

Immune Regulatory Effect of Newly Isolated *Lactobacillus delbrueckii* from Indian Traditional Yogurt

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Lactic acid bacteria (LAB) are microorganisms that are believed to provide health benefits. Here, we isolated LAB from Indian fermented foods, such as traditional Yogurt and Dosa. LAB from Yogurt most significantly induced TNF- α and IL-1 β production, whereas LAB from Dosa induced mild cytokine production. After 16S rRNA gene sequencing and phylogenetic analysis, a Yogurt-borne lactic acid bacterium was identified and classified as *Lactobacillus delbrueckii* subsp. *bulgaricus*, and it was renamed *L. delbrueckii* K552 for the further studies. Our data suggest that the newly isolated *L. delbrueckii* can be used for the treatment of immune deficiency disorders.

Keywords: Lactic acid bacteria, *Lactobacillus delbrueckii*, tumor necrosis factor-alpha, interleukin-1beta, Indian fermented foods

Lactic acid bacteria (LAB) are known for their health-promoting effects [3] and have effects on the production of cytokines and on cell proliferation in human intestinal epithelial cells [8]. In this study, LAB were isolated from Yogurt and Dosa, which are the most popular fermented products of South India. The fermented foods were spread on MRS agar plates, and 100 colonies were selected and cultured in MRS broth. The cultured LAB samples were numbered as Y-1 to Y-100 of Yogurt and nD1–nD100 of new Dosa samples. Live or heat-killed LAB and control *Staphylococcus aureus* were treated on THP-1, a monocyte-like cell line, for 4 h, and cytokine production was estimated by ELISA.

As shown in Fig. 1A, TNF- α was significantly increased by both live and heat-killed LAB from the Yogurt sample ($p < 0.01$ in live sample; $p < 0.001$ in heat-killed sample as compared with untreated). Similarly, IL-1 β production was

also increased by live and heat-killed LAB from Yogurt (Fig. 1B; $p < 0.001$ in live and heat-killed samples as compared with untreated), whereas LAB from Dosa did not induce them. Cytokine production by LAB from Yogurt was higher in heat-killed bacteria-treated cells than in live treated one. It is possible that heat-killed bacteria may expose more bioactive antigens on their cell wall surface. TNF- α production by Y-52 was dose dependent (Fig. 2A), and it was increased up to 6 h of culture and then decreased (Fig. 2B). Y-52-mediated IL-1 β was significantly increased ($p < 0.001$) with 1×10^6 bacteria (Fig. 2C) for 9 h culture (Fig. 2D). Cytokine induction in THP-1 cells by Y-52 was higher than *S. aureus*, indicating that Y-52 can be used as an immune regulator.

To identify and classify the Y-52 LAB, the 16S rRNA gene was sequenced and sequence alignment was performed using NCBI BLAST (<http://blast.ncbi.nlm.nih.gov>). The neighbor-

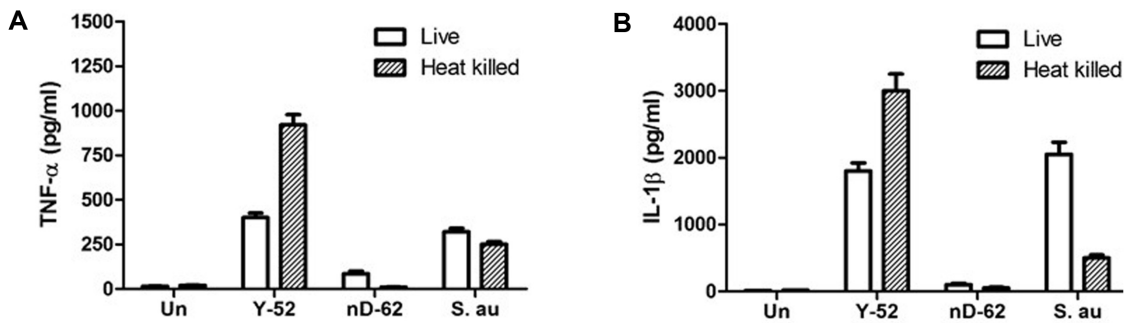


Fig. 1. Cytokine induction by newly isolated LAB.

THP-1 cells were stimulated with LAB isolated from Indian traditional fermented Yogurt and Dosa for 4 h. To make inactivated LAB, they were incubated in an 80°C water bath for 20 min. The production of TNF-α (A) and IL-1β (B) was estimated by ELISA using culture supernatants. Representative data from LAB (e.g., Y-52 from Yogurt and nD-62 from Dosa) are shown. *S. aureus* (S. au) was used as the control. Values are the mean ± SD of three independent experiments.

joining tree based on 16S rRNA gene sequences shows the phylogenetic relationships between *Lactobacillus* and Y-52, with the bar indicating 0.02 substitutions per nucleotide position (Fig. 3). Bootstrap analysis with 1,000 replicates was also conducted in order to obtain confidence levels for the branches. Most of the species in *Lactobacillus* were included in the phylogenetic tree. Y-52 was identified as

L. delbrueckii subsp. *bulgaricus*. Thus, Y-52 was renamed as *L. delbrueckii* K552 for the further studies.

Lactobacillus species have been used as probiotics for a long time in human history [4]. For example, *L. plantarum* reduces serum cholesterol, LDL-cholesterol, and triglyceride levels [10], and attenuates inflammatory bowel disease [6]. *L. rhamnosus* suppresses allergic sensitization and airway

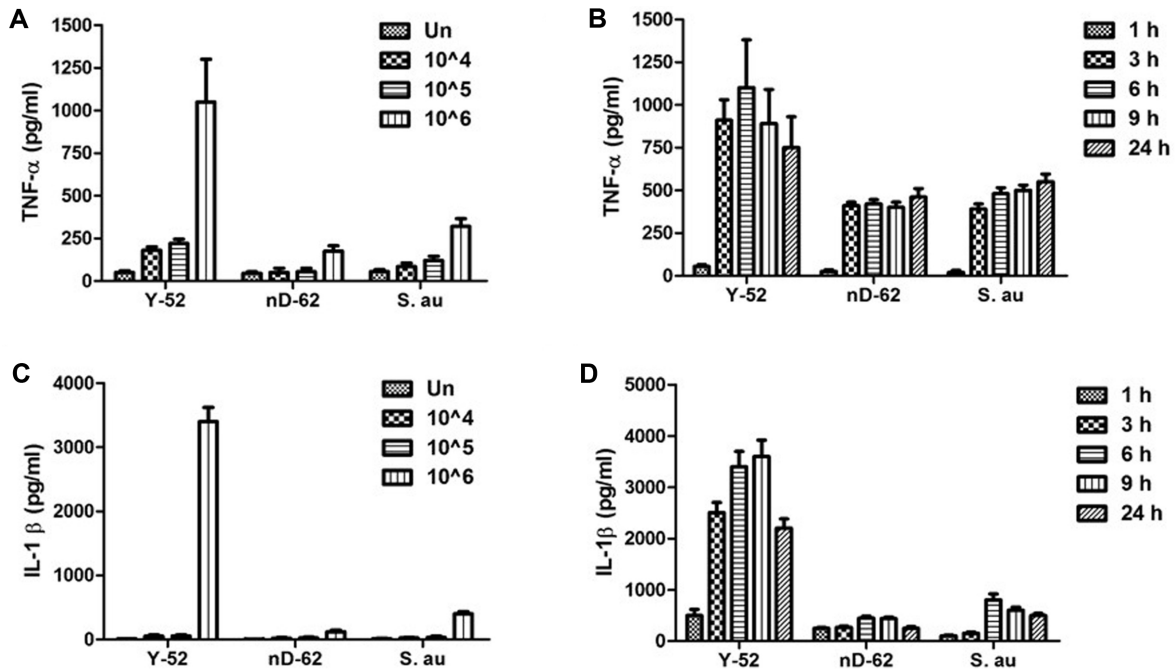


Fig. 2. Dose- or time-dependent effects of LAB on cytokine induction.

THP-1 cells were stimulated with the indicated dose of bacteria for 4 h and the levels of (A) TNF-α and (C) IL-1β production were analyzed using ELISA. Cells were incubated with 1×10^6 cells of LAB for the indicated time and the levels of (B) TNF-α and (D) IL-1β were analyzed using ELISA. Bacteria Y-52 and nD-62 were isolated from Yogurt and Dosa, respectively. *S. aureus* (S. au) was used as the control. Data are expressed as the mean ± SD.

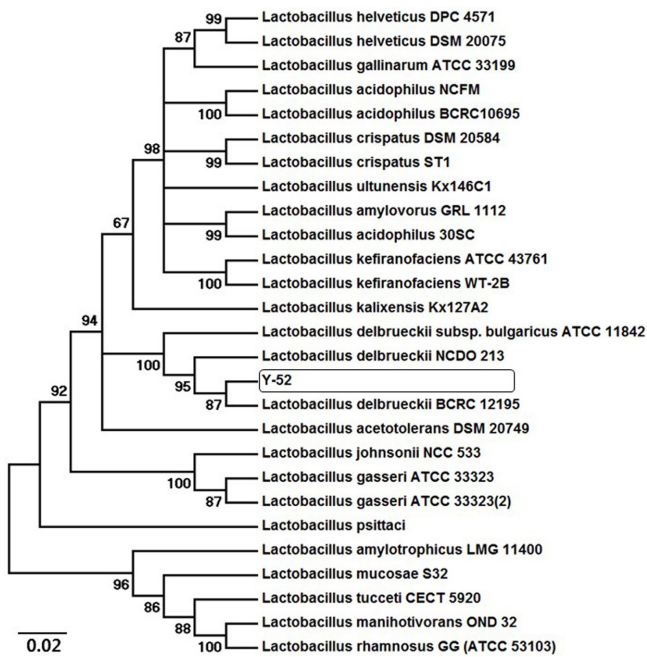


Fig. 3. Phylogenetic analysis of the Y-52 16S rRNA gene sequence compared with type strains belonging to *Lactobacillus*.

A phylogenetic tree was constructed by the MEGA6 program. Bootstrap analysis was performed using 1,000 replications. Bar, 0.02 substitutions per nucleotide position.

inflammation [2]. *L. delbrueckii* can protect against *Listeria monocytogenes* [1], and *L. sakei* has been suggested to have beneficial effects in children with atopic eczema-dermatitis syndrome [10]. The immunostimulating potency of lactobacilli appears to be species-specific [5]. For example, a previous study reported that *L. rhamnosus* and *L. plantarum* moderately induced the expression of co-stimulatory molecules and stimulated low-level production of cytokines and chemokines [8]. In contrast, *L. delbrueckii* was reported to stimulate the production of high levels of the pro- and anti-inflammatory cytokines TNF- α and IL-10, respectively, as well as NO in the mucosal immune system [1].

In this study, we evaluated the effects of newly isolated LAB from Indian traditional Yogurt on the TNF- α and IL-1 β induction in human THP-1 cells to identify which LAB have immune regulatory activity. Interestingly, Y-52 (*L. delbrueckii* K552) significantly increased TNF- α and IL-1 β , the induction of which was higher than *S. aureus* treatment. Although *S. aureus* treatment increased TNF- α and IL-1 β production, this pathogen should not be applied for the disease treatment since it has many toxins and side effects. On the other hand, *L. delbrueckii* is a harmless LAB.

Therefore, our data suggest that Y-52 isolated from traditional Indian fermented foods may be used for the treatment of immune disorders.

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