**Original Research / Orijinal Araştırma**

Turk J Immunol 2022;10(3):170-8
DOI: 10.4274/tji.galenos.2022.54154

# Evaluating the Effect of a Food-origin *Lactobacillus plantarum* Strain on Th17 Related Cytokines

## Gıda Kökenli *Lactobacillus plantarum* Suşunun Th17 ile İlişkili Sitokinler Üzerindeki Etkisinin Değerlendirilmesi

**Mehri Joghataei[1], Gholamreza Pouladfar[2], Fakhri Shahidi[1], Mehdi Kalani[2]**

[1]Ferdowsi University of Mashhad, Faculty of Agriculture, Department of Food Science and Technology, Mashhad, Iran
[2]Professor Alborzi Clinical Microbiology Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

**Cite as:** Joghataei M, Pouladfar G, Shahidi F, Kalani M. Evaluating the Effect of a Food-origin *Lactobacillus plantarum* Strain on Th17 Related Cytokines. Turk J Immunol 2022;10(3):170-8

**Received:** 01.01.2022 **Accepted:** 27.06.2022

**Corresponding Author:** Gholamreza Pouladfar, Professor Alborzi Clinical Microbiology Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran
**Phone:** +98 713 647 43 03 **E-mail:** pouladfar_ghr@hotmail.com **ORCID:** orcid.org/0000-0001-8676-4607

### Abstract

**Objective:** This study aimed to assess the impact of a wild strain of *Lactobacillus plantarum* (PL4) on the production of IL-10 and T helper (Th) 17 related cytokines, including interleukin (IL)-17A, IL-17F, IL-21, and IL-22, compared to a standard probiotic strain, *Lactobacillus plantarum*. PL4 was isolated from Lighvan cheese and its probiotic potential was proved before. Probiotics with immune regulatory activity might be effective in the case of Th17 cells and IL-17-related pathways, which are involved in the pathogenesis and are associated with a worse result of some diseases. These regulatory effects might be arising from different bacterial cell substances.

**Materials and Methods:** Peripheral blood mononuclear cells (PBMC) were treated with cell debris and cell extract of both strains with and without human anti-CD3/CD28 T-cell activator beads in order to determine immunomodulatory effects of the strains. A number of cytokines were assessed.

**Results:** Bacterial cell debris and extract had no stimulatory or suppressive effects on the Th17 related cytokines and only the debris of both strains increased IL-10 production significantly. When PBMC pretreated with bacterial cell debris or extracts was stimulated with anti-CD3/CD28 beads, both strains down-regulated IL-17F. PL4 extract could decrease IL-21 and IL-17A but conversely, its debris increased these cytokines.

**Conclusion:** Pretreatment with PL4 extract induced lower levels of IL-17A, IL-17F, IL-21 and IL-10 in stimulated PBMCs, implying an anti-inflammatory potential of this strain. Significant differences among the effects of the strains confirmed strain dependency of their immunomodulatory properties.

**Keywords:** *Lactobacillus plantarum*, immunomodulatory properties, Th17 related cytokines, PBMC

### Öz

**Amaç:** Bu çalışmada, yabani bir *Lactobacillus plantarum* (PL4) suşunun, interlökin (IL)-10 ve IL-17A, IL-17F, IL-21 ve IL-22 dahil olmak üzere T helper (Th) 17 ile ilişkili sitokinlerin üretimi üzerindeki etkisini, standart bir probiyotik suş olan *Lactobacillus plantarum* ile karşılaştırarak değerlendirmesi amaçlanmıştır. PL4, Lighvan peynirinden izole edilmiş ve probiyotik potansiyeli daha önce kanıtlanmıştır. İmmün düzenleyici aktiviteye sahip probiyotikler, patogenezde yer alan ve bazı hastalıkların daha kötü bir sonucu ile ilişkilendirilen Th17 hücreleri ve IL-17 ile ilgili yolaklar durumunda etkili olabilmekte ve bu düzenleyici etkiler, farklı bakteri hücre maddelerinden kaynaklanabilmektedir.

**Gereç ve Yöntem:** Periferik kan mononükleer hücreleri (PBMC), suşların immünomodülatör etkilerini belirlemek için, insan anti-CD3/CD28 T hücre aktivatör boncukları olan ve olmayan her iki suşun hücre debrisi ve hücre ekstraktı ile muamele edildi. Kültür süpernatanlarında sitokinlerin miktarları belirlendi.

**Bulgular:** Bakteriyel hücre debris ve ekstraktı, Th17 ile ilgili sitokinler üzerinde hiçbir uyarıcı veya baskılayıcı etki göstermedi ve sadece her iki suşun debrisi IL-10 üretimini anlamlı ölçüde artırdı. Bakteriyel hücre debrisi veya ekstraktı ile ön işleme tabi tutulan PBMC, anti-CD3/CD28

**ORCID:** M. Joghataei 0000-0002-1306-8667, G. Pouladfar 0000-0001-8676-4607, F. Shahidi 0000-0002-5257-8205, M. Kalani 0000-0002-7091-6466

boncukları ile uyarıldığında, her iki suş da IL-17F seviyelerini azalttı. PL4 ekstraktı, IL-21 ve IL-17A seviyelerini azaltmasının tersine PL4 ekstrat debrisi bu sitokinleri artırdı.

**Sonuç:** PL4 ekstraktı ile ön muamele, uyarılmış PBMC'lerde daha düşük IL-17A, IL-17F, IL-21 ve IL-10 seviyelerine neden olmuştur, bu bulgular bu suşun anti-enflamatuvar potansiyelini göstermektedir. Suşların etkileri arasındaki önemli farklılıklar, immünomodülatör özelliklerinin suş bağımlılığını doğrulamaktadır.

**Anahtar Kelimeler:** *Lactobacillus plantarum*, immünomodülatör özellikler, Th17 ile ilgili sitokinler, PBMC

## Introduction

Twenty years ago, Mosmann et al. (1) proposed that CD4 cells separated into two subgroups called T helper 1 (Th1) and Th2, and numerous illnesses were inferable to some extent to Th1/Th2 imbalance. At the point when a third subgroup, known as Th17, was recognized in 2005, how we might interpret the role of lymphocytes in human illness was updated (2). Th17 cells have recently attracted impressive consideration because they exhibit distinct effector functions from Th1 and Th2 cells. When activated, these cells produce various different proinflammatory cytokines, including interleukin (IL)-17A, IL-17F, IL-21, and IL-22, and are believed to be involved in the protection of the host from microorganisms that Th1 or Th2 are not appropriate for. Several investigations have revealed a strong relationship between Th17 hyperactivity and some human diseases and multiple inflammatory effects on epithelial, endothelial, and fibroblastic cells (3). According to Sarmiento-Monroy et al. (4), blockade of the IL-23/IL-17 axis utilizing designated treatments and medications that indirectly act on this pathway may have beneficial effects in patients with coronavirus disease-2019 (COVID-19) contamination. Recent data in humans and mice propose that over-the-top Th17 action plays a significant part in the pathogenesis of immune-mediated diseases such as inflammatory bowel disease (5). Regulation of Th17 cells is as of now seen as a possibly certain pharmacological treatment for a wide cluster of human infections related to distorted Th17 reactions (6).

IL-10 is quite possibly the main cytokine tracked down in the human immune response (7). The major biological functions of IL-10 are the limitation and arrest of inflammatory responses and the regulation of differentiation and proliferation of various immune cells. Subsequently, it is generally viewed as an immunosuppressive particle by restraining the production of pro-inflammatory mediators and increasing the production of anti-inflammatory mediators (8).

Probiotics are characterized by the FAO/WHO as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (9). *Lactobacilli* as the most popular member of probiotics, at population levels exceeding $10^8$ CFU per gram of feces, it exerts various health-promoting functions of which immune regulatory activity (10). Different clinical efficacies of strains can be attributed to their varying abilities to induce the promotion of CD4 T-cell subsets. A number of probiotic lactobacilli promote Th1 responses (11), while others show Th2-suppressive effect by the induction of regulatory cytokines, or vice versa (12). *Lactobacillus plantarum* (*L. plantarum*) is a major colonizer of the human gut that increase antibody responses to intestinal pathogens. The experimental evidence suggests that *L. plantarum* suppress the Th1 immune response in mice (13). In a study *L. plantarum*-fed-mice maintained a healthier state after being injected with lipopolysaccharides (LPS) compared to other bacterial strain fed-mice and in the LPS-only control mice which is likely due to lower production of tumor necrosis factor-alpha and IL-12 (10). Significantly increased level of IL-4 in the blood (13). According to Kawashima et al. (14), oral administration of *L. plantarum* strain YU with high IL-12 inducing effects inhibited the proliferation of influenza A virus in bronchoalveolar lavage fluids. They concluded that the strain had a beneficial effect against the virus replication by activating Th1 immune responses.

Several recent studies have shown the anti-inflammatory affacts some probiotic strains on Th17-associated diseases (15-17). There is increasing evidence that probiotics selectively target the Th17 lineage in the prevention and treatment of inflammatory and autoimmune diseases (18-20). It is widely accepted that the anti-inflammatory effects of probiotics in inflammatory bowel disease (IBD) are primarily related to the downregulation of pro-inflammatory Th17-associated cytokine production because they affected the polarization of T-cell responses (21). One of the ways in which probiotics can exert immunomodulatory activities is by increasing the production of IL-10 (22). Several researchers have announced that the consumption of probiotic strains/probiotic mixtures/fermented foods could have anti-inflammatory effects through the modulation of IL-10 (23-25).

Studies have also reported immunomodulatory effects due to cheese consumption in humans (26) and rats (27). Although it is not clear which components of cheese are responsible for the modulatory actions, we would consider lactic acid bacteria (LAB) as a possibility. Lighvan is a popular Iranian cheese, with a long history of safe use. Its natural microbiome consists of unique LAB with different properties (28).

Since LAB are often used both in clinical applications and food products with many of their characteristics proven to be strain specific, a thorough examination of the effect of each strain on immune response seems to be essential for their safety assessment and segregating some effective strains. Those are helpful to the safest and most effective treatments. In this study, we analyzed the impact of *L. plantarum* strain, isolated from Lighvan cheese, on Th17 cells by means of assessing its impact on Th17 related cytokines and IL-10 induction and compared it with that of a standard probiotic strain.

## Materials and Methods

### Bacterial Strains and Related Preparations

Two *L. plantarum* strains consisting of a standard well-known probiotic strain, *L. plantarum* ATCC 14917 (PATCC) and *L. plantarum* PL4, isolated from Lighvan cheese (PL4), were included in this study. According to our previous study, the latter was able to survive in human gastrointestinal tract and showed a good antimicrobial effect and acceptable adhesion to human epithelial cell line. Moreover, PL4 strain can significantly increase the apoptosis of HT-29 cells (colon cancer cell line), while having no effect on human umbilical vein endothelial cells (29). Both strains were grown under microaerophilic conditions at 37°C on Man Rogosa Sharp Agar medium (Liofilchem, Italy). Mid log cultures were collected by centrifugation (8000 × g for 3 min), washed, and then resuspended in 5 mL Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, France). The bacterial suspensions (adjusted to the 3 McFarland scale) were then sonicated (Dr. Hielscher, Germany) at 5-6 power levels and 30% duty for 5 min. Bacterial suspensions were then centrifuged at 8000 × g for 30 min to separate cell debris from cell extract. Supernatants containing the cell extract and the pellets containing the cell debris were taken for further investigations.

### Isolation of Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells (PBMC) were isolated from fresh peripheral blood of 4 healthy donors (males, mean age: 45 years) by Ficoll density gradient centrifugation using Lymphodex (Innotrain, Germany). All donors gave written informed consent for blood collection and consent forms and procedures were in compliance with all relevant federal guidelines and institutional policies (in accordance with the ethical standards of the institutional research committee of Shiraz University of Medical Sciences, IR.SUMS.REC). Cells harvested from the interface were washed twice, enumerated and resuspended in complete media (CM) containing RPMI-1640 culture medium, supplemented with 10% (v/v) fetal calf serum, penicillin (100 IU/mL) and streptomycin (100 µg/mL). Trypan blue staining was done to assess the viability of isolated cells, found to be more than 97%.

### PBMCs Stimulation with Bacterial Extracts and Debris and/or Anti-CD3/CD28

PBMCs were cultured at a density of 750.000 cell/well in CM. The cells were stimulated with *Lactobacillus* culture debris or *Lactobacillus* culture extracts (multiplicity of infection: 1) and one well remained unstimulated as control. To assess the impact of lactobacilli on the production of cytokines as a result of stimulation with anti-CD3/CD28, the cells were pretreated with *Lactobacillus* debris or extracts, 1hr prior to addition of Dynabeads™ Human T-activator CD3/CD28 (Gibco, Germany). All cells were incubated at 37°C in a humidified atmosphere with 5% (v/v) $CO_2$. After 48 h incubation, cell culture supernatants were harvested and stored at -70°C until cytokine assay.

### Cytokine Quantification in Culture Supernatants

The amounts of IL-10, IL-17A, IL-17F, IL-21 and IL-22 in culture supernatants were determined by LEGENDplex™ Human Th Cytokine Panel (13-plex) (Biolegend, Germany), according to manufacturer's instructions using flow cytometer (BD FACSCalibur). Data analysis was performed by LEGENDplex™ Data Analysis Software, version 8.0.

### Statistical Analysis

All the values were presented as mean ± standard deviation of at least three-time repetitions. All statistical analyses were performed using the SPSS 20 statistical package (SPSS, Chicago, USA). Treatment groups were compared with the appropriate control groups, and statistical significances were measured using the independent sample t-test. Significant differences among treatments were evaluated by two-way analysis of variance (ANOVA) with post-hoc LSD. P values less than 0.05 were considered as statistically significant.

## Results

### Cytokine Production in PBMCs Stimulated with Bacterial Cell Debris and Extracts

Table 1 shows the results of cytokine production in PBMCs after stimulation with debris or extract of *Lactobacillus* strains. The levels of all the above-mentioned cytokines except IL-10, in non-stimulated PBMCs and those stimulated with cell debris and extract of *Lactobacillus* strains, were under detection limit of the method used for cytokine quantification. As shown in Figure 1a, in comparison with the control, the debris of both strains significantly increased IL-10 level ($p<0.01$), while the extracts did not exert any remarkable effects.

**Cytokine Production in PBMC Pretreated with Bacterial Cell Debris and Extracts Stimulated with Anti-CD3/CD28 Beads**

The results of cytokines production by PBMCs, pretreated with *Lactobacillus* cell debris or extracts and stimulated with anti-CD3/CD28 beads, are summarized in Table 1. As shown in Figure 1b, neither cell debris nor extracts had significant effect on IL-22 produced by anti-CD3/CD28 beads treatment. Of *Lactobacillus* debris, PL4 significantly up-regulated IL-21 (p-value=0.047) and ATCC 14917 down-regulated IL-17F (p-value=0.039) production. However, no substantial effects were detected on the other cytokines. Among the extracts, both PL4 and PATCC decreased the levels of IL-17F (p-value=0.01, 0.009) and IL-10 (PL4: p-value=0.008; PATCC p-value=0.044) in pretreated PBMC. In addition, PL4 extract could also attenuate the effects of anti CD3/CD28 on IL-17A (p-value=0.05) and IL-21 (p-value=0.048) (Figure 1).

Comparison of PL4 and PATCC strains regarding their effects on cytokine production in anti CD3/CD28 treated PBMC, revealed that the cell debris of both strains had a comparable stimulating capacity to induce all cytokines with the exception of IL-21 (p-value=0.045) and IL-10 (p-value=0.018). The higher levels of these cytokines were induced by PL4 debris (Figure 2). Only the strain PL4 extract significantly reduced IL-21 and IL-17A production when PBMC was stimulated with anti CD3/CD28 beads (p-value=0.048, 0.05).

IL-10 can negatively affected the Th17 immune response. To identify whether an increase in IL-10 level could have an effect on Th17 related cytokines production, we analyzed IL-10 release by PBMC cells, pretreated or not with *Lactobacillus* cell debris or extract and stimulated with anti CD3/CD28 beads. As shown in Figure 3, the levels of IL-10 were not higher in any of the samples of PBMCs with lactobacilli, in which Th17 related cytokine levels were low.

A significant positive correlation between IL-17A and IL-22 (p-value=0.003) was found in the case of debris while for extracts (Figure 4), IL-17A correlated significantly with IL-21 (p-value=0.002). Only when PBMC was stimulated with anti CD3/CD28 alone, a negative significant correlation was noticed between IL-17A and IL-10 (p-value=0.002).

## Discussion

In this study, two strains of *L. plantarum* were assessed for their abilities to affect the production of Th17 related cytokines such as IL-21, IL-22, IL-17A and IL-17F as well as IL-10 by PBMC. According to our previous study, these two strains had probiotic potential (27) and it would be valuable to determine how they might affect the immune system. Both strains modulated almost similar patterns of cytokines, but significant differences were observed between their effects on several cytokine productions. Above all, strain PL4 extract showed a higher anti-inflammatory effect than its standard counterpart. These finding are consistent with many studies reporting the genera/species or even strain specific immunomodulatory effects of probiotic strains (30,31).

Taking into account the effect of manufacturing process and the gastro-enteric passage on the bacterial viability, the results obtained in this study might be attributable to the effect of bacterial cell debris and extract released by the dead bacteria ingested during the probiotic administration. It was shown that LAB metabolites could suppress immune activation (32) and some researchers reported that the regulatory effects of probiotics could be owing to the induction of immune cell apoptosis (33,34). Definitely, in our study, any observed immunomodulatory effects of both strains could not be the result of metabolites of LAB or associated with their apoptotic activity, as we used bacterial cell debris and extracts, and lack of apoptotic effect by these strains on human normal cells was proven before (29).

**Table 1.** The *in vitro* effects of *Lactobacillus* cell debris and extracts on the production of cytokines (pg/mL) by PBMC in the absence or presence of anti-CD3/CD28 beads

| | IL-17A | IL-17F | IL-21 | IL-22 | IL-10 |
|---|---|---|---|---|---|
| Control (no stimuli) | <1.3 | <0.69 | <1.03 | <0.18 | 79.7 ± 14.0 |
| Strain PL4 debris | <1.3 | <0.69 | <1.03 | <0.18 | 578.1 ± 219.8 |
| Strain PL4 extract | <1.3 | <0.69 | <1.03 | <0.18 | 135.5 ± 38.8 |
| Strain PATCC debris | <1.3 | <0.69 | <1.03 | <0.18 | 240.1 ± 68.6 |
| Strain PATCC extract | <1.3 | <0.69 | <1.03 | <0.18 | 82.4 ± 56.7 |
| Stimulated with anti-CD3/CD28 | | | | | |
| anti-CD3/CD28 beads | 1093.4 ± 242.2 | 284.7 ± 68.6 | 177.0 ± 37.0 | 488.2 ± 196.5 | 1473.6 ± 558.3 |
| Strain PL4 debris | 1174.8 ± 403.5 | 166.6 ± 143.3 | 296.7 ± 62.0 | 559.6 ± 259.9 | 1453.0 ± 200.3 |
| Strain PL4 extract | 717.8 ± 67.8 | 128.8 ± 26.2 | 116.5 ± 4.4 | 352.4 ± 126.4 | 402.8 ± 86.7 |
| Strain PATCC debris | 949.4 ± 167.6 | 120.3 ± 82.4 | 209.3 ± 5.8 | 513.2 ± 66.6 | 853.1 ± 11.8 |
| Strain PATCC extract | 1141.2 ± 419.7 | 93.1 ± 33.4 | 153.4 ± 39.1 | 450.7 ± 127.7 | 526.8 ± 122.2 |

IL: Interleukin, PBMC: Peripheral blood mononuclear cells

When incubated with PBMC alone, bacterial cell debris and extract exhibited no stimulating or suppressive effects on Th17 related cytokines. This can be explained by the fact that probiotics re-stablish the immune milieu to normal in unusual states but have no effect when the environment is healthy (35). Previous research revealed that increased expression of mucosal IL-10, one of the key mediators of Th2 and Th1 immune responses (36), as a result of probiotic treatment, could enhance tolerance toward the resident gut bacteria and would modulate the subsequent inflammatory responses (37). In agreement with this study, our results showed that *L. plantarum* cell debris could significantly increase IL-10 concentration in cell culture supernatant. Likewise, Lammers et al. (38) revealed that genomic material extracted from several genus of probiotic bacteria with high GC content, had stimulatory effect on

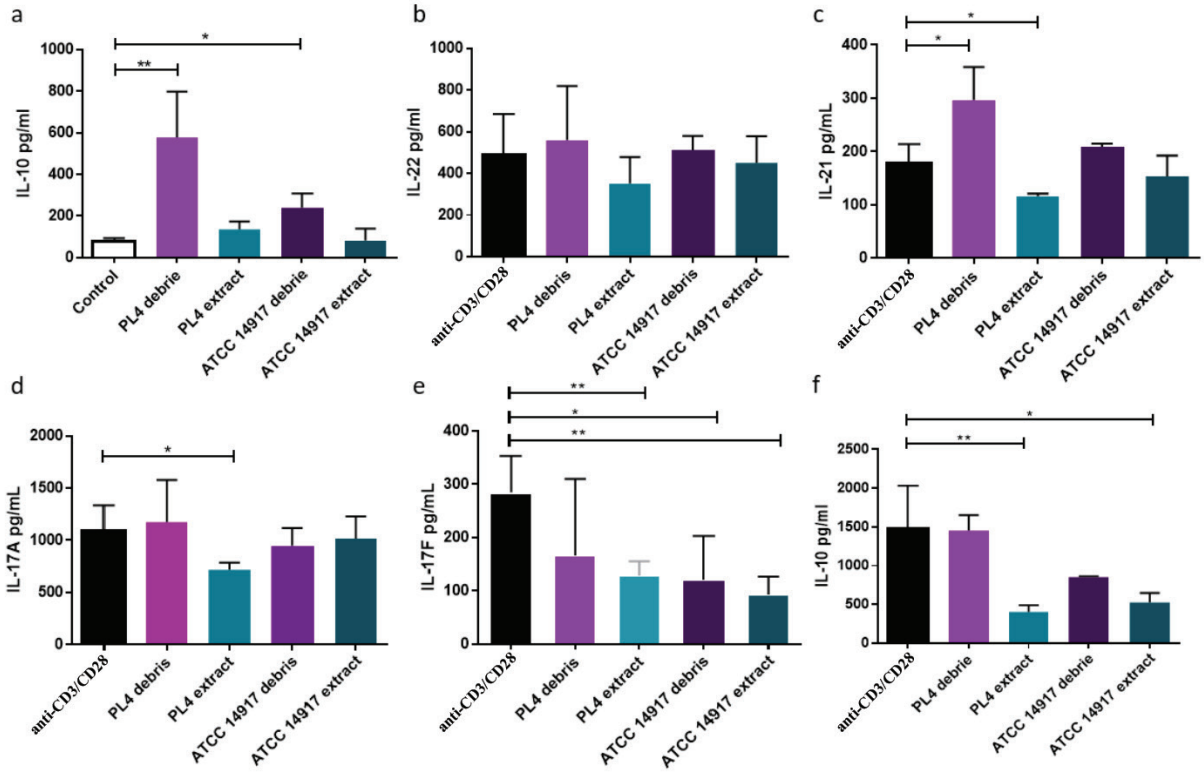


**Figure 1.** The bars represent IL-10 production when PBMC was treated with bacterial cell debris and extracts alone (a) as well as the production of IL-22 (b), IL-21 (c), IL-17A (d), IL-17F (e) and IL-10 (f) by PBMC (mean ± SD) pretreated with bacterial cell debris and extracts and stimulated with anti-CD3/CD28 beads. Values are mean ± SD of at least three replicates. (*) and (**) indicate a significant difference, compared to the control or anti-CD3/CD28 stimulation alone at $p<0.05$ and $p<0.01$, relatively

*PBMC: Peripheral blood mononuclear cells, IL: Interleukin, SD: Standard deviation*

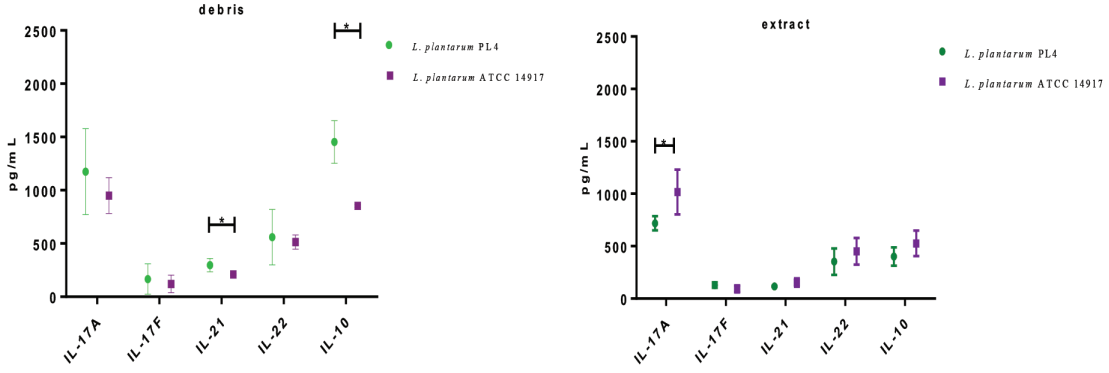


**Figure 2.** Comparison of the effects of pretreatment with each *Lactobacillus* strain cell debris or extract on cytokine production by PBMC stimulated with anti-CD3/CD28 beads. (*) indicates a significant difference between the effect of debris or extracts of the two strains at $p<0.05$

*PBMC: Peripheral blood mononuclear cells*

human PBMCs to produce more IL-10. According to Meng et al. (39) *L. plantarum* KLDS1.0318 8, a newly identified probiotic, could increase the secretion of IL-2, IL-6, and IFN-γ in cyclophosphamide-treated mice and helped maintain a balance between Th1 and Th2 type cytokines. In contrast, Lee et al. (40) reported that dead nano-sized *L. plantarum* nF1 isolated from kimchi (a traditional Korean fermented cabbage) promoted Th1 and Th17 immune responses compared to Th2 immune response in murine primary splenocytes. Although the key factors of these distinct immunological properties are not revealed, there is evidence that the composition of cell surface molecules or the content of DNA fragments can determine whether and how bacteria induce specific effects (41). Grangette et al. (23) and Horie et al. (42) demonstrated that lipoteichoic acid composition and copy number of CpG motifs, which are strain specific character, determined the mode of immune-stimulatory effect (proinflammatory or anti-inflammatory) of *Lactobacillus* cells. This may provide a logical explanation for the sometimes-conflicting evidence presented in the literature regarding the immune-modulating impact of different closely related species or strains. However, determination of the factor in cell debris of these strains involved in IL-10 induction remains to be investigated.

To investigate the anti-inflammatory properties of the strains, PBMCs pretreated with bacterial cell debris or extract were stimulated with anti-CD3/CD28 beads, the closest stimuli to physiological stress. The results suggested that when PBMC were pretreated with *Lactobacillus* strains cell debris and exposed to anti-CD3/CD28, cytokine-inducing effects did not change that much and inflammatory responses were somewhat modulated only in the case of bacterial cell extracts pretreatment. Indeed, extracts could decrease the concentration of all cytokines more effectively than their corresponding debris, and yet, the anti-inflammatory effects of strain PL4 extract was more significant. Down-regulated levels of IL-21 and IL-17A in PBMCs, stimulated with anti-CD3/CD28 beads in the presence of PL4 but not PATCC extract, implies that PL4 has more anti-inflammatory

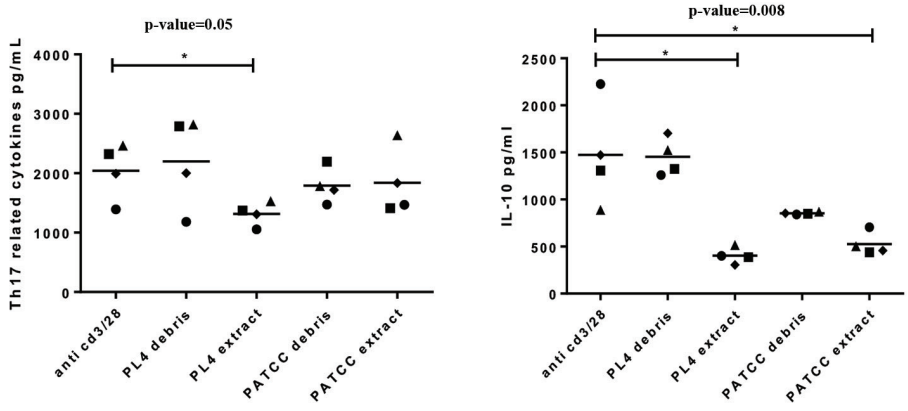


**Figure 3.** PBMC stimulated with anti-CD3/CD28 bead alone or pretreated with different bacterial strains cell debris or extract. Each symbol represents a different PBMC donor. Lines represent mean values. (*) indicates a significant difference, compared to anti-CD3/CD28 stimulation alone at $p<0.05$

*PBMC: Peripheral blood mononuclear cells*

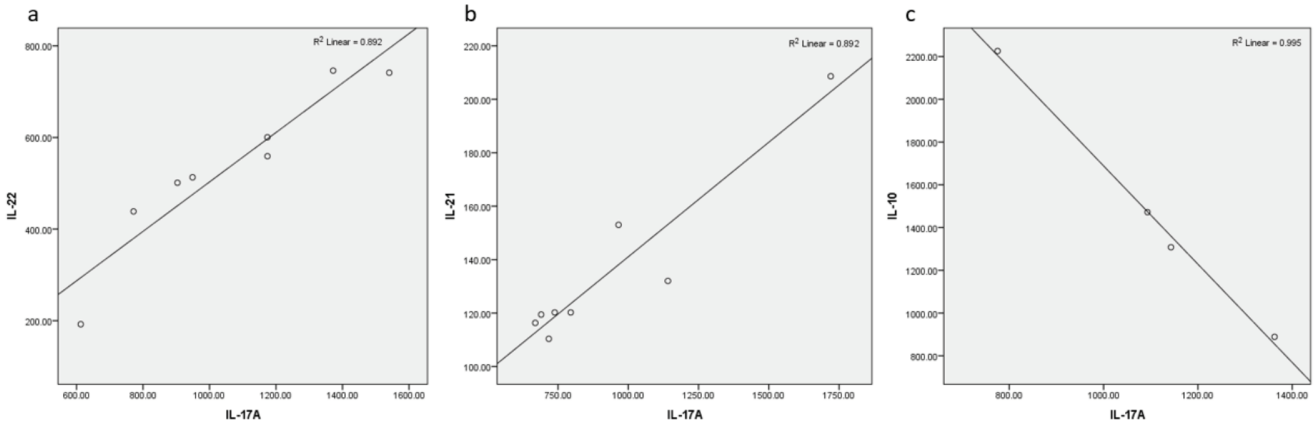


**Figure 4.** Significant correlations observed between cytokines produced, in the case of pretreatment with debris (a), extracts (b) and anti-CD3/CD28 stimulation without any pretreatment (c)

potential compared to PATCC strain. Furthermore, a significant difference between the effect of debris of these strains on IL-21 and IL-10 lends support to their strain-dependent behaviors.

Several researchers reported a reduction in Th17 cells and their corresponding proinflammatory cytokines, following the use of probiotic in animal models and in colonic tissue from patients with IBD (36,43). Similarly, Di Giacinto et al. (44) reported that 3-week probiotic administration in mice was associated with a significant increase and subsequent decrease in the production of IL-10 and pro-inflammatory cytokines, respectively, by lamina propria mononuclear cells treated with anti-CD3/CD28. COVID-19 has emerged as a pandemic disease with high mortality and development of an effective treatment is an urgent need. What happens in severe acute respiratory syndrome in COVID-19 patients is the polarization of T-cells toward the Th17 promotion and Th1 suppression, which contributes to an uncoordinated composition of the immune response. Several investigators found that IL-17 was elevated in COVID-19 patients in intensive care, compared to non-intensive-care and controls. It is suggested that blocking IL-17 could be a potentially beneficial therapeutic strategy for severe COVID19 patients (45,46). Taken together, these findings highlight the importance of IL-17 blockage by biological drugs that are already available and we suggest that some probiotic materials like PL4 extract might be effective in attenuation of IL-17 production. Further investigations are advised to make the strain PL4 applicable for clinical purposes.

Recent studies have been conducted to elucidate whether the anti-inflammatory impacts of probiotics in Th17-related diseases may be the outcome of their effects on Th cells balance via the controlling effect of IL-10 (47-49). Our results showed that anti-inflammatory properties of strain PL4 extract were not due to the suppressive effect of IL-10. Similar to our results, Chiba et al. (50) declared that certain probiotics might regulate pro-inflammatory responses via mechanisms other than the induction of regulatory T-cells. However, pathways through which these bacterial cell debris and extract affect cytokine production remains to be investigated.

The differentiation of Th17 cells follows the exposure of naive $CD4^+$ T-cells to antigen-presenting cell-derived polarizing cytokines such as IL-21 (51), while innate cells such as γδ T-cells (52), and natural killer cells (53) by producing IL-17A and IL-22 can influence the innate response via the induction of chemokines and antimicrobial proteins (54). In this study, the positive significant correlation between IL-17A/IL-22 (r=0.944) and IL-17A/IL-21 (r=0.945) in the presence of bacterial cell debris and extract, respectively, might suggest different patterns of immune stimulation by *Lactobacillus* bacterial cell debris and extract through innate immune response or adaptive Th17 cells function.

### Study Limitations

This research work has some limitations. Due to the lack of financial resources and sufficient time, it was not possible to increase the number of people examined. Detailed investigation and determination of the effective factors in bacterial cell debris and extract is one of the most important next steps that are recommended.

## Conclusion

The immunomodulatory properties of lactobacilli for each individual strain should be carefully evaluated before considering potential probiotic use, as capability of these two *L. plantarum* strains to modulate immune response via Th17 related cytokines was strain dependent.

We observed that strain PL4, isolated from Lighvan cheese with proven probiotic properties, was more potent than *L. plantarum* standard strain (ATCC 14917) in the induction of anti-inflammatory effect, here defined as reduced release of Th17 related cytokines, which might be helpful in attenuating the excessive production of these cytokines in the case of IBD or COVID-19 infection. This regulatory effect was not exerted as a result of modulation of IL-10 production. Bacterial cell debris and extract had no stimulatory effect on the production of Th17 related cytokines implying their modulatory role in healthy status. It seems that the regulation of Th17 mediated immune response by LAB is modulated by the involvement of many different immune cells and mediators while its mechanisms still remains to be discovered. So, more studies are needed to exactly verify the effect of cell wall components, DNA fragments, soluble proteinaceous factors or other different cytoplasmic bacterial components existing in cell debris and extracts. Once again, it was proven that food origin isolates might have significant effects than it was proven for well known strains.

### Ethics

**Ethics Committee Approval:** All procedures performed in the study were in accordance with the ethical standards of the institutional research committee (Shiraz University of Medical Sciences, IR.SUMS.REC) and with the 1964 Helsinki declaration (approval number: 1396/310, date: 05.03.2018).

**Informed Consent:** All donors gave written informed consent for blood collection and consent forms and procedures were in compliance with all relevant federal guidelines and institutional policies.

**Peer-review:** Externally peer-reviewed.

**Authorship Contributions**

Surgical and Medical Practices: M.J., M.K., Concept: M.J., M.K., Design: G.P., F.S., Data Collection or Processing: M.J., M.K., Analysis or Interpretation: M.J., Literature Search: M.J., F.S., Writing: M.J., G.P., F.S., M.K.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declare that they have no relevant financial.

## References


1. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 1986;136:2348-57.
2. Bettelli E, Korn T, Kuchroo VK. Th17: the third member of the effector T cell trilogy. Curr Opin Immunol. 2007;19:652-7.
3. Guglani L, Khader SA. Th17 cytokines in mucosal immunity and inflammation. Curr Opin HIV AIDS. 2010;5:120-7.
4. Sarmiento-Monroy JC, Parra-Medina R, Garavito E, Rojas-Villarraga A. T Helper 17 Response to Severe Acute Respiratory Syndrome Coronavirus 2: A Type of Immune Response with Possible Therapeutic Implications. Viral Immunol. 2021;34:190-200.
5. Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, et al. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science. 2006;314:1461-3.
6. Zhang W, Liu X, Zhu Y, Liu X, Gu Y, Dai X, et al. Transcriptional and posttranslational regulation of Th17/Treg balance in health and disease. Eur J Immunol. 2021;51.9:2137-2150.
7. Glocker EO, Kotlarz D, Klein C, Shah N, Grimbacher B. IL-10 and IL-10 receptor defects in humans. Ann N Y Acad Sci. 2011;1246:102-107.
8. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy--review of a new approach. Pharmacol Rev. 2003;55:241-69.
9. Borchers AT, Selmi C, Meyers FJ, Keen CL, Gershwin ME. Probiotics and immunity. J Gastroenterol. 2009;44:26-46.
10. Land MH, Rouster-Stevens K, Woods CR, Cannon ML, Cnota J, Shetty AK. Lactobacillus sepsis associated with probiotic therapy. Pediatrics. 2005;115:178-181.
11. Veckman V, Miettinen M, Matikainen S, Lande R, Giacomini E, Coccia EM, et al. Lactobacilli and streptococci induce inflammatory chemokine production in human macrophages that stimulates Th1 cell chemotaxis. J Leukoc Biol. 2003;74:395-402.
12. You J, Yaqoob P. Evidence of immunomodulatory effects of a novel probiotic, Bifidobacterium longum bv. infantis CCUG 52486. FEMS Immunol Med Microbiol. 2012;66:353-62.
13. Hong YF, Kim H, Kim HR, Gim MG, Chung DK. Different immune regulatory potential of Lactobacillus plantarum and Lactobacillus sakei isolated from Kimchi. J Microbiol Biotechnol. 2014;24:1629-1635.
14. Kawashima T, Hayashi K, Kosaka A, Kawashima M, Igarashi T, Tsutsui H, et al. Lactobacillus plantarum strain YU from fermented foods activates Th1 and protective immune responses. Int Immunopharmacol. 2011;11:2017-24.
15. Jan RL, Yeh KC, Hsieh MH, Lin YL, Kao HF, Li PH, et al. Lactobacillus gasseri suppresses Th17 pro-inflammatory response and attenuates allergen-induced airway inflammation in a mouse model of allergic asthma. Br J Nutr. 2012;108:130-9.
16. Mimura T, Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot IC, et al. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. Gut. 2004;53:108-14.
17. Bibiloni R, Fedorak RN, Tannock GW, Madsen KL, Gionchetti P, Campieri M, et al. VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. Am J Gastroenterol. 2005;100:1539-46.
18. Tanabe S. The effect of probiotics and gut microbiota on Th17 cells. Int Rev Immunol. 2013;32:511-25.
19. Hedin C, Whelan K, Lindsay JO. Evidence for the use of probiotics and prebiotics in inflammatory bowel disease: a review of clinical trials. Proc Nutr Soc. 2007;66:307-15.
20. Damaskos D, Kolios G. Probiotics and prebiotics in inflammatory bowel disease: microflora 'on the scope'. Br J Clin Pharmacol. 2008;65:453-67.
21. Sanders ME, Guarner F, Guerrant R, Holt PR, Quigley EM, Sartor RB, et al. An update on the use and investigation of probiotics in health and disease. Gut. 2013;62:787-96.
22. Lavasani S, Dzhambazov B, Nouri M, Fåk F, Buske S, Molin G, Thorlacius H, et al. A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing regulatory T cells. PLoS One. 2010;5:e9009.
23. Grangette C, Nutten S, Palumbo E, Morath S, Hermann C, Dewulf J, et al. Enhanced antiinflammatory capacity of a Lactobacillus plantarum mutant synthesizing modified teichoic acids. Proc Natl Acad Sci U S A. 2005;102:10321-6.
24. Mastrangeli G, Corinti S, Butteroni C, Afferni C, Bonura A, Boirivant M, et al. Effects of live and inactivated VSL#3 probiotic preparations in the modulation of in vitro and in vivo allergen-induced Th2 responses. Int Arch Allergy Immunol. 2009;150:133-43.
25. de Moreno de Leblanc A, Perdigón G. The application of probiotic fermented milks in cancer and intestinal inflammation. Proc Nutr Soc. 2010;69:421-8.
26. Ibrahim F, Ruvio S, Granlund L, Salminen S, Viitanen M, Ouwehand AC. Probiotics and immunosenescence: cheese as a carrier. FEMS Immunol Med Microbiol. 2010;59:53-9.
27. Lollo PC, Cruz AG, Morato PN, Moura CS, Carvalho-Silva LB, Oliveira CA, et al. Probiotic cheese attenuates exercise-induced immune suppression in Wistar rats. J Dairy Sci. 2012;95:3549-58.
28. Edalatian MR, Habibi NMB, Mortazavi A, Mayo B. The biodiversity and evolution of lactic flora during ripening of the Iranian semisoft Lighvan cheese. Int J Dairy Technol. 2012;65:81-9.
29. Joghataei M, Shahidi F, Pouladfar G, Mortazavi SA, Ghaderi A. Probiotic potential comparison of Lactobacillus strains isolated from Iranian traditional food products and human feces with standard probiotic strains. J Sci Food Agric. 2019;99:6680-8.
30. Isaacs K, Herfarth H. Role of probiotic therapy in IBD. Inflamm Bowel Dis. 2008;14:1597-605.
31. Mileti E, Matteoli G, Iliev ID, Rescigno M. Comparison of the immunomodulatory properties of three probiotic strains of Lactobacilli using complex culture systems: prediction for in vivo efficacy. PLoS One. 2009;4:e7056.

32. Ligaarden SC, Axelsson L, Naterstad K, Lydersen S, Farup PG. A candidate probiotic with unfavourable effects in subjects with irritable bowel syndrome: a randomised controlled trial. BMC Gastroenterol. 2010;10:16.

33. Peluso I, Fina D, Caruso R, Stolfi C, Caprioli F, Fantini MC, et al. Lactobacillus paracasei subsp. paracasei B21060 suppresses human T-cell proliferation. Infect Immun. 2007;75:1730-7.

34. Angulo S, Morales A, Danese S, Llacuna L, Masamunt MC, Pultz N, et al. Probiotic sonicates selectively induce mucosal immune cells apoptosis through ceramide generation via neutral sphingomyelinase. PLoS One. 2011;6:e16953.

35. Daniluk U, Alifier M, Kaczmarski M. Probiotic-induced apoptosis and its potential relevance to mucosal inflammation of gastrointestinal tract. Adv Med Sci. 2012;57:175-82.

36. Singh A, Sarangi AN, Goel A, Srivastava R, Bhargava R, Gaur P, et al. Effect of administration of a probiotic preparation on gut microbiota and immune response in healthy women in India: an open-label, single-arm pilot study. BMC Gastroenterol. 2018;18:85.

37. Schreiber S, Heinig T, Thiele HG, Raedler A. Immunoregulatory role of interleukin 10 in patients with inflammatory bowel disease. Gastroenterology. 1995;108:1434-44.

38. Lammers KM, Brigidi P, Vitali B, Gionchetti P, Rizzello F, Caramelli E, et al. Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells. FEMS Immunol Med Microbiol. 2003;38:165-172.

39. Meng Y, Li B, Jin D, Zhan M, Lu J, Huo G. Immunomodulatory activity of Lactobacillus plantarum KLDS1.0318 in cyclophosphamide-treated mice. Food Nutr Res. 2018;62.

40. Lee HA, Kim H, Lee KW, Park KY. Dead Lactobacillus plantarum Stimulates and Skews Immune Responses toward T helper 1 and 17 Polarizations in RAW 264.7 Cells and Mouse Splenocytes. J Microbiol Biotechnol. 2016;26:469-76.

41. Wells, Jerry M. Immunomodulatory mechanisms of lactobacilli. Microbial cell factories. BioMed Central. 2011;10:1-15.

42. Horie A, Tomita Y, Ohshio K, Fujiwara D, Fujii T. Characterization of genomic DNA of lactic acid bacteria for activation of plasmacytoid dendritic cells. BMC Microbiol. 2019;19:88.

43. Chen L, Zou Y, Peng J, Lu F, Yin Y, Li F, et al. Lactobacillus acidophilus suppresses colitis-associated activation of the IL-23/Th17 axis. J Immunol Res. 2015;2015:909514.

44. Di Giacinto C, Marinaro M, Sanchez M, Strober W, Boirivant M. Probiotics ameliorate recurrent Th1-mediated murine colitis by inducing IL-10 and IL-10-dependent TGF-beta-bearing regulatory cells. J Immunol. 2005;174:3237-46.

45. Wu D, Yang XO. TH17 responses in cytokine storm of COVID-19: An emerging target of JAK2 inhibitor Fedratinib. J Microbiol Immunol Infect. 2020;53:368-370.

46. Cafarotti S. Severe Acute Respiratory Syndrome-Coronavirus-2 Infection and Patients With Lung Cancer: The Potential Role of Interleukin-17 Target Therapy. J Thorac Oncol. 2020;15:e101-e103.

47. Gad M, Ravn P, Søborg DA, Lund-Jensen K, Ouwehand AC, Jensen SS. Regulation of the IL-10/IL-12 axis in human dendritic cells with probiotic bacteria. FEMS Immunol Med Microbiol. 2011;63:93-107.

48. Shida K, Nanno M, Nagata S. Flexible cytokine production by macrophages and T cells in response to probiotic bacteria: a possible mechanism by which probiotics exert multifunctional immune regulatory activities. Gut Microbes. 2011;2:109-14.

49. Roselli M, Finamore A, Nuccitelli S, Carnevali P, Brigidi P, Vitali B, et al. Prevention of TNBS-induced colitis by different Lactobacillus and Bifidobacterium strains is associated with an expansion of gammadeltaT and regulatory T cells of intestinal intraepithelial lymphocytes. Inflamm Bowel Dis. 2009;15:1526-36.

50. Chiba Y, Shida K, Nagata S, Wada M, Bian L, Wang C, et al. Well-controlled proinflammatory cytokine responses of Peyer's patch cells to probiotic Lactobacillus casei. Immunology. 2010;130:352-62.

51. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol. 2009;27:485-517.

52. Umemura M, Yahagi A, Hamada S, Begum MD, Watanabe H, Kawakami K, et al. IL-17-mediated regulation of innate and acquired immune response against pulmonary Mycobacterium bovis bacille Calmette-Guerin infection. J Immunol. 2007;178:3786-96.

53. Cella M, Fuchs A, Vermi W, Facchetti F, Otero K, Lennerz JK, et al. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. Nature. 2009;457:722-5.

54. Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, et al. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat Med. 2008;14:282-9.