Original Article

Anti-inflammatory Effects of Spores Derived from Probiotic Strains *Bacillus subtilis* natto and *Bacillus coagulans* Hammer on Human Intestinal Epithelial Cells *In Vitro*

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Abstract

Background: Probiotic administration can be an effective treatment against intestinal inflammation. This study aimed to assess the potential effects of spores isolated from probiotic strains *Bacillus subtilis* natto and *Bacillus coagulans* Hammer on inflammation induced by lipopolysaccharide (LPS) in human colon epithelial cells *in vitro*.

Materials and Methods: The viability of HT-29 cells treated with spores derived from *B. subtilis* natto and *B. coagulans* Hammer (MOI 10, 100, 1000), as well as LPS (10 µg/ml) was assessed. The anti-inflammatory effects of spores were examined on HT-29 cells that were pre-stimulated with LPS. The expression level of *IL-6* and *TLR4* genes in HT-29 cells was quantified after 24 h using RT-qPCR.

Results: There was no significant reduction in the viability of HT-29 cells after exposure to LPS and various MOIs of probiotic spores. Stimulation of HT-29 cells with LPS significantly increased the expression level of *IL-6* and *TLR4* in comparison to control ($P < 0.0001$). Spores isolated from both probiotic strains, *B. subtilis* natto and *B. coagulans* Hammer, caused a significant reduction in the gene expression of *IL-6* and *TLR4* in HT-29 cells compared to LPS control ($P < 0.0001$).

Conclusion: The findings of this study suggest that probiotics-derived spores may exert anti-inflammatory effects through interference with the LPS signaling pathway in colon cancer HT-29 cell line.

Keywords: *Bacillus subtilis* natto, *Bacillus coagulans* Hammer, Anti-inflammatory effect, Lipopolysaccharide, HT-29 cells

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Introduction

The human gastrointestinal tract consists of a plethora of microorganisms, including bacteria, fungi, viruses, and archaea, which form the most intricate microbial consortium within the host[1]. These microbial

companions have established a symbiotic relationship with the host, wherein diverse beneficial microbes and their metabolic byproducts influence gastrointestinal homeostasis and modulate susceptibility to disease[2]. The profound genetic and metabolic potentials of the gut microbiome highlight the pervasive influence of this community of microbes across diverse facets of human physiology, encompassing physical health, development, aging, and pathogenesis of diseases[3-5]. Thus, judicious manipulation of the gut microbiota via microbiome-oriented strategies offers promising therapeutic options to improve human health[6, 7].

Probiotics are live microorganisms that, in adequate quantities, can fortify the individual's immune defenses against prospective ailments or provide health benefits for treating patients[7-9]. These beneficial microorganisms exert their therapeutic influence by modulating the equilibrium of the gut microbiota, which is a fundamental determinant of overall health[10]. An accumulating body of evidence has illuminated that probiotics exert their immunomodulatory effects predominantly through the enhancement of intestinal barrier integrity and attenuation of inflammatory processes[11-14]. As ongoing research continues to reveal the link between probiotics and human physiology, their incorporation into clinical practice holds substantial promise to start a new epoch of preventive and therapeutic modalities to improve health outcomes and heighten well-being.

Lipopolysaccharide (LPS) is the key component of the Gram-negative bacterial cell wall, which stands as an important contributor in activating inflammatory response and helps the persistence of bacterial infection[15]. Empirical evidence substantiates the adverse effects of LPS on the equilibrium of the gut microbiota and its potential to disrupt gut cellular function, thereby eliciting immune reactions[16]. At the core of this interaction lies toll-like receptor (TLR)-dependent pathways, which are integral components of the innate immune system charged with detecting and responding to microbial entities, namely LPS[17]. Upon engagement with *TLR4*, LPS initiates the secretion of proinflammatory cytokines and perpetuates immune dysregulation in the gut environment[18]. Furthermore, LPS can compromise intestinal barrier integrity, impeding its role as a critical defense mechanism against pathogenic agents and toxins[19]. Within the realm of probiotics, extensive studies have been dedicated to the positive impact of *Bacillus subtilis* natto and *Bacillus coagulans* Hammer on gastrointestinal diseases[20]. These microbes can exist as either vegetative cells or enter dormant states and produce spores[21]. It has been suggested that the diminutive size of spores can facilitate direct interactions with intestinal epithelial cells and the immune system, thereby potentially averting the activation of inflammatory mediators[22].

This study aimed to investigate the anti-inflammatory effects of spores derived from probiotic strains *B. subtilis* natto and *B. coagulans* Hammer on the expression of *IL-6* and *TLR4* genes in the LPS-stimulated HT-29 cell line.

Methods

Bacterial growth conditions and spore isolation: Probiotic strains, including *B. subtilis* natto and *B. coagulans* Hammer, were obtained from World Intellectual Resource Co., Taiwan, and Natures Only, INC., USA, respectively. Briefly, a suspension of each strain was primed in brain heart infusion broth (Merck, Darmstadt, Germany). Subsequently, 100 µl of each suspension was plated onto Muller Hinton agar (Merck, Darmstadt, Germany) and incubated under aerobic conditions at 37°C for 24 h. Bacterial colonies were further incubated at room temperature for an additional week to enforce the process of sporulation.

A loop full of *B. subtilis* natto as well as *B. coagulans* Hammer colonies was suspended in phosphate-buffered saline (PBS, pH 8) to extract the spores. Subsequently, the suspension was treated with 50 µg/ml lysozyme prepared in Tris-HCl (pH 7.5) at 37°C for 30 min. Furthermore, cell debris was removed by washing lysates with PBS and centrifugation (12,000 × g, 10 min) five times. The purified spores were stored at -20°C for further cell experiments[22].

Cell culture: The HT-29 cell line (ATCC HTB-38) was acquired from the Research Institute of Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Cells were maintained in high-glucose Dulbecco's modified Eagle's minimal essential medium (H-DMEM, Gibco, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Gibco, USA), 100 U/ml

penicillin (Sigma-Aldrich, USA), and 100 U/ml streptomycin (Sigma-Aldrich, USA), and incubated in a 5% CO_2 humidified atmosphere at 37°C. The media were renewed every 24 hours to reach 80% confluent.

Cell viability assay: To evaluate the cytotoxic effects of varying concentrations of spores derived from *B. subtilis* natto and *B. coagulans* Hammer, as well as LPS on HT-29 cells, cell viability assay with MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide) was performed using the commercial Cell Proliferation Kit I (Sigma-Aldrich, St. Louis, Missouri, USA) per the manufacturer's instruction. Briefly, HT-29 cells were seeded in a 96-well plate and incubated at 37°C to reach confluency. Subsequently, cells were washed with PBS and exposed to 10 µg/ml LPS (*E. coli* O111:B4, Sigma, USA) for 1, 3, 6, 12, 18, and 24 h periods. In addition, HT-29 cells were separately treated with varying concentrations of spores (MOI 10, 100, 1000) derived from *B. subtilis* natto and *B. coagulans* Hammer for 24 hours.

Following treatment, 0.5 mg/ml MTT solution was added to each well; then, the plates were incubated at 37°C for four hours. The reaction ceased when cells were contaminated with 200 µl of dimethyl sulfoxide (DMSO, Thermo Fisher Scientific Inc., MA, USA) for 15 min. The wells without cells served as blank. The optical density (OD) of each well was calculated using an ELISA microplate reader (BioTek, USA) at 550 nm, with a reference wavelength of 600 nm. to evaluate the viability of HT-29 cells.

Treatment of HT-29 cells with LPS and spores: According to the results of the cell viability assay, the highest concentrations of spores of *B. subtilis* natto and *B. coagulans* Hammer, which had no significant impact on the viability of HT-29 cells (MOI 1000), were applied in downstream cell treatment assays. HT-29 cells were treated with 10 µg/ml LPS at 37°C for three hours. Following incubation, cells were treated with spores for 24 h. All experiments were carried out in triplicate.

Total RNA extraction, cDNA synthesis and RT-qPCR: The adherent cells were trypsinized with 0.25% trypsin-EDTA solution (Sigma, USA) and washed with PBS. The total RNA of HT-29 cells was extracted using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The quality and concentration of obtained RNA samples were determined by NanoDrop spectrophotometer (ND-1000, Thermo Scientific, USA). Moreover, cDNA synthesis was done using PrimeScript™ RT reagent Kit (Takara, Japan) as per the manufacturer's instructions. The cDNA samples were preserved at -20°C until further use.

RT-qPCR assay was performed using Rotor-Gene® Q (Qiagen, GmbH, Germany) real-time PCR system and BioFACT™ 2X Real-Time PCR Master Mix (BIOFACT CO., Ltd. Daejeon, South Korea), with β-actin serving as the reference gene. The oligonucleotide sequences used to quantify the expression level of *IL-6* and *TLR4* genes are presented in Table 1[23,24]. The experiments were run in triplicate.

Statistical Analysis: The relative expression level of target genes was measured by the $2^{-\Delta\Delta Ct}$ method. Statistical analysis was conducted using GraphPad Prism software (v.10). The one-way analysis of variance (ANOVA) was carried out to determine the statistical significance between groups. The data were presented as the mean of three independent experiments, and error bars show the standard deviations (SD). Differences were statistically significant when $P < 0.05$.

Ethics approval and consent to participate: The study was approved by the Institutional Ethics Review Committee of the Research Institute for Gastroenterology and Liver Diseases at Shahid Beheshti University of Medical Sciences (IR.SBMU.RIGLD.REC.1399.011).

Results

Viability of HT-29 cells: MTT assay was performed to determine the cytotoxic effect of LPS and spores derived from *B. subtilis* natto and *B. coagulans*

Table 1. Oligonucleotide sequences used in this study.

Target gene	Oligonucleotide sequences (5′–3′)	P.S (bp)
IL-6	F: GCACTGGCAGAAAACAACCT R: TCAAACTCCAAAAGACCAGTGA	119
TLR4	F: ATGCATGGATCAGAAACTCAGCAA R: AAACTTCCTGGGGAAAAACTCTGG	104
β-actin	F: ATGTGGCCGAGGACTTTGATT R: AGTGGGGTGGCTTTTAGGATG	107

PS (Product size (bp))

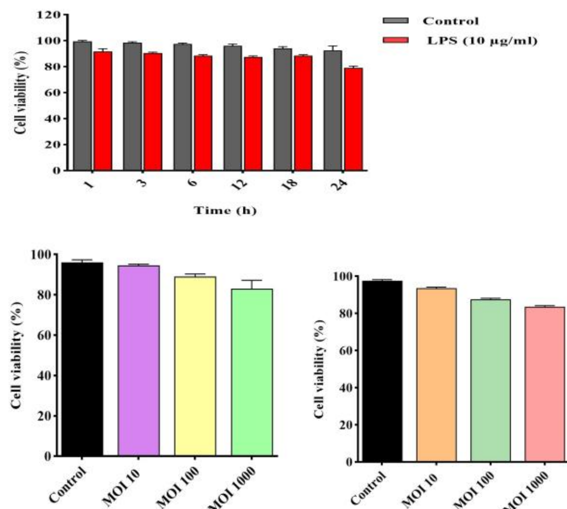

Figure 1. Cell viability of HT-29 cells determined by MTT assay. *(A)* HT-29 cells treated with LPS at concentration of 10 µg/ml for 1, 3, 6, 12, 18, 24 h time points. *(B)* HT-29 cells treated with varying MOIs (10, 100, 1000) of *B. subtilis* natto spores for 24 h. *(C)* HT-29 cells treated with varying MOIs (10, 100, 1000) of *B. coagulans* Hammer spores for 24 h. Data are presented as mean ± SD from three independent experiments.

Hammer on HT-29 cells. As shown in Figure 1, although LPS (at different time points) and spores (at various MOIs) caused a minor reduction in the viabilityof HT-29 cells, no significant cytotoxic effects were detected in comparison to untreated control cells.

Spores isolated from *B. subtilis* natto and *B. coagulans* Hammer decreased the gene expression of *IL-6* and *TLR4*: As shown in Figure 2, LPS significantly induced the expression of *IL-6* in HT-29 cells compared to untreated control ($P < 0.0001$). In contrast, treatment of HT-29 cells with *B. subtilis* natto and *B. coagulans* Hammer ($P < 0.0001$) spores reduced the expression level of the *IL-6* gene in LPS-stimulated HT-29 cells after 24 h. As presented in Figure 3, *B. subtilis* natto and *B. coagulans* Hammer spores similarly caused a significant reduction in the gene expression of *TLR4* in HT-29 cells compared to LPS control ($P < 0.0001$). Also, spores of *B. subtilis* natto demonstrated a greater impact on reducing the expression level of *IL-6* and *TLR4* genes in LPS-stimulated HT-29 cells compared to spores of *B. coagulans* Hammer. This difference was detected to be statistically significant ($P < 0.05$).

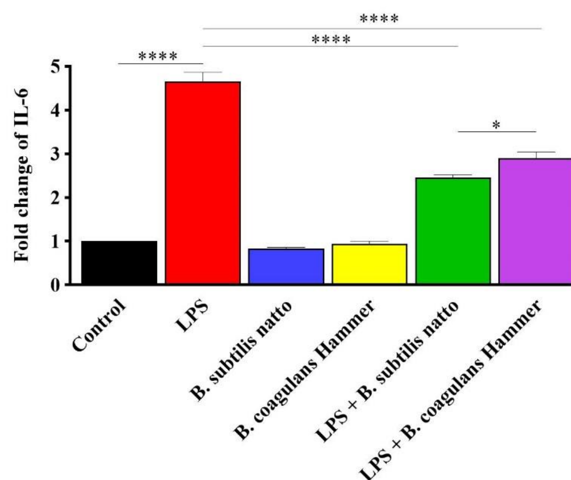

Figure 2. Relative gene expression of *IL-6* by using RT-qPCR assay in HT-29 cells upon treatment with *B. subtilis* natto and *B. coagulans* Hammer spores, and LPS for 24 h. Genes expression data were normalized to *β*-actin as the reference gene. Data are presented as mean ± SD from three independent experiments. A *P* value of <0.05 was considered significant (**P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001) by one-way ANOVA statistical analysis.

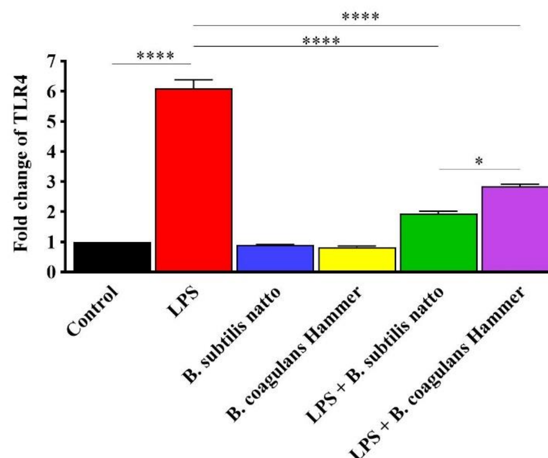

Figure 2. Relative gene expression of *TLR4* by using RT-qPCR assay in HT-29 cells upon treatment with *B. subtilis* natto and *B. coagulans* Hammer spores and LPS for 24 h. Genes expression data were normalized to *β*-actin as the reference gene. Data are presented as mean ± SD from three independent experiments. A *P* value of <0.05 was considered significant (**P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001) by unpaired student's t test and one-way ANOVA statistical analysis.

Discussion

The present study demonstrated the immunomodulatory potential of spores obtained from *B. subtilis* natto and

B. coagulans Hammer against LPS-induced inflammation in HT-29 cells. Recently, bacterial spores as probiotics have shown promising advantages over live bacterial cells[25]. Spores are composed of a robust and protective coat, such as proteins and other molecules that safeguard genetic material and essential cellular components [26]. Furthermore, early colonization by *Bacillus* spores can potentially trigger immunomodulatory responses within the gut[27]. Accordingly, the interaction between certain spore coat ligands and immune receptors, such as TLRs on intestinal epithelial cells, could induce an immunomodulatory effect in the inflamed gastrointestinal tract in response to enteric pathogens or their antigens[28].

A previous study illustrating the interplay between *B. subtilis* spores and TLRs revealed that *B. subtilis* spores elicited nonspecific cellular immune responses, indicative of innate immunity stimulation in mice[29]. The spores obtained from *B. subtilis* can promote the development of the gut-associated lymphoid tissue (GALT) and pre-immune antibody complex after growth and sporulation in the murine gut[30]. Previous research has indicated that orally ingested *B. subtilis* spores are immunogenic and can induce *TLR1* and *TLR2*; however, the correlation of this immune activity with the probiotic function remains incompletely understood [31].

The complex interaction between LPS and *TLR4* within the gastrointestinal milieu participates in orchestrating inflammatory cascades that are crucial for gut homeostasis[32]. Upon LPS recognition by *TLR4*, downstream signaling cascades are activated, culminating in the activation of NF-κB and MAPK pathways, perpetuating an inflammatory condition within the gut microenvironment[33]. Moreover, the crosstalk between LPS and *TLR4* extends beyond conventional signaling pathways, influencing the gut epithelial integrity and microbiota composition, thus shaping a delicate balance between gut inflammation and immune tolerance[34]. Consequently, understanding the nuanced interplay between LPS and *TLR4* holds significant implications for elucidating the pathogenesis of gastrointestinal disorders and underscores potential therapeutic strategies targeting this intricate axis.

The NF-κB signaling pathway operates downstream of the *TLR4*/MYD88-dependent signal transduction cascade. Following external stimulation, activation of the *TLR4*/MYD88 signaling pathway regulates the expression of target genes such as *IL-1β*, *IL-6*, and *TNF-α*[35]. Previous studies have demonstrated that *Bacillus* SC06 markedly downregulates *TNF-α* and *IL-1α* gene expression in the intestinal mucosa while upregulating *IL-6* and *IL-8* transcription, thereby enhancing the integrity of the intestinal mucosa to bolster the barrier function of intestinal epithelial cells and immune response[36]. Our study findings reveal a significant increase in the expression of inflammatory proteins, including *TLR4* and *IL-6*, in response to LPS stimulation. Notably, supplementation with probiotic spores substantially mitigates this response, suggesting a potential inhibition of NF-κB activation and downstream proinflammatory factor expression, thus safeguarding cells from LPS-induced inflammation. It is noteworthy that both spores of *B. subtilis* natto and *B. coagulans* Hammer were capable of reducing the gene expression of *IL-6* and *TLR4* compared to LPS control. In a study using acute lung injury (ALI) murine models, *IL-6*, *IL-1β*, and *TNF-α* exhibited significant elevation in the experimental LPS group compared to the control group, accompanied by a decrease in IL-4 content. Conversely, supplementation with TL3 reversed these trends markedly. Wang, Y. et al. confirmed the ability of *B. coagulans* Hammer to enhance immune function by inhibiting proinflammatory cytokine secretion, increasing anti-inflammatory cytokine secretion, and mitigating oxidative stress through the regulation of the *TLR4*/MyD88/NF-κB and Nrf2 signal pathways, along with modulation of intestinal microflora[36]. The findings of the present study revealed that *B. subtilis* natto spores more significantly reduced the gene expression of *TLR4* in LPS-stimulated HT-29 cells in comparison with spores of *B. coagulans* Hammer.

Conclusion

The present study showed that spores isolated from *B. subtilis* natto and *B. coagulans* Hammer can modulate the gene expression of *IL-6* and *TLR4* in HT-29 cells in vitro. Overall, it can be suggested that these spores might have great potential as probiotics with immunomodulatory effects. However, additional *in vivo* research is warranted to analyze the various

interactions of these beneficial spores with immune cells in both human and animal gastrointestinal tracts. Further elucidation of the underlying mechanisms governing these effects is crucial for understanding the therapeutic potential of spore-based probiotics in modulating host immune response and promoting gut health.

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Conflict of interest

The authors further declare that they have no conflict of interest.

References

1. Coker OO. Non-bacteria microbiome (virus, fungi, and archaea) in gastrointestinal cancer. Journal of Gastroenterology and Hepatology. 2022 Feb;37(2):256-62.
2. Cheng D, Song J, Xie M, Song D. The bidirectional relationship between host physiology and microbiota and health benefits of probiotics: A review. Trends in Food Science & Technology. 2019 Sep 1; 91:426-35.
3. Loh JS, Mak WQ, Tan LK, Ng CX, Chan HH, Yeow SH, Foo JB, Ong YS, How CW, Khaw KY. Microbiota–gut–brain axis and its therapeutic applications in neurodegenerative diseases. Signal Transduction and Targeted Therapy. 2024 Feb 16;9(1):37.
4. Warne RW, Kirschman L, Zeglin L. Manipulation of gut microbiota during critical developmental windows affects host physiological performance and disease susceptibility across ontogeny. Journal of Animal Ecology. 2019 Jun;88(6):845-56.
5. Salarieh N, Emami Meibodi A, Alipour S, Azimirad M, Looha MA, Asadzadeh Aghdaei H, Yadegar A, Shahrokh S, Zali MR. Characterization of the mucosal microbiota in patients with nodular lymphoid hyperplasia with concurrent irritable bowel syndrome compared to healthy controls. Molecular Biology Reports. 2023 Jan;50(1):145-55.
6. Mills S, Stanton C, Lane JA, Smith GJ, Ross RP. Precision nutrition and the microbiome, part I: current state of the science. Nutrients. 2019 Apr 24;11(4):923.
7. Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EM, Sanders ME, Shamir R, Swann JR, Szajewska H, Vinderola G. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nature Reviews Gastroenterology & Hepatology. 2021 Sep;18(9):649-67.
8. Stavropoulou E, Bezirtzoglou E. Probiotics in medicine: a long debate. Frontiers in immunology. 2020 Sep 25; 11:2192.
9. Milner E, Stevens B, An M, Lam V, Ainsworth M, Dihle P, Stearns J, Dombrowski A, Rego D, Segars K. Utilizing probiotics for the prevention and treatment of gastrointestinal diseases. Frontiers in Microbiology. 2021 Aug 9; 12:689958.
10. Liu Y, Wang J, Wu C. Modulation of gut microbiota and immune system by probiotics, pre-biotics, and post-biotics. Frontiers in nutrition. 2022 Jan 3; 8:634897.
11. Salarieh N, Emami Meibodi A, Alipour S, Azimirad M, Looha MA, Asadzadeh Aghdaei H, Yadegar A, Shahrokh S, Zali MR. Characterization of the mucosal microbiota in patients with nodular lymphoid hyperplasia with concurrent irritable bowel syndrome compared to healthy controls. Molecular Biology Reports. 2023 Jan;50(1):145-55.
12. Ghavami SB, Yadegar A, Aghdaei HA, Sorrentino D, Farmani M, Mir AS, Azimirad M, Balaii H, Shahrokh S, Zali MR. Immunomodulation and generation of tolerogenic dendritic cells by probiotic bacteria in patients with inflammatory bowel disease. International journal of molecular sciences. 2020 Aug 29;21(17):6266.
13. Heil E. *Probiotic Use for the Primary Prevention of Clostridioides difficile infection* (Doctoral dissertation).
14. Luongo D, Treppiccione L, Sorrentino A, Ferrocino I, Turroni S, Gatti M, Di Cagno R, Sanz Y, Rossi M. Immune-modulating effects in mouse dendritic cells of lactobacilli and bifidobacteria isolated from individuals following omnivorous, vegetarian and vegan diets. Cytokine. 2017 Sep 1; 97:141-8.
15. Li X, Hu D, Tian Y, Song Y, Hou Y, Sun L, Zhang Y, Man C, Zhang W, Jiang Y. Protective effects of a novel Lactobacillus rhamnosus strain with probiotic characteristics against lipopolysaccharide-induced intestinal inflammation in vitro and in vivo. Food & function. 2020;11(7):5799-814.
16. Candelli M, Franza L, Pignataro G, Ojetti V, Covino M, Piccioni A, Gasbarrini A, Franceschi F. Interaction between lipopolysaccharide and gut microbiota in inflammatory bowel diseases. International journal of molecular sciences. 2021 Jun 10;22(12):6242.
17. Nabavi-Rad A, Sadeghi A, Asadzadeh Aghdaei H, Yadegar A, Smith SM, Zali MR. The double-edged sword of probiotic supplementation on gut microbiota structure in Helicobacter pylori management. Gut Microbes. 2022 Dec 31;14(1):2108655.
18. Fakharian F, Thirugnanam S, Welsh DA, Kim WK, Rappaport J, Bittinger K, Rout N. The role of gut dysbiosis in the loss of intestinal immune cell functions and viral pathogenesis. Microorganisms. 2023 Jul 21;11(7):1849.
19. Yoo JY, Groer M, Dutra SV, Sarkar A, McSkimming DI.

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20. Mazkour S, Shekarforoush SS, Basiri S. The effects of supplementation of Bacillus subtilis and Bacillus coagulans spores on the intestinal microflora and growth performance in rat. Iranian journal of microbiology. 2019 Jun;11(3):260.
21. Adibpour N, Hosseininezhad M, Pahlevanlo A, Hussain MA. A review on Bacillus coagulans as a Spore-Forming Probiotic. Applied Food Biotechnology. 2019 Jan 1;6(2):91-100.
22. Azimirad M, Alebouyeh M, Naji T. Inhibition of lipopolysaccharide-induced interleukin 8 in human adenocarcinoma cell line HT-29 by spore probiotics: B. coagulans and B. subtilis (natto). Probiotics and antimicrobial proteins. 2017 Mar; 9:56-63.
23. Noori M, Azimirad M, Eslami G, Looha MA, Yadegar A, Ghalavand Z, Zali MR. Surface layer protein A from hypervirulent Clostridioides difficile ribotypes induce significant changes in the gene expression of tight junctions and inflammatory response in human intestinal epithelial cells. BMC microbiology. 2022 Oct 27;22(1):259.
24. Lee JY, Lee JD, Phipps S, Noakes PG, Woodruff TM. Absence of toll-like receptor 4 (TLR4) extends survival in the hSOD1 G93A mouse model of amyotrophic lateral sclerosis. Journal of neuroinflammation. 2015 Dec; 12:1-9.
25. Shinde T, Vemuri R, Shastri MD, Perera AP, Tristram S, Stanley R, Eri R. Probiotic Bacillus coagulans MTCC 5856 spores exhibit excellent in-vitro functional efficacy in simulated gastric survival, mucosal adhesion and immunomodulation. Journal of Functional Foods. 2019 Jan 1; 52:100-8.
26. Clair G, Esbelin J, Mallea S, Bornard I, Carlin F. The spore coat is essential for Bacillus subtilis spore resistance to pulsed light, and pulsed light treatment eliminates some spore coat proteins. International journal of food microbiology. 2020 Jun 16; 323:108592.
27. Freedman KE, Hill JL, Wei Y, Vazquez AR, Grubb DS, Trotter RE, Wrigley SD, Johnson SA, Foster MT, Weir TL. Examining the gastrointestinal and immunomodulatory effects of the novel probiotic Bacillus subtilis DE111. International journal of molecular sciences. 2021 Feb 28;22(5):2453.
28. Tobita K, Meguro R. Bacillus subtilis BN strain promotes Th1 response via Toll-like receptor 2 in polarized mouse M1 macrophage. Journal of Food Biochemistry. 2022 Feb;46(2): e14046.
29. Permpoonpattana P, Hong HA, Khaneja R, Cutting SM. Evaluation of Bacillus subtilis strains as probiotics and their potential as a food ingredient. Beneficial microbes. 2012 Mar 22;3(2):127-36.
30. Tavares Batista M, Souza RD, Paccez JD, Luiz WB, Ferreira EL, Cavalcante RC, Ferreira RC, Ferreira LC. Gut adhesive Bacillus subtilis spores as a platform for mucosal delivery of antigens. Infection and immunity. 2014 Apr;82(4):1414-23.
31. Hong HA, Duc LH, Cutting SM. The use of bacterial spore formers as probiotics. FEMS microbiology reviews. 2005 Sep 1;29(4):813-35.
32. Wang K, Yang X, Wu Z, Wang H, Li Q, Mei H, You R, Zhang Y. Dendrobium officinale polysaccharide protected CCl4-induced liver fibrosis through intestinal homeostasis and the LPS-TLR4-NF-κB signaling pathway. Frontiers in Pharmacology. 2020 Mar 12; 11:240.
33. Fan J, Liu S, Ai Z, Chen Y, Wang Y, Li Y, Li X, Xiao S, Wang Y. Fermented ginseng attenuates lipopolysaccharide-induced inflammatory responses by activating the TLR4/MAPK signaling pathway and remediating gut barrier. Food & Function. 2021;12(2):852-61.
34. Yu P, Ke C, Guo J, Zhang X, Li B. Lactobacillus plantarum L15 alleviates colitis by inhibiting LPS-mediated NF-κB activation and ameliorates DSS-induced gut microbiota dysbiosis. Frontiers in Immunology. 2020 Oct 2; 11:575173.
35. Li T, Li F, Liu X, Liu J, Li D. Synergistic anti-inflammatory effects of quercetin and catechin via inhibiting activation of TLR4–MyD88-mediated NF-κB and MAPK signaling pathways. Phytotherapy Research. 2019 Mar;33(3):756-67.
36. Wang Y, Lin J, Cheng Z, Wang T, Chen J, Long M. Bacillus coagulans TL3 Inhibits LPS-Induced Caecum Damage in Rat by Regulating the TLR4/MyD88/NF-κB and Nrf2 Signal Pathways and Modulating Intestinal Microflora. Oxidative Medicine and Cellular Longevity. 2022;2022(1):5463290.