBG), were assessed at baseline, at day 45 and at day 90 (end of the study).

RESULTS:

Probiotic intake significantly improved lactobacilli colonization on vaginal epithelial cells. The results demonstrated a significant difference in LBG score when comparing day 0 to day 45 ($p=0.000016$) and to day 90 ($p=0.001415$) in women treated with *L. plantarum*P17630. No evidence of improvement in LBG score was recorded in women who received placebo ($p<0.005$). The increased lactobacilli adhesion was associated with the improvement of clinical signs such as redness, swelling, and discharge.

CONCLUSIONS:

This study demonstrates that the administration of oral probiotic *Lactobacillus plantarum* P17630 improves vagina colonization of acid lactic bacteria (an increase of vaginal LBG score) and suggests the use of this oral product to successfully prevent episodes of vulvovaginal candidiasis. The clinical study described in this article is registered in the ISRCTN registry with trial registration ID ISRCTN12669692.

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Exopolysaccharide from *Lactobacillus plantarum* LRCC5310 offers protection against rotavirus-induced diarrhea and regulates inflammatory response.

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We aimed to determine the effects of *Lactobacillus* strains against rotaviral infections. Rotaviruses are the major causative agent of acute gastroenteritis in infants and children worldwide. However, to date, no specific antiviral drugs for the treatment of rotavirus infection have been developed. We identified 263 *Lactobacillus* strains from 35 samples of the traditional Korean fermented vegetable food, kimchi. Among them, *Lactobacillus plantarum* LRCC5310, more specifically the exopolysaccharides produced by these cells, were shown to have an antiviral effect against human rotavirus Wa strain in vitro. In vivo, the oral administration of exopolysaccharides for 2 d before and 5 d after mouse infection with the murine rotavirus epidemic diarrhea of infant mice strain led to a decrease in the duration of diarrhea and viral shedding and prevented the destruction of enteric epithelium integrity in the infected mice. We demonstrated here that the exopolysaccharides extracted from *L. plantarum* LRCC5310 can be used for the effective control of rotavirus infection.

KEYWORDS: *Lactobacillus plantarum*; diarrhea; exopolysaccharide; rotavirus

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***Lactobacillus plantarum* lipoteichoic acid inhibits biofilm formation of *Streptococcus mutans*.**

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Dental caries is a biofilm-dependent oral disease and *Streptococcus mutans* is the known primary etiologic agent of dental caries that initiates biofilm formation on tooth surfaces. Although some *Lactobacillus* strains inhibit biofilm formation of oral pathogenic bacteria, the molecular mechanisms by which lactobacilli inhibit bacterial biofilm formation are not clearly understood. In this study, we demonstrated that *Lactobacillus plantarum* lipoteichoic acid (Lp.LTA) inhibited the biofilm formation of *S. mutans* on polystyrene plates, hydroxyapatite discs, and dentin slices without affecting the bacterial growth. Lp.LTA interferes with sucrose decomposition of *S. mutans* required for the production of exopolysaccharide, which is a main component of biofilm. Lp.LTA also attenuated the binding of fluorescein isothiocyanate-conjugated dextran to *S. mutans*, which is known to have a high affinity to exopolysaccharide on *S. mutans*. Dealanylated Lp.LTA did not

inhibit biofilm formation of *S. mutans* implying that D-alanine moieties in the Lp.LTA structure were crucial for inhibition. Collectively, these results suggest that Lp.LTA attenuates *S. mutans* biofilm formation and could be used to develop effective anticaries agents.

Isolates of *Lactobacillus plantarum* and *L. reuteri* display greater antiproliferative and antipathogenic activity than other *Lactobacillus* isolates

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Purpose. Lactic acid bacteria (LAB) have been associated with many beneficial effects in human digestive physiology. The aim of this study was to evaluate such effect, including attachment, antiproliferation and anti-pathogenic/antibacterial/antimicrobial properties of LAB isolated from healthy humans.

Methodology. Thirteen isolates, obtained from fecal samples of healthy individuals, were identified by phenotypic and molecular methods. Human colon adenocarcinoma cell line HT-29 and the cell proliferation kit II (XTT) assay were used for examination of the *Lactobacillus* adherence and antiproliferative activity, respectively. In addition, the inhibitory effect of *Lactobacillus* isolates against pathogenic bacteria was examined.

Results. Out of 13 *Lactobacillus* isolates, 5 (38%) isolates were non-adhesive, 4 (31%) were adhesive and 4 (31%) were strongly adhesive. Amongst the isolated lactobacilli, *L. reuteri* showed the highest degree of inhibitory effect against the attachment of the enteropathogens. The XTT assay showed that 3 different isolates had the strongest antiproliferative activity with the maximum effect observed by *L. plantarum* isolates.

Conclusion. Our results described that different *Lactobacillus* species isolated from normal fecal samples had different degrees of antiproliferative and anti-pathogenic/antibacterial/antimicrobial activities. However, no isolates showed all of the examined properties concurrently, suggestive that a combination of *Lactobacillus* species is needed for an active biological defense system.

***Lactobacillus plantarum* CECT 7527, 7528 and 7529: probiotic candidates to reduce cholesterol levels**

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BACKGROUND Hypercholesterolaemia plays a key role in the development and progression of coronary artery disease. There are numerous drugs available to treat this condition but they are often expensive and can have unwanted side effects. Therefore, a screening of lactic acid bacteria to select candidate probiotic strains to reduce cholesterol levels was carried out. Three *Lactobacillus plantarum* strains (CECT 7527, 7528 and 7529) were selected as potential probiotics to reduce cholesterol levels after conducting several *in vitro* tests for demonstrating the functionalities of the strains according to international guidelines.

RESULTS The three strains showed a high ability to survive under gastrointestinal tract conditions and to adhere to intestinal cells. Regarding lipid metabolism, the strains showed a great production of bile salt hydrolase, especially when combined. Moreover, the strains assimilated cholesterol directly from the medium. Part of the cholesterol present in the medium was removed via binding onto the bacterial cellular surface. Finally, the three strains, especially CECT 7529, produce large quantities of propionic and butyric acids.

CONCLUSION Combined, these characteristics suggest that these strains could be excellent candidates for reducing high blood cholesterol levels. © 2013 Society of Chemical Industry

Anti-inflammatory Properties of Gastric-derived *Lactobacillus plantarum* XB7 in the Context of *Helicobacter pylori* Infection

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Background *Helicobacter pylori* colonization of the gastric epithelium induces interleukin-8 (IL-8) production and inflammation leading to host cell damage. We searched for gastric-derived *Lactobacillus* with the ability to suppress *H. pylori*-induced inflammation.

Materials and methods Conditioned media from gastric-derived *Lactobacillus* spp. were tested for the ability to suppress *H. pylori*-induced IL-8 production in AGS gastric epithelial cells. IL-8 protein and mRNA levels were measured by ELISA and qPCR, respectively. The changes on host cell signaling pathway were analyzed by Western blotting and the anti-inflammatory effect was tested in a Sprague–Dawley rat model.

Results Conditioned media from *L. salivarius* B101, *L. rhamnosus* B103, and *L. plantarum* XB7 suppressed IL-8 production and IL-8 mRNA expression in *H. pylori*-induced AGS cells without inhibiting *H. pylori* growth. Conditioned media from LS-B101, LR-B103, and LP-XB7 suppressed the activation of NF- κ B in AGS cells, while strain LP-XB7 also suppressed c-Jun activation. The anti-

inflammatory effect of LP-XB7 was further assessed *in vivo* using a *H. pylori*-infected Sprague–Dawley rat model. Strain LP-XB7 contributed to a delay in the detection and colonization of *H. pylori* in rat stomachs, attenuated gastric inflammation, and ameliorated gastric histopathology. Additionally, the administration of LP-XB7 correlated with the suppression of TNF- α and CINC-1 in sera, and suppression of CINC-1 in the gastric mucosa of *H. pylori*-infected rats.

Conclusions These results suggest that *L. plantarum* XB7 produces secreted factors capable of modulating inflammation during *H. pylori* infection, and this probiotic *Lactobacillus* strain shows promise as an adjunctive therapy for treating *H. pylori*-associated disease.

Effect of Lactobacillus on body weight and body fat in overweight subjects: a systematic review of randomized controlled clinical trials

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Gut microbiota is important for maintaining body weight. Modulation of gut microbiota by probiotics may result in weight loss and thus help in obesity treatment. The aim of this systematic review was to evaluate the effects of *Lactobacillus* on weight loss and/or fat mass in overweight adults. A search was performed on the Medline (PubMed) and Scopus electronic databases using the search terms: ‘probiotics’, ‘*Lactobacillus*’, ‘obesity’, ‘body weight changes’, ‘weight loss’, ‘overweight’, ‘abdominal obesity’, ‘body composition’, ‘body weight’, ‘body fat’ and ‘fat mass’. In the total were found 1567 articles, but only 14 were included in this systematic review. Of these nine showed decreased body weight and/or body fat, three did not find effect and two showed weight gain. Results suggest that the beneficial effects are strain dependent. It can highlight that *Lactobacillus plantarum* and *Lactobacillus rhamnosus* when combined with a hypocaloric diet, *L. plantarum* with *Lactobacillus curvatus*, *Lactobacillus gasseri*, *Lactobacillus amylovorus*, *Lactobacillus acidophilus* and *Lactobacillus casei* with phenolic compounds, and multiple species of *Lactobacillus*.

Observational Prospective Clinical Study on Lactobacillus plantarum in Women with Bacterial Vaginosis/Vaginitis

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Background - We have analysed the role of Lactobacillus plantarum P 17630 in women with bacterial vaginosis/ aerobic vaginitis.

Methods - We conducted an observational, prospective, multicenter study on women with a diagnosis of bacterial vaginosis/aerobic vaginitis treated or not treated with Lactobacillus plantarum P 17630 >100.000.000 UFC. Eligible for the study were women aged 18 years or more with one or more moderate/severe clinical symptom: itching, vaginal burning and dryness (evaluated according to a self reported four level scale: absent, mild, moderate and severe), diagnosis of bacterial vaginosis or aerobic vaginitis. At study entry women were asked about presence and intensity (on a four level scale: absent, mild, moderate and severe) of itching, vaginal burning and dryness and leucorrhea. The same information was also collected at follow up visit 15 days after study entry. Women were proposed together with specific treatment the use of Lactobacillus plantarum P 17630, according to the following schedule: 1 vaginal capsule for 6 consecutive days, then a capsule per week. For the purposes of this study, resolution of the clinical infection was defined as: absence of clue cells and negative results for at least 2 Amsel criteria (for bacterial vaginosis) and / or absence of clinical symptoms and vaginal pH normal or score Donders < 3 (for aerobic vaginitis) and/or culture negative.

Results - A total of 94 patients were enrolled: of those 48 (51.1%) were treated with Lactobacillus plantarum. At the follow up visit, 40 women treated with Lactobacillus plantarum reported clinical resolution (83.3%). The corresponding value in no treated women was 22 women (47.8%) (P<0.05). No adverse event was reported in both groups.

Conclusion - This observational study suggests that Lactobacillus plantarum given in association with specific treatment may improve clinical resolution in women with BV and aerobic vaginitis.

Keywords: Bacterial vaginosis; Aerobic vaginitis; Lactobacillus plantarum

The Probiotic Bacterium Lactobacillus Plantarum 299v Improves Vascular Endothelial Function and Decreases Inflammatory Biomarkers in Men With Established Cardiovascular Disease

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Introduction: There is a significant unmet clinical need to identify novel therapies to prevent and treat the progression of cardiovascular disease. This requires identification of additional contributory processes to the pathogenesis of heart disease so that mechanism-based interventions may be developed.

Hypothesis: Our objective was to translate the recently discovered mechanistic link in rats between changes in the intestinal microbiota and the pathogenesis of cardiovascular disease to humans.

Methods: Twenty male adult patients with stable coronary heart disease on stable doses of vasoactive medications and HMG-CoA reductase inhibitors consumed a probiotic drink containing *Lactobacillus plantarum* 299v (3 oz. serving containing 20 billion colony forming units) once a day for 42 days. Vascular endothelial function was measured at the beginning and end of the study period by brachial flow-mediated dilation.

Results: The probiotic improved brachial artery flow mediated dilation, a surrogate marker of cardiovascular risk, by 33% from $3.55 \pm 1.96\%$ to $4.73 \pm 2.33\%$ with a trend towards decreased LDL-cholesterol by 8% from 96 ± 11 mg/dl to 88 ± 10 mg/dl. Probiotic therapy decreased circulating levels of the inflammatory cytokines IL-8 by 24% (17 ± 2 to 13 ± 1 pg/ml) and IL-12 by 20% (56 ± 7 to 45 ± 6 pg/ml). Whole microbiome analysis based on abundance of 2,206 taxa using hierarchical clustering revealed no separation of the overall intestinal microbial population between pre- and post-probiotic treatment samples. Bacterial genus richness ranged from 334 to 530 and archaeal ranged from 8 to 25, with no significant differences between pre- and post-probiotic samples. *Lactobacillus* and *Bacillus* species were enriched in post-probiotic samples. *Lactobacillus plantarum* 299v levels were elevated in post-probiotic samples, confirming patient compliance.

Conclusions: Our discovery of a relationship between *Lactobacillus plantarum* 299v, improved vascular endothelial function and decreased inflammation indicates intestinal microbiota may be developed as therapies to prevent and treat the progression of cardiovascular disease.

Immunogenic Properties of *Lactobacillus plantarum* Producing Surface-Displayed *Mycobacterium tuberculosis* Antigens

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Tuberculosis (TB) remains among the most deadly diseases in the world. The only available vaccine against tuberculosis is the bacille Calmette-Guérin (BCG) vaccine, which does not ensure full protection in adults. There is a global urgency for the development of an effective vaccine for preventing disease transmission, and it requires novel approaches. We are exploring the use of lactic acid bacteria (LAB) as a vector for antigen delivery to mucosal sites. Here, we demonstrate the successful expression and surface display of a *Mycobacterium tuberculosis* fusion antigen (comprising Ag85B and ESAT-6, referred to as AgE6) on *Lactobacillus plantarum*. The AgE6 fusion antigen was targeted to the bacterial surface using two different anchors, a lipoprotein anchor directing the protein to the cell membrane and a covalent cell wall anchor. AgE6-producing *L. plantarum* strains using each of the two anchors induced antigen-specific proliferative responses in lymphocytes purified from TB-positive donors. Similarly, both strains induced immune responses in mice after nasal or oral immunization. The impact of the anchoring strategies was reflected in dissimilarities in the immune responses generated by the two *L. plantarum* strains *in vivo*. The present study comprises an initial step toward the development of *L. plantarum* as a vector for *M. tuberculosis* antigen delivery.

IMPORTANCE This work presents the development of *Lactobacillus plantarum* as a candidate mucosal vaccine against tuberculosis. Tuberculosis remains one of the top infectious diseases worldwide, and the only available vaccine, bacille Calmette-Guérin (BCG), fails to protect adults and adolescents. Direct antigen delivery to mucosal sites is a promising strategy in tuberculosis vaccine development, and lactic acid bacteria potentially provide easy, safe, and low-cost delivery vehicles for mucosal immunization. We have engineered *L. plantarum* strains to produce a *Mycobacterium tuberculosis* fusion antigen and to anchor this antigen to the bacterial cell wall or to the cell membrane. The recombinant strains elicited proliferative antigen-specific T-cell responses in white blood cells from tuberculosis-positive humans and induced specific immune responses after nasal and oral administrations in mice.

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Impact of *Lactobacillus plantarum* Sortase on Target Protein Sorting, Gastrointestinal Persistence, and Host Immune Response Modulation

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Sortases are transpeptidases that couple surface proteins to the peptidoglycan of Gram-positive bacteria, and several sortase-dependent proteins (SDPs) have been demonstrated to be crucial for the interactions of pathogenic and nonpathogenic bacteria with their hosts. Here, we studied the role of sortase A (SrtA) in *Lactobacillus plantarum* WCFS1, a model *Lactobacillus* for probiotic organisms. An isogenic *srtA* deletion derivative was constructed which did not show residual SrtA activity. DNA microarray-based transcriptome analysis revealed that the *srtA* deletion had only minor impact on the full-genome transcriptome of *L. plantarum*, while the expression of SDP-encoding genes remained completely unaffected. Mass spectrometry analysis of the bacterial cell surface proteome, which was assessed by trypsinization of intact bacterial cells and by LiCl protein extraction, revealed that SrtA is required for the appropriate subcellular location of specific SDPs and for their covalent coupling to the cell envelope, respectively. We further found that SrtA deficiency did not affect the persistence and/or survival of *L. plantarum* in the gastrointestinal tract of mice. In addition, an *in vitro* immature dendritic cell (iDC) assay revealed that the removal of surface proteins by LiCl strongly affected the proinflammatory signaling properties of the SrtA-deficient strain but not of the wild type, which suggests a role of SDPs in host immune response modulation.

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Preventive Effects of Probiotic Bacteria *Lactobacillus plantarum* and Dietary Fiber in Chemically-induced Mammary Carcinogenesis

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Aim: The purpose of the present study was to evaluate the chemopreventive efficacy of a new probiotic bacterial strain, *Lactobacillus plantarum* LS/07 (PRO), prebiotic oligofructose-enriched inulin (PRE) and PRO-PRE combination in a rat model of breast cancer.

Materials and Methods: Mammary carcinogenesis was induced by 7,12-dimethylbenz[a]anthracene (DMBA). Daily oral administration of PRO (at a dose of 8.4×10^8 c.f.u./rat) and PRE (in the diet, 20 g/kg) started two weeks before the first DMBA dose and lasted until the end of the experiment (16 weeks).

Results: Administration of PRO, PRE and PRO-PRE combination significantly suppressed the tumor frequency, increased Cd4⁺ T-cells in tumor tissue and reduced serum tumor necrosis factor- α concentration. In PRO and PRO-PRE groups, the decline of Cd8⁺ T-cells in blood and their increase in tumor tissue was observed.

Conclusion: Long-term administration of Lactobacillus plantarum LS/07 with and without inulin is effective against breast cancer, at least partially, through immunomodulatory mechanisms.

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