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Role of Native Probiotic *Lactobacillus* Species via TGF-β Signaling Pathway Modulation in CRC

Amin Sepehr[1], Shadi Aghamohammad[1], Roya Ghanavati[2], Malihe Talebi[3], Mohammad Reza Pourshafie[1*], Mahdi Rohani[1*]

[1]Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran; [2]Behbahan Faculty of Medical Sciences, Behbahan, Iran; [3]Department of Microbiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background: Colon microbiome composition in CRC patients undergoes remarkable changes. The present study was designed to assess the impact of *Lactobacillus* mixture on the regulating the CRC by influencing the TGF-β signaling pathway in both in vitro (HT-29 cancer cells) and in vivo (BALB/c mice) models.

Methods: In this study, the antiproliferative effect of a native potential probiotic *Lactobacillus* mixture on HT-29 cancer cells was evaluated using the MTT assay method. Also, qRT-PCR was performed to assess the RNA expression level of genes associated with the TGF-β signaling pathway at three levels: receptor, regulatory, and inhibitory SMADs. Finally, the in vivo assays were investigated by three groups of mice: a naive group (PBS), a disease group (AOM/DSS + PBS), and a treatment group (AOM/DSS + *Lactobacillus* mixture in PBS).

Results: The MTT results showed a significant decrease in proliferation of HT-29 cancer cells after 120 h of treatment. Furthermore, qRT-PCR demonstrated the downregulation of the *smad2/3* gene expression in HT-29-treated cells and also reduction in the level of *smad4* gene expression. In addition, in the mouse model, the *tgf-βR1* gene was downregulated in the group treated with AOM/DSS/*Lactobacillus*, but not the AOM/DSS group. A downregulation of *smad4* gene expression was also observed in *in vivo* models.

Conclusion: The obtained results suggest that our novel probiotic *Lactobacillus* mixture could have a positive impact on the inhibition of the CRC progression by downregulating the TGF-β signaling pathway.
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Keywords: Colorectal cancer, Lactobacillus, Probiotics, TGF-β

Corresponding Authors:
Mohammad Reza Pourshafie
Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran; Tel.: (+98-21) 66405535; E-mail: m_pour@pasteur.ac.ir, pour62@yahoo.com

Mahdi Rohani
Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran; Tel.: (+98-21) 64112230; E-mail: kia.rohani@gmail.com

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List of Abbreviations:
AOM: azoxymethane; **CRC:** colorectal cancer; **DMEM:** Dulbecco's Modified Eagle Medium; **DSS:** dextran sulfate sodium; **H&E:** Hematoxylin and Eosin; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **PBS:** phosphate-Buffered Saline; **qRT-PCR:** quantitative reverse transcription polymerase chain reaction; **SMAD:** small mothers against decapentaplegic; **TGF-β:** transforming growth factor beta

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INTRODUCTION

Colorectal cancer is one of the most prevalent types of neoplasia in people. Despite remarkable progress in diagnosing and treating this illness, CRC continues to be a major cause of death in women and men. Regrettably, the worldwide burden of CRC is predicted to surge by 60% until the year 2030[1,2]. According to the Global Cancer Observatory, the number of new CRC cases was estimated to be more than 1.9 million in 2020, and it is predicted to exceed over 3.1 million in 2040[3].

Carcinogenesis is mainly attributed to the disruption of different signaling pathways. Among various pathways, the TGF-β pathway is involved in cell-to-cell communication and has been conserved throughout human evolution. It has been established that the proper function of this pathway is essential for the development and differentiation of cells[4]. The abnormal TGF-β signaling pathway and mutations in the genes associated with this pathway, affect cell tumorigenicity in various cancers. Of note, disruption of the TGF-β superfamily signaling pathway has frequently been identified in CRC[5]. Earlier studies have indicated that mutations in components of the TGF-β signaling pathway are present in 27% and 87% of non-hypermutated and hypermutated neoplasia. Additionally, activating this pathway in CRC mainly increases invasion and metastasis. Furthermore, function of TGF-β pathway in the tumor microenvironment often hampers tumor immunity and facilitates the survival of cancer cells[6].

The TGF-β gene family comprises two receptors, *tgf-βR1* and *tgf-βR2*, that perceive the TGF-β signal input[7]. Other components of this pathway include SMAD proteins, which trigger a SMAD signaling cascade associated with cell proliferation[8]. Indeed, TGF-βR-mediated phosphorylation of SMAD2 and SMAD3 induces complex formation with the SMAD4, which is then translocated to the cell nucleus. The SMAD4 complex, a downstream mediator of this pathway, binds to specific regulatory DNA sequence elements and is involved in controlling target gene transcription[9]. Conversely, inhibitory SMADs, specifically SMAD6 and SMAD7, play a negative regulatory role in TGF-β signaling. SMAD7 binds to the activated TGF- βRI and prevents the activation of the SMAD cascade, while SMAD6 can directly bind to receptors and prevent the formation of an R-CO-SMAD heterodimer complex[10].

The status of the colon microbiome is intricately connected to the advancement and growth of gastrointestinal cancer, which includes CRC[11]. The gut microbiota may influence colorectal carcinogenesis through various mechanisms, including microbial factors such as opportunistic metabolites, regulation of the inflammatory process, and immune response in the tumor-associated microenvironment[12]. Studies have reported that the gut microbiota in CRC patients, compared to healthy individuals, is different in terms of bacterial number and species[13,14]. The most common type of gut microbiota with health-promoting properties is lactic acid bacteria, which have a beneficial effect when consumed in sufficient quantities[15]. As a specific example, the amount of *L. plantarum* and *L. acidophilus* significantly increases in healthy individuals compared to patients suffering from CRC[16].

In our previous investigation, we isolated two strains of *L. plantarum* and one strain of *L. brevis* and *L. rhamnosus* from the feces of healthy Iranian volunteers[17]. A combination of these strains has been shown to have anti-inflammatory effects by modulating the Wnt and Notch signaling pathways[18,19]. Based on this background, this study evaluated the impact of our native potential probiotic mixture on CRC progression by modulating the TGF-β signaling pathway to understand how it affects other signaling pathways.

MATERIALS AND METHODS

Cocktail preparation

In the previous study conducted by our team, *L. rhamnosus* and *L. brevis*, as well as two strains of *L. plantarum*, were collected from the stool samples of healthy volunteers with average ages between 20 and 36 years[17]. In the next step, bacterial isolates were grown in a De Man, Rogosa, and Sharpe medium (Sigma Aldrich, UK) and incubated at 37 °C overnight. The bacteria were collected through the process of centrifugation (room temperature, 4000 ×g, 10 min) and adjusted to a suitable concentration (0.5 Mc Farland) by using RPMI-1640 medium[18]. The strains were combined in a tube to create a mixture of *Lactobacillus*.

Cell culture

The anticancer properties of the probiotic mixture were investigated using the HT-29 cell line, which was acquired from the Pasteur Institute of Iran, Tehran. The HT-29 cells were cultivated in a controlled environment (5% CO_2 and 37 °C) using a Dulbecco's Modified Eagle Medium medium enriched with a high glucose concentration.

MTT assay

The antiproliferative effect of the probiotic mixture on the HT-29 cell growth was assessed by the MTT assay kit (BIO-IDEA, Iran). The HT-29 cells were seeded at a cell density of 5×10^3 cells/well and then treated with live *Lactobacillus* mixture at a multiplicity of infection of 100 in an incubator containing a CO_2 concentration of 5% at 37°C for 24, 72, and 120 h. This study used untreated HT-29 cells at corresponding time points as

negative controls. The cells underwent two rounds of washing with PBS (pH 7.4). Subsequently, the culture medium was replaced by a fresh medium every 6 h for 72 h. The number of viable cells in each well was detected by the Trypan blue before incubating cells with MTT solution at a defined concentration (0.5 mg/ml) at 37 °C for about 4 h. Then blue formazan crystals were formed and dissolved in DMSO (50 µl) for 20 minutes. The microplate reader (Bio-Rad, USA) was utilized to determine the cell viability at 570 nm. The analysis for each group was replicated three times. To analyze the viability, we used the following formulation in which the OD sample, OD medium, and OD control indicate the absorbance of the treated cells, background, and control cells, respectively.

$$\text{Proliferation of cells} = \frac{(OD\ sample - OD\ medium)}{(OD\ control - OD\ medium)} \times 100$$

Animal treatment with *Lactobacillus* mixture

This study used 15 female BALB/c mice (six to eight weeks) for all investigations. The mice were housed in polycarbonate cages (each contained five mice) in a suitable room with controlled conditions and kept in quarantine for one week under a 12/12 h light-dark cycle. The animals were categorized into three different groups: (1) naive (ordinary dietary foods + PBS administration), (2) disease control (PBS gavage + AOM/ DSS-induced mice, and (3) treatment (AOM/DSS-induced mice + *Lactobacillus* mixture in PBS). The naive and disease control groups were administered by 0.2 mL of PBS daily, whereas the treatment group received 0.2 mL of *Lactobacillus* mixture in PBS, orally. Orogastric administration of PBS or *Lactobacillus* mixture began seven days before tumor induction and carried on daily. For induction of CRC, disease control and treatment groups were injected with a single dose of AOM (10 mg/kg; Sigma-Aldrich) as a carcinogenic agent intraperitoneally. After seven days, water containing 2% DSS was administered to the disease control and treatment groups for five days. Following a 14-day recovery period in which the DSS water was replaced with DSS-free water. Mice were administered two more rounds of water containing 2% DSS[20] and were sacrificed 64 days after AOM injection by rapid cervical dislocation. Subsequently, the distal parts of the colon were removed and cleaned with ice PBS. The segments of the colon were frozen for future examinations.

Tumor assessment and histopathological examinations

Colon samples were washed with PBS. Then distal colon segments were stained with H&E based on the defined protocol[20]. An experienced pathologist classified all tumors based on the indexes used for classifying mouse colon tumors. The stage of cancers was detected through an Olympus-BX51 microscope at a magnification of ×100. The tumor stage was determined based on the examination of several factors, including the tumor primary location, tumor size, involvement of regional lymph nodes, and the presence of multiple tumors.

Measurement of the expression of TGF-β signaling pathway genes

The qRT-PCR was employed to measure the gene expression related to the TGF-β signaling pathway. For this purpose, the total RNA from each group was extracted using a total RNA extraction kit, following the manufacturer's instructions. The RNA quality and quantity were evaluated by assessing the absorbance at 260/280 nm. By following the protocols, we generated complementary DNA templates utilizing the PrimeScript cDNA synthesis kit (Takara Bio, Japan). Then in various mouse groups gene expression were quantified by employing SYBR Premix Ex Taq (Takara Bio).

Primers used in this study were obtained from the online Primer Bank website (http://pga.mgh.harvard.edu/ primerbank) (Table 1). Each experiment was conducted using a StepOnePlus™ Real-Time PCR system. The *GAPDH* gene was used as a normalizer.

Statistical analysis

For comparison among multiple groups, the One-way ANOVA method was conducted through GraphPad Prism version 8. The student's t-test was employed to compare the two groups. Statistical significance was determined for *p* values less than 0.05.

RESULTS

***Lactobacillus* species inhibited the proliferation of cancer cells**

The inhibitory effects of the *Lactobacillus* mixture were examined in vitro on HT-29 cancer cells using the MTT assay. The obtained results demonstrated that the *Lactobacillus* species significantly inhibited the multiplication of CRC cells by 72% when treated for 24 to 120 h (Fig. 1). Based on the MTT obtained results, an incubation time of 24 to 120 h was selected as the most appropriate time for the treatment of HT-29 cells with a *Lactobacillus* mixture (10^8 CFU/mL) for further study.

***Lactobacillus* mixture reduced the carcinogenesis rate in mice**

Animal experiment results revealed that administering our native potential probiotic mixture of *Lactobacillus*

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Table 1. The sequences, length, primer ID, and annealing temperature of primers used in this study

Gene	Sequences	Primer bank ID	Amplicon size (bp)	Tm (°C)
tgf-βR1-human	F: GCTGTATTGCAGACTTAGGACTG R: TTTTTGTTCCCACTCTGTGGTT	195963411c2	90	60.7 60.2
tgf-βR1-mice	F: ATATCTGCCATAACCGCACTG R: CTGAAATGAAAGGGCGATCTAGT	40254607c2	81	60.5 60.1
tgf-βR2-human	F: AAGATGACCGCTCTGACATCA R: CTTATAGACCTCAGCAAAGCGAC	133908633c2	119	60.9 60.7
tgf-βR2-mice	F: AACATGGAAGAGTGCAACGAT R: CGTCACTTGGATAATGACCAACA	239787880c2	90	60 60.5
smad2-human	F: CCGACACACCGAGATCCTAAC R: GAGGTGGCGTTTCTGGAATATAA	118572580c2	125	61.9 60.1
smad2-mice	F:AAGCCATCACCACTCAGAATTG R: CACTGATCTACCGTATTTGCTGT	357197176c1	100	60.6 60.1
smad3-human	F: TGGACGCAGGTTCTCCAAAC R: CCGGCTCGCAGTAGGTAAC	223029439c1	90	62.4 62.1
smad3-mice	F: AGGGGCTCCCTCACGTTATC R: CATGGCCCGTAATTCATGGTG	254675248c1	77	62.9 61.4
smad4-human	F: CCACCAAGTAATCGTGCATC R: TGGTAGCATTAGACTCAGATGGG	195963400c3	76	61.0 60.9
smad4-mice	F: ACACCAACAAGTAACGATGCC R: GCAAAGGTTTCACTTTCCCCA	28201436a1	83	60.8 61.0
smad6-human	F: CCTCCCTACTCTCGGCTGTC R: GGTAGCCTCCGTTTCAGTGTA	236465444c1	90	63.0 61.2
smad6-mice	F: GCAACCCCTACCACTTCAGC R: GTGGCTTGTACTGGTCAGGAG	12836011a1	90	62.8 62.1
smad7-human	F: TTCCTCCGCTGAAACAGGG R:CCTCCCAGTATGCCACCAC	299890804c1	116	61.6 61.8
smad7-mice	F: GGGCTTTCAGATTCCCAACTT R: AGGGCTCTTGGACACAGTAGA	111154104c2	115	60.2 62.0
gapdh-human	F: CTGGGCTACACTGAGCACC R: AAGTGGTCGTTGAGGGCAATG	378404907c3	101	62.0 62.9
gapdh-mice	F: TGACCTCAACTACATGGTCTACA R: CTTCCCATTCTCGGCCTTG	126012538c2	85	60.2 60.2

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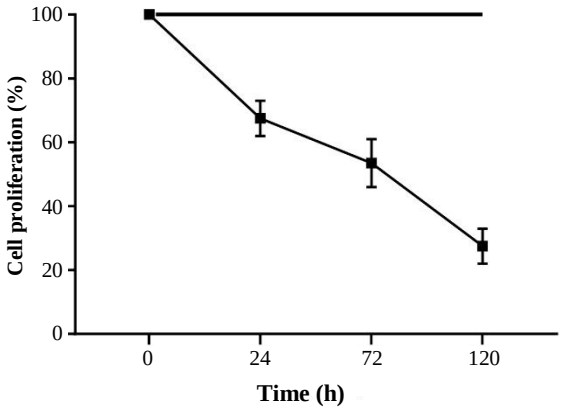

Fig. 1. Effects of *Lactobacillus* mixture at a multiplicity of infections of 100 on the viability of HT-29 cell line after 24, 72, and 120 h. Data were represented as mean ± SD ($p < 0.05$).

to mice, reduced colon tumor numbers. In the treated mice, tumor formation was more observed in the distal region of the colon compared to the other areas, and the colon length decreased in this group compared to the PBS group (7.5 in PBS group and 6.5 in treated group). However, the AOM/DSS-consuming mice indicated greater tumor nodules in comparison to the AOM/DSS/*Lactobacillus* mixture group (Fig. 2A). The decrease in cancer development was obtained in the AOM/DSS/*Lactobacillus*-induced group relative to the cancer mice (Fig. 2C). Indeed, AOM/DSS is a colitis-associated CRC model. The H&E examination revealed that inflammation in AOM/DSS/*Lactobacillus*-induced mice was less pronounced compared to the DSS group (Fig. 2B). Furthermore, different factors, including crypt abscess, muscle thickening, degree of inflammation, and crypt architecture, were used to analyze CRC status. Only two mice from the group treated with our native potential probiotic *Lactobacillus* mixture were found to be in the adenocarcinoma stage (Fig. 2C). Overall, the treatment with our native potential probiotic *Lactobacillus* mixture has been shown to effectively reduce the rate of carcinogenesis in mice using the AOM/DSS/*Lactobacillus* model of CRC.

Lactobacillus mixture modulates the expression of TGF-β signaling pathway genes

The qRT-PCR was performed to evaluate the expression level of genes involved in the TGF-β signaling pathway receptors, regulatory SMADs, and inhibitory SMADs. The results demonstrated that the *Lactobacillus* mixture remarkably downregulated the *tgf-βR(1/2)* expression in HT-29 cancer cells compared to the control cells during 24 to 120 h incubation in the in vitro model. Data from the in vivo model confirmed the in vitro results. Indeed, the expression of *tgf-βR1* was notably suppressed in the AOM/DSS/*Lactobacillus* mice compared to the AOM/DSS mice; however, it was not statistically significant for *tgf-βR2* (Fig. 3). On the other hand, treatment with a *Lactobacillus* mixture downregulated TGF-β regulatory SMADs at the mRNA level compared to the untreated controls. Indeed, the expression of *smad2/3* genes was effectively suppressed after treatment with the *Lactobacillus* mixture, resulting in the downregulation of the *smad4* gene ($p < 0.05$). Consistent with our in vitro results, the expression of the *smad4* gene effectively decreased in the AOM/DSS/*Lactobacillus*-treated mice compared to the AOM/DSS mice in the in vivo model. The results are depicted in Figure 4.

Inhibitory effects of *Lactobacillus* mixture on SMAD gene expression

Furthermore, the results demonstrated a reduction of the smad7 mRNA level in the AOM/DSS/Lactobacillus-induced group compared to the AOM/DSS-induced mice. However, the change in the level of this gene was not statistically significant in HT-29 cancer cells. Treatment with the *Lactobacillus* mixture increased the *smad6* gene expression in HT-29 cells in both models (Fig. 5).

DISCUSSION

CRC is a fatal malignancy with high clinical importance and ranks among the primary factors contributing to mortality. The stability and safety of chemoradiotherapy and synthetic drugs used to treat cancer are in doubt. Indeed, these therapies impair the quality of life or contribute to the development of drug resistance, which are not affordable for many patients[21]. Researchers are trying to find new strategies with high safety and efficiency for preventing and treating CRC. In this regard, manipulation of the gut microbiota by probiotics to improve the safety of cancer treatment and reduce its side-effect profile has been explored in a few studies[22]. Hence, it is crucial to identify promising probiotics, determine optimal administration dosages, and understand the underlying molecular pathways of their effectiveness.

The underlying mechanisms for the anticancer effects of probiotics are multifaceted, including altering the tumor microenvironment through the production of metabolites and factors, suppressing the growth of gut microbiota involved in the production of mutagens, and protecting DNA from oxide damage as well as regulating the immune system[23]. Besides, the quorum-sensing system facilitates the attachment and accumulation of bacteria to membrane receptors. Subsequently, it transmits messages to cancer cells,

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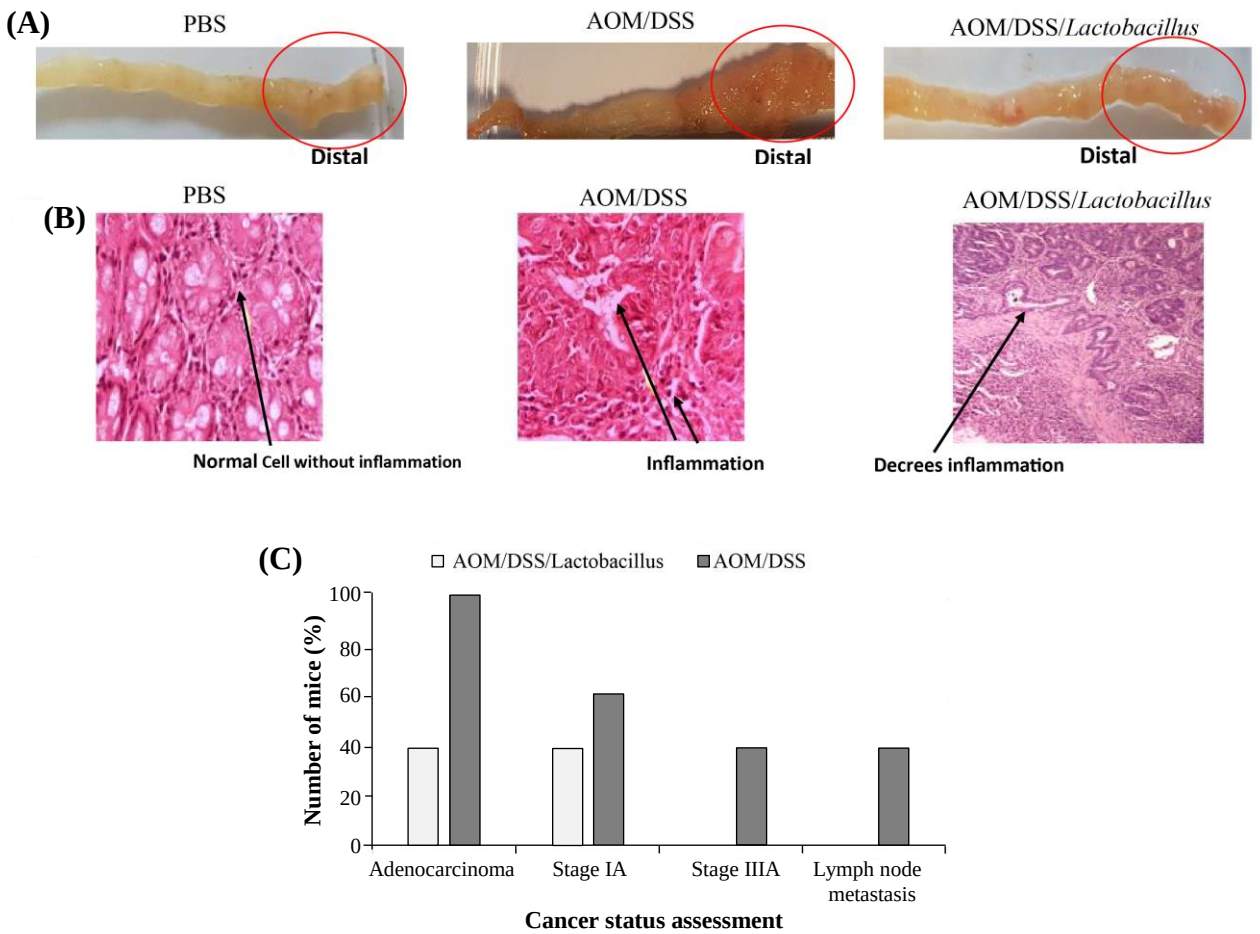

Fig. 2. Histopathological evaluation of colic tumors in mice. (A) Macroscopic appearance of colic tumors; a large number of tumor nodules were observed in the AOM/DSS-consuming group compared to the AOM/DSS/*Lactobacillus* mixture group in the distal. (B) Representative H&E-stained images of distal colon tissues in the PBS-treated mice, AOM/DSS/*Lactobacillus*-treated mice, and AOM/DSS-treated mice; inflammation in AOM/DSS/L.M mice were fewer compared to the AOM/DSS group (scale bars: 100 µm). (C) Analysis of cancer status in mouse colon tissue. To analyze CRC status, five mice from each treated group were examined for different factors, including crypt abscess, muscle thickening, degree of inflammation, and crypt architecture. PBS: PBS-treated mice as negative control; AOM/DSS/*Lactobacillus*-treated mice: AOM/ DSS/*Lactobacillus*-treated mouse; AOM/DSS: AOM/ DSS-treated mouse (n = 5 mice) in an in vivo experiment.

modifying vital signaling pathways that regulate the cell growth and programmed cell death. These pathways include Wnt, Notch, and TGF-β[24]. These signaling pathways in the colonic crypt starts with elevated levels of Notch and Wnt expression during the initial stages of proliferation, and followed by progressing towards the differentiation with increased TGF-β signaling.[25]. In our previous studies, the *Lactobacillus* mixture demonstrated the antiproliferative and inhibitory effects in CRC through the modulation of Notch and Wnt signaling pathways[18,19]. While the inhibitory role of the *Lactobacillus* mixture used in our study was confirmed in the earliest stages of the cell proliferation , little was known about its effect on the differentiated stage. In this study, the anticancer activity of our native potential probiotic *Lactobacillus* mixture was evaluated by modulating the TGF-β signaling pathway and its regulatory genes involved in antiproliferation in both in vitro and in vivo models.

The results from the MTT assay revealed that the *Lactobacillus* mixture caused notable growth inhibition in HT-29 cancer cells after 120 h of incubation. The antiproliferative effect of this *Lactobacillus* mixture was approved in our previous study by inducing apoptosis (early and late) and necrosis after 120 h incubation using the flow cytometry method[19]. Other studies have also shown that consuming *Lactobacillus* strains can hamper proliferation by activating pro-apoptotic pathways in CRC cells[26,27]. The present investigation demonstrated that live potential *Lactobacillus* produces antiapoptotic molecules in tumors that could disrupt mitochondrial membrane integrity by releasing antimicrobial peptides

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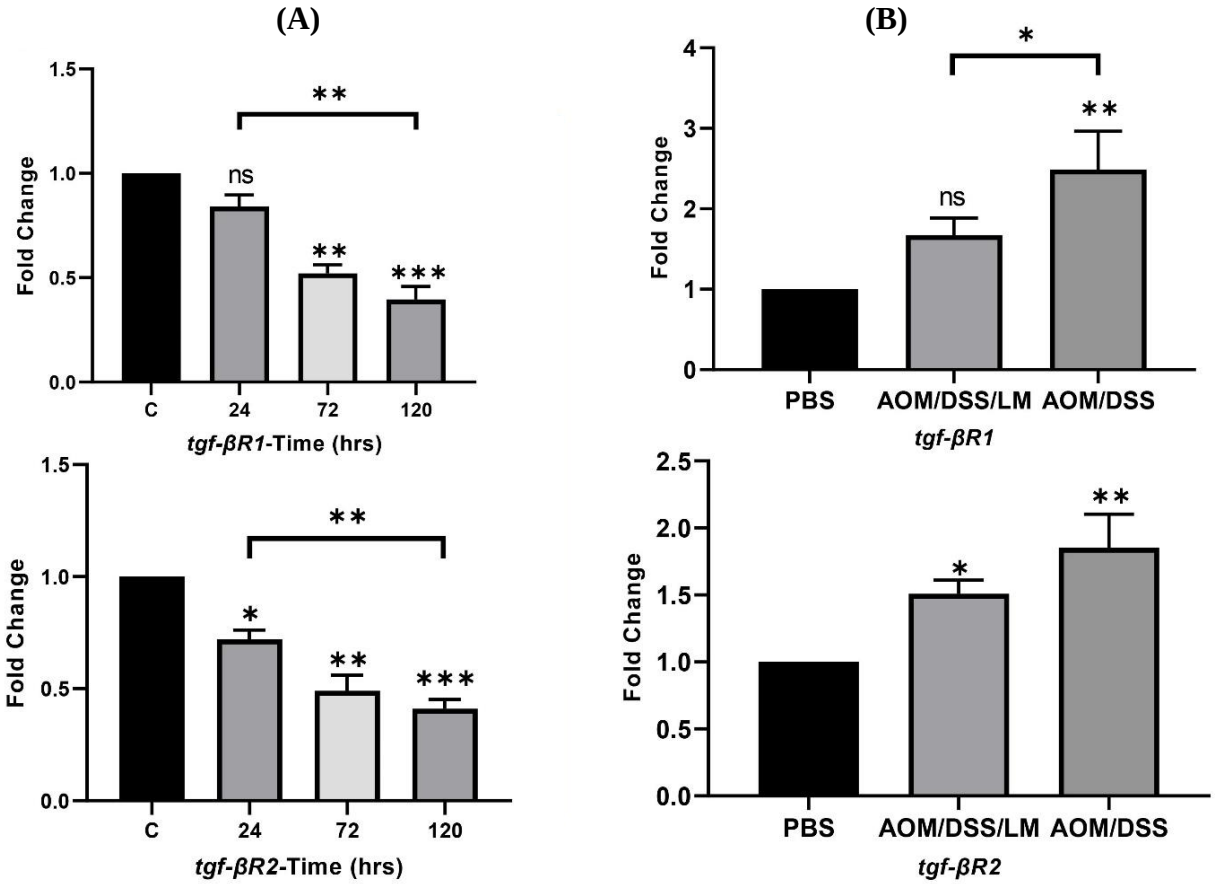

Fig. 3. Relative fold change of *tgf-βR1* and *tgf-βR2* genes in the presence of *Lactobacillus* mixture in (A) HT-29 cancer cells and (B) murine model that were compared with the control group. Control (c; untreated HT-29 cells in corresponding time point); PBS: PBS-treated mice as control; AOM/DSS/*Lactobacillus*: AOM/DDS/*Lactobacillus*-treated mouse; AOM/DSS: AOM/DDS-treated mouse. Data were normalized with the *GAPDH* gene. Results were expressed as mean, and error bars (SD). Statistical analysis was performed using a one-way ANOVA test compared with control+ ($^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$). ns: not significant.

and metabolites. Furthermore, animal studies have shown that the intake of *Lactobacillus* strains leads to decreased number and size of colon tumors in mouse models[28].

The results of real-time PCR revealed that remarkably treatment with the *Lactobacillus* mixture downregulatesthe expression of *tgf-βR1/2* compared to the untreated HT-29 cells. Moreover, the *Lactobacillus* mixture significantly suppressed the overexpression of *tgf-βR1* in *Lactobacillus*-treated mice compared to the disease control; however, this result was not notable for *tgf-βR2*. Indeed, the *Lactobacillus* mixture affected cell proliferation by suppressing *tgf-βR1* in vivo and in vitro. Some studies have shown that *tgf-βR1* overexpression is significantly associated with the risk of developing CRC in humans and may have a major role in tumor metastasis[29,30]. There is also a widespread belief that *tgf-βR1* significantly facilitates ECM remodeling, potentially influencing the interaction between tumor cells and the differentiation of matrix/epithelial cells[31]. In addition, the polymorphic variant in the promoter region of *tgf-βR2* has previously been reported in patients with various types of cancer[32].

In the present study, *smad6* as inhibitory SMADs increased after treatment with the *Lactobacillus* mixture in HT-29 cancer cells. Additionally, *smad6* mRNA level was elevated in *Lactobacillus*-treated mice compared to the disease control group. Indeed, *smad6*, as the primary inhibitory SMAD, competes with R-SMADs for binding to *tgf-βR* and preventing TGF-β signaling[33]. The *smad7*, which has an inhibitory effect on the interaction between R-SMAD and *tgf-βR1*, is another I-SMAD evaluated in this investigation. The qRT-PCR demonstrated that *smad7* mRNA expression decreased in the disease group compared to the naïve in the in vivo model. In addition, the *Lactobacillus* mixture increased *smad7* mRNA level in the treated mice. However, regarding *smad7*, the results were found to be insignificant. Indeed, the dissimilarities between both models regarding *smad7* expression might be attributed

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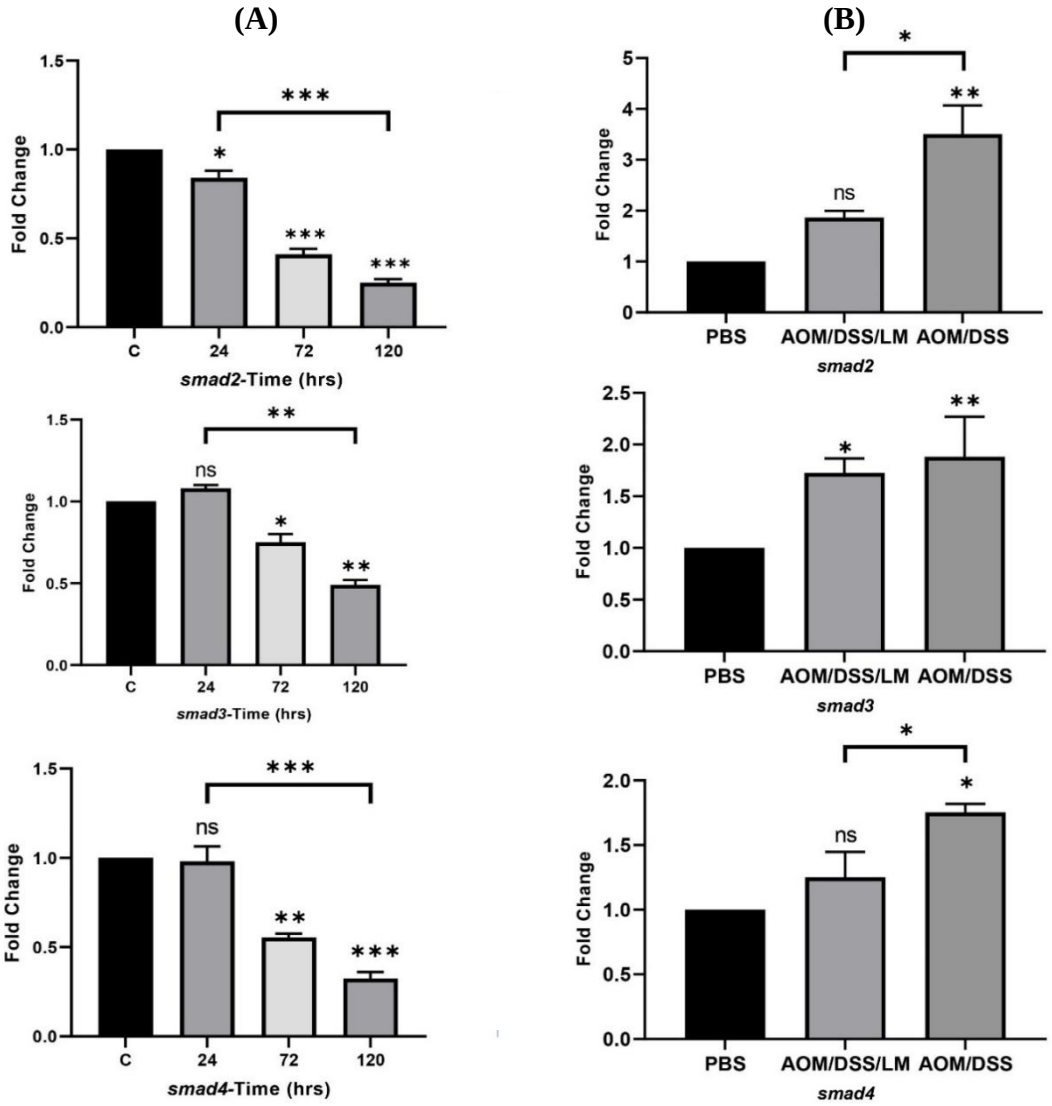

Fig. 4. Relative fold change of *smad2*, *smad3*, and *smad4* genes in the presence of *Lactobacillus* mixture in (A) in HT29 cancer cells and (B) murine model that were compared with the control group. Control (c; untreated HT-26 cells); PBS: PBS-treated mice as control; AOM/DSS/*Lactobacillus*: AOM/DDS/*Lactobacillus*-treated mouse; AOM/DSS: AOM/SSD-treated mouse. Data were normalized with the *GAPDH* gene. Results were expressed as mean, and error bars (SD). Statistical analysis was performed using a one-way ANOVA test ($^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$). ns: not significant.

to the impact on the physiological system, the involvement of AOM or DSS interventions, and the cross-regulation with an alternative pathway such as β-catenin. In the present study, the real-time PCR results showed that R-SMADs (*smad2/3*) was significantly overexpressed, which led to the overexpression of the *smad4* in the treated group in in vivo and in vitro models. The study performed by Yu and Feng showed that *smad4* overexpression in CRC results in blocking tumor-suppressive responses[34]. Consistent with our results, the study by Jia *et al.* revealed that the upregulation of *smad4* was remarkable in 209 sporadic CRC patients[35]. Actually, this pathway serves as a promoter of cell proliferation in the later phases of tumor progression and metastasis[34].

CONCLUSION

Substantial evidence supports the positive impact of probiotics in reducing the advancement of cancer, including CRC, through various signaling pathways. Dysregulation of signaling cascades results in the acquisition of a malignant phenotype. However, their relative importance in CRC is not completely understood. This investigation showed that our native

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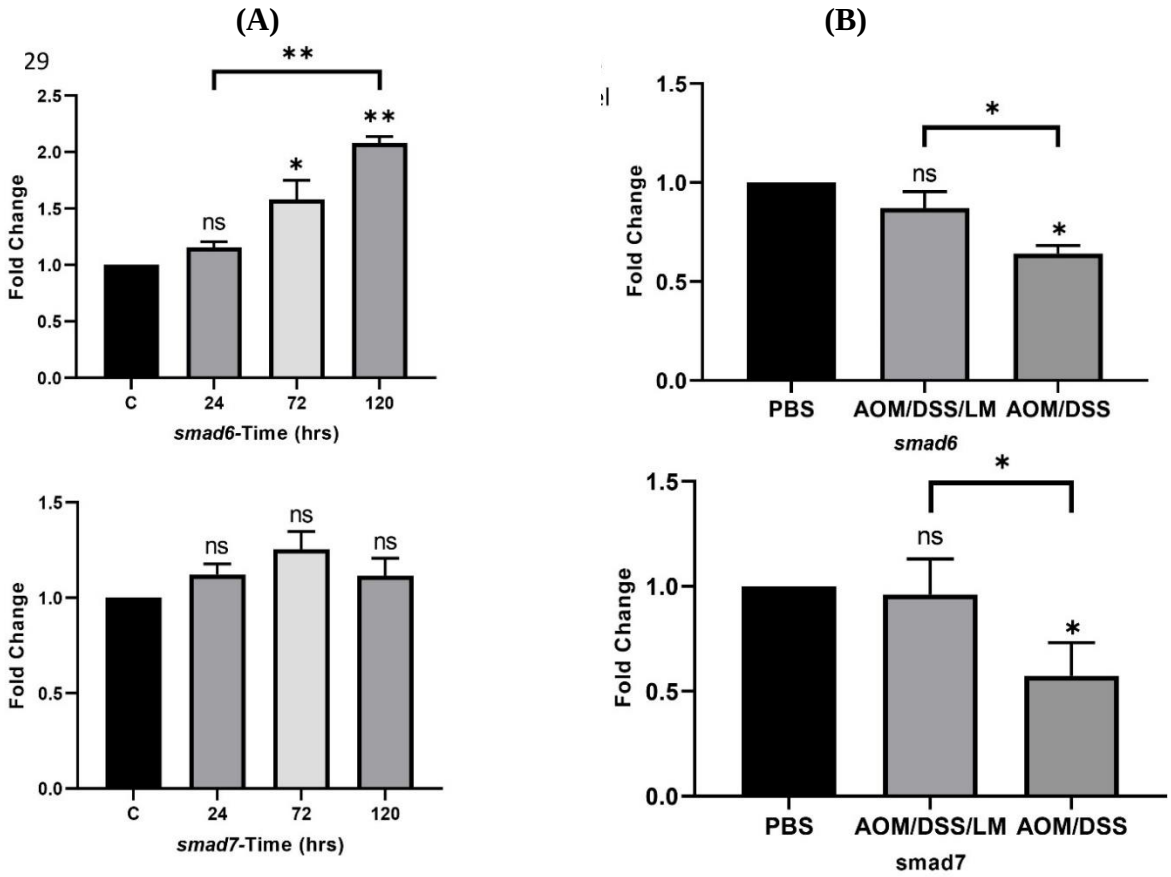

Fig. 5. Relative fold change of *smad6* and *smad7* genes in the presence of *Lactobacillus* mixture in (A) HT-29 cancer cells and (B) murine model that were compared with the control group. Control (c; untreated HT-26 cells); PBS: PBS-treated mice as control; AOM/DSS/*Lactobacillus*: AOM/DDS/*Lactobacillus*-treated mouse; AOM/DSS: AOM/DDS-treated mouse. Data were normalized with the *GAPDH* gene. Results were expressed as mean, and error bars (SD). Statistical analysis was performed using a one-way ANOVA test ($^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$). ns: not significant.

potential probiotic *Lactobacillus* mixture affects CRC by modulating the TGF-β signaling pathway in both models. Besides our previous studies, the results of this study revealed that consumption of our potential probiotics could be beneficial in improving colon health. Although our results support the beneficial effects of this native potential probiotic mixture, additional studies about how this mixture affects other signaling pathway elements are needed to find new strategies for combating CRC incidence and progression.

DECLARATIONS

Acknowledgments

The authors appreciate the cooperation of all personnel in the Bacteriology Laboratory at the Pasteur Institute of Iran, Tehran. We declare that no artificial intelligence (AI)- assisted technologies were used in the production of the submitted work.

Ethical approval

All the experimental procedures in this study were conducted in accordance with the Institutional Animal Care guidelines of the Declaration of Helsinki and approved by the Research Ethics Committee of Iran University of Medical Sciences, Tehran, Iran (ethical code: IR.IUMS.FMD.REC.1397.066).

Consent to participate

Not applicable.

Consent for publication

All authors reviewed the results and approved the final version of the manuscript.

Authors' contributions

AS: wrote the manuscript and contributed to experiments; SA: prepared all the tables and figures; RG: contributed to experiments; MT: contributed to data analysis; MRP and MR: supervised the experiment and manuscript revision.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Supplementary information

The online version does not contain supplementary material.

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