Golestan University of Medical Sciences

Jorjani Biomedicine Journal

Online ISSN: 2645-3509

Investigating the Effects of *Lactobacillus Plantarum* Strain ATCC 8014 on Gene Expression of NF-κB, TLR-4, and BCL-2 in Oral Rat Cancer Induced by 4-Nitroquioline 1-Oxide

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Article Type:

Original article

Article History:

Received: 30 Jul 2022
Revised: 1 Oct 2022
Accepted: 9 Oct 2022
Published: 10 Nov 2022

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DOI: 10.29252/jorjanibiomedj.10.4.12

Abstract

Background and Objectives: Oral cancer relates to oral and intestinal microbiota composition. *Lactobacillus* species are considered probiotic bacteria due to their ability to modulate human immune responses and therapeutic effects. This study aimed to investigate the effects of *Lactobacillus plantarum* (*L. plantarum*) on TLR4 gene expression and its downstream pathways in a mouse model of oral cancer.

Material and Methods: This experimental study was conducted in the Stem Cell Research Center, Faculty of Modern Technologies, Golestan University of Medical Sciences, Gorgan, Iran, in 2021. *L. plantarum* strain ATCC 8014 was used in this study. Twenty-eight male Wistar rats were divided into four groups (n = 7). 4-Nitroquinoline 1-oxide (4NQO) was used to establish oral cancer in rats. A pathological examination was adopted to confirm cancer establishment. Rats were treated with probiotic *L. plantarum* before and after cancer development. After extracting RNA from blood and synthesizing cDNA, the ability of lactobacilli to modulate the expression of TLR4 genes was investigated by Real-Time PCR Methods (RT-PCR).

Results: In the present study, it was observed that after causing cancer and treating the animal with *L. plantarum*, the expression of the TLR4 gene decreased significantly (*P*-value < 0.05), which might, in turn, affect the downstream pathways, which included the decrease in the expression of BCL-2 and NF-κB genes. Accordingly, the expression of the NF-κB gene was significantly reduced in rats received *L. plantarum*gavaged for two weeks after cancer induction.

Conclusion: According to the obtained results, probiotic *L. plantarum* significantly affects the gene expression of NF-κB and TLR4 in cancerous rats. It was also shown that *L. plantarum* was more efficient in reducing TLR-4 and NF-κB genes expression when it was gavaged for 14 days after tumor induction.

Keywords: *Lactobacillus plantarum* [MeSH]; Probiotic [MeSH]; Toll-Like Receptors [MeSH]; Apoptosis [MeSH]

 Publisher: Golestan University of Medical Sciences.

Highlights

- Oral Squamous Cell Carcinoma was developed with 4NQO in the animal model.
- *L. plantarum* treatment in cancerous rats decreases TLR-4 gene expression.

Introduction

Oral cancer is the sixth type of cancer worldwide, specifically related to the combination of oral and intestinal microbiota (1). Despite recent advances in cancer treatment, such as chemotherapy and immunotherapy, oral cancer remains the second leading cause of cancer-related deaths. Based on this, exploring new and effective methods for treating oral cancer is suggested. Although the association between some bacterial species and oral cancer has already been established, the complexity of the relationship between cancer and oral microbiota remains unexplained (2–4). As a result, there is a lack of new biomarkers for the early detection of oral cancer. In this context, the administration of probiotics has recently been considered a promising cancer prevention strategy due to their immunological effects. In this field, little research has been done on the effects of probiotics in the development of oral cancer. Today, probiotics are used as an adjunctive treatment to improve the effects of chemotherapy and immunotherapy. Probiotics have different effects on normal and cancer cells. Recently, the therapeutic potential of lactobacilli has been reported in research, but the molecular mechanisms of their anticancer effects are not yet fully understood and require further research and investigation (5).

Anticancer effects of probiotics are due to regulation of immune response, induction of programmed death (apoptosis), and their antioxidant properties. In comparison, the daily consumption of specific probiotic strains can restore microbiota balance and a person's health and inhibit the colonization of pathogenic microorganisms in the intestine. In addition, probiotic bacteria have many characteristics (6). According to the research done, probiotics using different mechanisms include short-chain fatty acid production, reducing toxic compounds, inhibiting mutagenic agents, strengthening the immune system, producing hydrogen peroxide (H2O2), producing bacteriocin, competing with pathogenic bacteria, and effects Anticancer have beneficial roles in human health (7).

New studies show that the best way to inhibit or suppress cell proliferation is to induce programmed cell death because it will not cause inflammation in nearby cells and will be an immune factor for tumor suppression. Studies show that probiotic bacteria such as *Lactobacillus* strains induce the mitochondrial pathway of programmed death in cancer cells (8). Therefore, Pattern Recognition Receptors (PRRs) are essential in properly functioning the innate immune system. These receptors are proteins mainly expressed by the cells of the innate immune system, such as dendritic cells, macrophages, monocytes, neutrophils, and epithelial cells, with two molecular classes called Pathogen-Associated Molecular Patterns (PAMP) that are associated with pathogens. Microbial pathogens are associated with Damage-Associated Molecular Patterns (DAMPs) that are identified with components of host cells that are released as a result of damage or cell death (9).

Different OSCC biomarkers have been evaluated, and Toll-Like Receptors (TLRs), anti-apoptotic B-cell lymphoma/leukemia-2 (Bcl-2), and NF-κB proteins are among the most important markers. TLRs are a class of proteins that play a key role in the innate immune system. TLRs bind to lipopolysaccharide of gram-negative bacteria, teichoic acid of gram-positive bacteria, and beta-glucan of fungi, which activate immune cell responses. Once activated, TLRs recruit other immune cells to mediate the antigen-induced signal transduction pathway. The recruited proteins are then responsible for the subsequent activation of other downstream proteins, including protein kinases, which further amplify the signal and ultimately lead to the up-regulation or suppression of genes that trigger inflammatory responses and the transcription of other genes. Recent studies have shown that TLRs are highly

expressed and active in many types of cancer; however, the role of toll-like receptors in Oral Squamous Cell Carcinoma (OSCC) is unclear (10). It has been shown that strong TLR-4 expression correlated with deeper OSCC tumor invasion and with higher tumor grade and long growth time of the tumor (11, 12). This indicates TLR-4 overexpression as a biomarker of OSCC. As a crucial regulator of inflammation and immune responses, the NF-κB transcription factor regulates the transcription of target genes closely related to cell survival, cell proliferation, apoptosis, invasion, and metastasis (13, 14). However, the significance of the expressions of both NF-κB and TLR-4 in the occurrence, development, and prognosis of oral SCC remains unclear.

BCL-2 proteins are one of the most anti-apoptotic proteins expressed in OSCC, and their overexpression contributes to anticancer drug resistance (15). BCL-2 is an anti-apoptotic protein that regulates cell cycle control through apoptosis. Bcl-2 expression varies in oral cancers. Teni et al. showed that overexpression of BCL-2 in patients with OSCC is correlated with oral cancer development (16). Furthermore, it has been shown that inhibition of BCL-2 protein leads to in vitro oral tumor regression (17). Accordingly, it can be concluded that probiotic therapy could be a suitable option for oral cancer treatment (18); however, its contribution to oral cancer therapy by affecting TLR-4, NF-κB, and BCL-2 proteins expression remains unclear.

To elucidate the antitumor effects of *L. plantarum* on OSCC development in vivo, the inhibitory activity of this probiotic bacterium on 4NQO-induced oral carcinogenesis was investigated in male Wistar rats. In addition, the effect of *L. plantarum's* expression of TLR-4, NF-κB, and Bcl-2 in tongue tissue was assessed. The present study showed a significant effect of *L.plantarum* on inducing apoptosis and increasing the expression of TLR-4 and NF-kB genes in cancer cells.

Materials and Methods

This experimental study was conducted at the Stem cell Research Center, Faculty of Advanced Medical Technologies of Gorgan University of Medical Sciences, Iran, from April to March 2021. In this study, the probiotic *L. plantarum* was purchased from the microbial collection center of Iran's biological reserves. This strain was cultured for growth and enrichment on broth medium (MRS) and incubated at 37°C for 24 hours, after which a suspension of bacteria was prepared using Phosphate-Buffered Saline (PBS) and some biochemical tests were done to confirm probiotic potential of this bacterium. 4NQO (Safirazma, Tehran, Iran) was used to establish an animal model of oral cancer (19). The amount of 20 ppm of 4NQO was dissolved in propylene glycol. Rats received 4NQO 3 times a week for 16 weeks, and each time a fresh solution was used.

Laboratory Animals

Twenty-eight male Wistar rats with an average weight of 150 grams were purchased from Pasteur Amol Institute, Mazandaran, Iran. They were kept at a temperature of 22±1°C with a light/dark cycle of 12.12 hours. The rats were divided into four groups of seven: group 1, including control rats, group 2, cancer group rats treated with 4NQO, and group 3, rats gavage with 108 cfu/kg *L. plantarum* for one week after purchase and before carcinogenesis. Group 4 was treated with 108 cfu/kg *L. plantarum* 14 days after carcinogenesis. All animal procedures were sent to the research ethics committee of Islamic Azad University of Damghan, which approved the experimental protocol (license number: 1401.002).

Real-Time PCR assay

Whole blood samples of rats were taken before and after cancer development and before and after probiotic bacteria gavage for real-time PCR (RT-PCR) (Yektatajhiz, Tehran, Iran). Total RNA was extracted for quantitative measurement of TLR4, NF-κB, and BCL-2 gene expression using the RNA extraction column kit and then converted to cDNA using the cDNA synthesis kit (Yektatajhiz, Tehran, Iran) for quantitative RT-PCR (qRT-

PCR). In this study, the GAPDH gene was used as the control gene. The sequence of primers used for RT-PCR is shown in Table 1. The gene expression in different groups was investigated and compared, and the

effect of *L.plantarum* was determined.

Table 1. Characteristics of the primers used for gene expression assay.

Primer	Orientation	5' to 3' sequence	Amplicon size (bp)
BCL-2	F	GGGTCATGTGTGTGGAGAG	181
	R	AGCCAGGAGAAATCAAACAG	
NF-κB	F	TTCCCCTGTACGATAGTCGG	77
	R	GTGCTAGAAGCTGGAGGATG	
TLR4	F	GCTTCTCCAATTTCTCACAAC	201
	R	AGGTCATTTTTGTCTCCACAG	
GAPDH	F	AGCTCATTTCCTGGTATGACA	125
	R	TTGCTCTCAGTATCCTTGC	

Statistical analysis

The data of gene expression levels of TLR-4 and Bcl-2 in control and 4NQO-treated tongue cells of rats were assessed by ΔCt and analyzed using the Student t-test. The results were considered statistically significant if the P value was less than 0.001, 0.01, or 0.05. SPSS software and GraphPad Prism 4 software were used to analyze the data.

Results

In this study, according to some biochemical tests, probiotic potential of *L.plantarum* was confirmed. This gram-positive bacterium was catalase-negative and none motile which fermented sugars such as lactose, glucose, mannitol, sucrose, fructose, xylose, and arabinose. Arginine hydrolysis was negative for this bacterium. Different pH resistance by *L. plantarum* was observed which is shown in table 2. This bacterium could easily grow in bile salt and after 5 hours its growth took an upward trend (*P-value*<0.05) (Chart.1).

Table 2. The effect of different pH (3, 3.5, and 4) on the survival percentage of *LactobacillusPlantarum* strain

PH	4	3.5	3
Grade growth	Excellent growth	Good growth	Poor growth
Lactobacillus Plantarum	%99	%87	%42

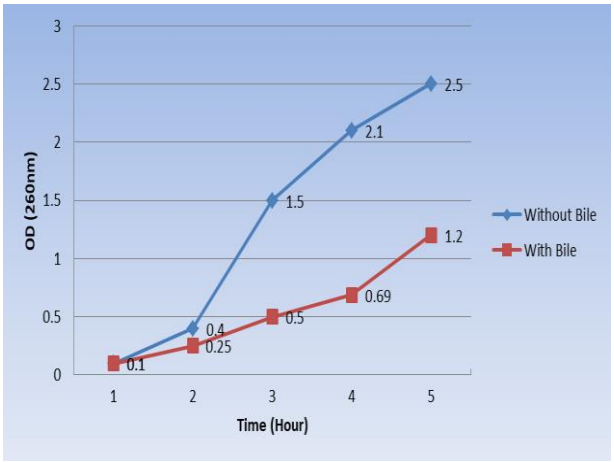

Chart 1. The ability *Lactobacillus plantarum* to grow in the presence of bile salt

General observations of animals

None of the rats died during the study. All animals appeared healthy until macroscopic tumors appeared on the Tongue. A decrease in the body weight of rats was observed from day 0 to week 16. However, improved body weight was observed in cancer rats with oral gavage of *L.plantarum*.

Pathological results

Gross changes, including leukoplakia, erosion, ulceration, and papillary appearance on the posterior dorsum of the Tongue, appeared during carcinogenesis (Figure 1). Accordingly, histopathological findings ranged from Hyperplasia (HP), mild to moderate Dysplasia

(mmDP), severe Dysplasia (sDP), and Carcinoma In Situ (ISC) to well-differentiated invasive Squamous Cell Carcinoma (SCC). The severity of the lesions corresponds to the duration of administration. In rats treated with 4NQO for 9, 13, and 16 weeks, the incidence of tongue cancer was 50.0%, 62.5%, and 77.8%, respectively.

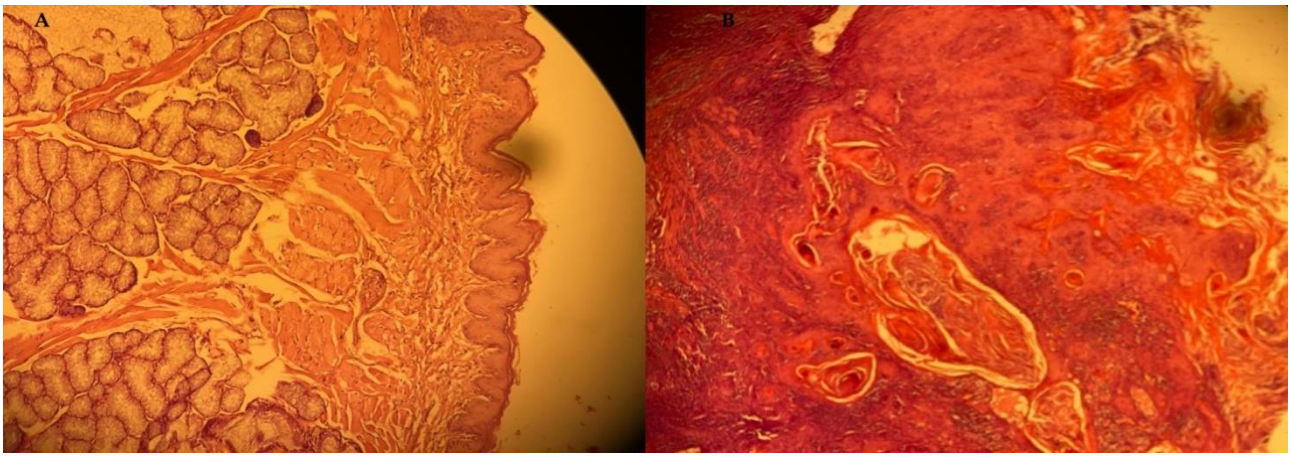

Figure 1. Pathological findings of rats' Tongue's mass before and after 4NQO administration. A) A microscopic finding of the normal epithelium of rat tongue. B) Sections from tongue mass in rats treated with 4NQO revealed a malignant epithelial neoplasm originating from squamous, arranged in nests and individuals invaded to subepithelium and muscularis layers. Tumoral cells show atypical features and form keratin pearls. Also, severe lymphocytic infiltration around invaded nests is seen. The mass was diagnosed with well-differentiated SCC.

Gene expression results

The results (Chart2) of this research showed that the expression of the BCL-2 gene decreased significantly in group 3 (*P*-value<0.001). Also, an increase in BCL-2 gene expression was observed in rats of treatment group 4 (*P*-value<0.01). In addition, a slight increase in BCL-2 gene expression was observed in group 2 cancer rats, which was not significant. The decrease in BCL-2 gene expression observed in treatment group 3 was significantly different from untreated cancer rats and group 4 (*P*-value <0.001). No significant change in Ct was observed between group 4 and untreated cancer group 2.Based on this, TLR4 gene expression was evaluated in cancer rats treated with *L.plantarum* before and after becoming cancerous and compared to control group 1 (*P*-value< 0.001). However, no significant changes in TLR4 gene expression were observed between group 2 and group 3. The decrease in TLR4 gene expression observed in untreated cancer group 2 significantly differed from groups 3 and 4 treated with *L.plantarum* (*P*-value<0.001). Also, the increase in TLR4 gene expression was higher in group 4 than in group 3 (*P*-value <0.05).

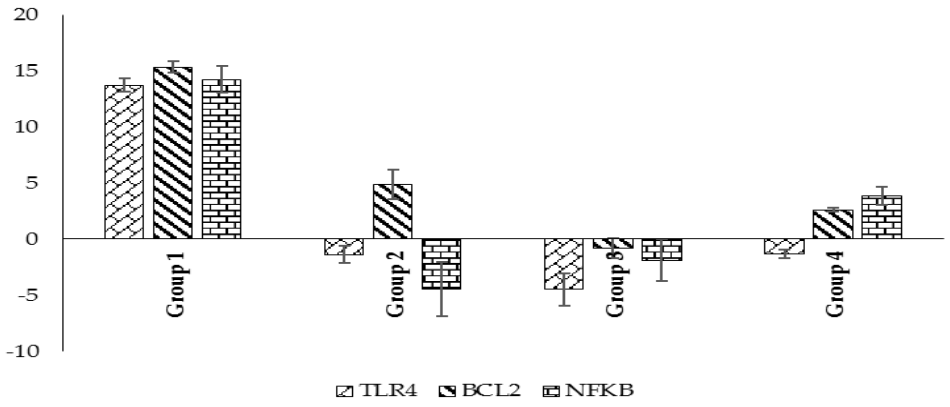

Chart 2. ΔCt analysis of gene expression. The GAPDH housekeeping gene was used as a control for data analysis

Probiotics are an emerging option for cancer prevention. The efficacy of probiotics in preventing other cancers and the correlation mechanism are still under investigation. On the other hand, research has shown that in each type of cancer, the expression of certain types of TLR increases, which can be used as a marker, and the expression of TLRs, in turn, increases the expression of downstream pathways such as NF-KB and BCL-2, which also prevent apoptosis. They become cancerous in cells.

In 2017, Cheng and colleagues showed that the *Lactobacillus rhamnosus* LGG strain alone was ineffective on oral squamous cell carcinoma cells. But the use of these two at the same time caused growth-inhibiting effects. Flow cytometry analysis showed that HSC-3 cancer cells treated with LGG (geniposide (1.0x103 CFU/ml LGG and 50 μg/ml) had a higher apoptosis rate than cells in other treatment groups (20).

In 2013, ming zhang and colleagues showed that a new probiotic, *Lactobacillus salivarius,* was isolated in Bama, China, which showed strong antitoxin properties in a preliminary assay. 4 nitroquinoline 1 oxide was used to develop an oral cancer model to study the anticancer activity of *L. salivarius* REN in vivo. The results showed that oral administration of probiotic *L. salivarius* REN or its secretions could effectively suppress oral cancer caused by 4-nitroquinoline 1-oxide in the early and postoperative stages. *L. salivarius* REN treatment significantly reduced the expression of Proliferating Cell Nuclear Antigen (PCNA) and induced apoptosis in a dose-dependent manner (19).

In 2021, mokhtari and his colleagues studied the effects of the supernatant of *Lactobacillus fermentum* and *Lactobacillus crispatus*, on HN5 cancer cells. The results showed that these *lactobacilli* do not prevent the development of oral cancer cells and acidic environment has the greatest effect on reducing the growth of oral cancer cells. Conclusion Considering the different effects of*lactobacilli* on different types of cancer, the effect of *L. crispatus* and *L. fermentum* on other oral cancer cell lines may be different from what was reported in this study (21).

In 2020, van noor fatiha noted in his review article that he found four probiotics in search of 744 articles on this disease that showed potential therapeutic effects in oral cancer, including *Acetobactersyzygii*, AJ2, *Lactobacillus plantarum*, and *Lactobacillus salivariusREN*. Among them, the use of *L. salivariusREN* leads to a 95% reduction in the risk of oral cancer (22).

John miyaguchi and colleagues stated in 2018 that solid tumors create an anaerobic environment. They evaluated the antitumor effect of the obligate anaerobic strain KK378, derived from *Lactobacillus casei*, using rats bearing head and neck cancer. After tumor formation, *L.caseiKK*378 was administered directly into the tumor, and tumor size and serum cytokine levels were analyzed. Their research showed that rats injected with 108 cfu of *L.casei KK378* showed reduced tumor growth compared to PBS control. Also, this research showed that direct injection of *lactobacillus* into the tumor could be a potential strategy for treating head and neck squamous cell cancer (23).

In 2009, rydberg investigated TLRs and their association with oral squamous cells and reported that TLR2, TLR3, and TLR5 were present in primary Head and Neck Squamous Cells (HNSCCs). This research showed that TLR agonists cause a strong response in this disease, which is characterized by inflammation and cell death, and finally, this research showed that the TLR system should be considered an important target in antitumor immunotherapy in the future (24).

In 2020, kamarajan and colleagues discussed the relationship between TLR signaling pathway and probiotics in oral malignancy. Their data showed that periodontal pathogens contribute to a highly aggressive cancer phenotype through crosstalk between MYD88/TLR and integrin signaling (25).

After comparing the results of the previous studies mentioned above and this study, it was found that the results were entirely consistent. According to

the data obtained from the current research, the probiotic *Lactobacillus plantarum* has a remarkable effect in inducing apoptosis in an animal model of oral cancer and is a strong therapeutic candidate for the treatment of oral SCCs by activating the mucosal innate immune response. This is due to the increase of PAMPs associated with lactobacilli, mainly recognized by PRRs, DC-SIGN, and TLR4, which leads to the activation of the NF-κB signaling pathway and the death of cancer cells. However, there is some limitation to the present study. Accordingly, the gene expression downstream of NF-κB and TLR-4 was not assessed. Furthermore, the decreased expression of BCL-2 might be attributed to enhancing apoptosis of cancer cells, which should be evaluated by measuring apoptosis in rats gavaged with *L. plantarum*.

Conclusion

By reducing the expression of TLRs, NF-κB, and BCL-2, probiotics may regulate downstream pathways, which will cause apoptosis in cancer cells and improve tumor tissue. This idea can be used in the future of probiotics and other medical treatments to speed up the recovery of the disease. The results of the present study suggest that probiotic *L. plantarum* can reduce the expression of OSCC's markers, including TLR-4, NF-κB, and BCL-2 in oral rat cancer induced by 4-Nitroquioline 1-Oxide.

Acknowledgment

The authors are thankful to all the laboratory personnel of Golestan University of Medical Sciences and the animal house staff of that university.

Funding source(s)

Financial support for this study was provided by a grant from the Islamic Azad University, Damghan, Iran.

Ethics approvals and consent to participate

The current study was carried out with the approval of the Vice-Chancellor for Research and Technology's Ethics Committee, Islamic Azad University, Damghan, Iran, with the approval code of 1401.002.

Conflict of interest

The authors declare that they have no competing interests.

References

1. Cancer O, Lesions P. Oral Cancer and Precancerous Lesions. CA Cancer J Clin [Internet]. 2002 [cited 2022]; 52(4):195-215. [DOI] [PMID] [Google Scholar]

2. Sedighi M, ZahediBialvaei A, Hamblin MR, Ohadi E, Asadi A, Halajzadeh M, Lohrasbi V, Mohammadzadeh N, Amiriani T, Krutova M, Amini A. Therapeutic bacteria to combat cancer; current advances, challenges, and opportunities. Cancer medicine. 2019; 8(6):3167-81. [DOI] [PMID] [Google Scholar]

3. Bhatt AP, Redinbo MR, Bultman SJ. The role of the microbiome in cancer development and therapy. CA: a cancer journal for clinicians. 2017; 8;67(4):326-44. [DOI] [PMID] [PMCID] [Google Scholar]

4. Rebersek M. Gut microbiome and its role in colorectal cancer. BMC cancer. 2021;21(1):1-3. [DOI] [PMID] [PMCID] [Google Scholar]

5. Hirayama K, Rafter J. The role of probiotic bacteria in cancer prevention. Microbes and infection. 2000; 1;2(6):681-6. [Google Scholar]

6. Yang X, Da M, Zhang W, Qi Q, Zhang C, Han S. Role of *Lactobacillus* in cervical cancer. Cancer Management and Research. 2018;10:1219. [Google Scholar]

7. Hemaiswarya S, Raja R, Ravikumar R, Carvalho I.S. Mechanism of action of probiotics. Braz. Arch. Biol. Technol. 2013; 56 (1): 113-119. [DOI].

8. Asoudeh-Fard A, Barzegari A, Dehnad A, Bastani S, Golchin A, Omidi Y. *Lactobacillus plantarum* induces apoptosis in oral cancer KB cells through upregulation of PTEN and downregulation of MAPK signalling pathways. BioImpacts: BI. 2017; 7(3):193. [DOI] [PMID] [PMCID] [Google Scholar]

9. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010; 19;140(6):805-20. [Google Scholar]

10. Palani CD, Ramanathapuram L, Lam-Ubol A, Kurago ZB. Toll-like receptor 2 induces adenosine receptor A2a and promotes human squamous carcinoma cell growth via extracellular signal regulated kinases ½. Oncotarget. 2018; 23;9(6):6814. [DOI] [PMID] [PMCID] [Google Scholar]

11. Mäkinen LK, Atula T, Häyry V, Jouhi L, Datta N, Lehtonen S, Ahmed A, Mäkitie AA, Haglund C, Hagström J. Predictive role of Toll-like receptors 2, 4, and 9 in oral tongue squamous cell carcinoma. Oral oncology. 2015; 1;51(1):96-102. [DOI] [PMID] [Google Scholar]

12. Li L, Zhou Z, Mai K, Li P, Wang Z, Wang Y, Cao Y, Ma X, Zhang T, Wang D. Protein overexpression of toll-like receptor 4 and myeloid differentiation factor 88 in oral squamous cell carcinoma and clinical significance. Oncology letters. 2021; 1;22(5):1-1. [view at publisher] [DOI] [PMID] [PMCID] [Google Scholar]

13. Zhang T, Ma C, Zhang Z, Zhang H, Hu H. NF-κB signaling in inflammation and cancer. MedComm. 2021;2(4):618-53. [DOI] [PMID] [PMCID] [Google Scholar]

14. Xia Y, Shen S, Verma IM. NF-κB, an active player in human cancers. Cancer immunology research. 2014;2(9):823-30. [view at publisher] [DOI] [PMID] [PMCID] [Google Scholar]

15. Arumugam J, Jeddy N, Ramamurthy A, Thangavelu R. The expression of Bcl-2 in oral squamous cell carcinoma—A review. Journal of Orofacial Sciences. 2017; 1;9(2):71. [DOI] [Google Scholar]

16. Teni T, Pawar S, Sanghvi V, Saranath D. Expression of bcl-2 and bax in chewing tobacco-induced oral cancers and oral lesions from India. Pathology Oncology Research. 2002;8(2):109-14. [DOI] [PMID] [Google Scholar]

17. Gibson SA, Pellenz C, Hutchison RE, Davey FR, Shillitoe EJ. Induction of apoptosis in oral cancer cells by an anti-bcl-2 ribozyme delivered by an adenovirus vector. Clinical cancer research. 2000;6(1):213-22. [view at publisher] [Google Scholar]

18. Zanetta P, Ormelli M, Amoruso A, Pane M, Azzimonti B, Squarzanti DF. Probiotics as Potential Biological Immunomodulators in the Management of Oral Lichen Planus: What's New?. International Journal of Molecular Sciences. 2022; 23;23(7):3489. [view at publisher] [Google Scholar]

19. Zhang M, Wang F, Jiang L, Liu R, Zhang L, Lei X, Li J, Jiang J, Guo H, Fang B, Zhao L. *Lactobacillus SalivariusREN* Inhibits Rat Oral Cancer Induced by 4-Nitroquioline 1-Oxide*Lactobacillus Salivarius REN* Inhibits Oral Cancer. Cancer Prevention Research. 2013; 1;6(7):686-94. [view at publisher] [DOI] [PMID] [Google Scholar]

20. Cheng Z, Xu H, Wang X, Liu Z. *Lactobacillus* raises in vitro anticancer effect of geniposide in HSC-3 human oral squamous cell carcinoma cells. ExpTher Med. 2017; 1;14(5):4586-94. [view at publisher] [DOI] [Google Scholar]

21. Mokhtari S, Atarbashi-Moghadam S, Motevaseli E, Ghafouri-Fard S, Hesampour A. *Lactobacillus fermentum* and *Lactobacillus crispatus* Do Not Have Cytotoxic Effects on HN5 Oral Squamous Cell Carcinoma Cell Line. International Journal of Dentistry. 2021; 28;2021. [DOI] [PMID] [PMCID] [Google Scholar]

22. Kamaluddin WN, Rismayuddin NA, Ismail AF, Aidid EM, Othman N, Mohamad NA, Arzmi MH. Probiotic inhibits oral carcinogenesis: A systematic review and meta-analysis. Archives of oral biology. 2020; 1;118:104855. [Google Scholar]

23. Miyaguchi J, Shiga K, Ogawa K, Suzuki F, Katagiri K, Saito D, Ikeda A, Horii A, Watanabe M, Igimi S. Treatment with *Lactobacillus* retards the tumor growth of head and neck squamous cell carcinoma cells inoculated in mice. The Tohoku Journal of Experimental Medicine. 2018; 245(4):269-75. [view at publisher] [DOI] [PMID] [Google Scholar]

24. Rydberg C, Månsson A, Uddman R, Riesbeck K, Cardell LO. Toll-like receptor agonists induce inflammation and cell death in a model of head and neck squamous cell carcinomas. Immunology. 2009;128(1pt2):e600-11. [DOI] [PMID] [PMCID] [Google Scholar]

25. Kamarajan P, Ateia I, Shin JM, Fenno JC, Le C, Zhan L, Chang A, Darveau R, Kapila YL. Periodontal pathogens promote cancer aggressivity via TLR/MyD88 triggered activation of Integrin/FAK signaling that is therapeutically reversible by a probiotic bacteriocin. PLoS pathogens. 2020; 1;16(10):e1008881. [DOI] [PMID] [PMCID] [Google Scholar]

How to cite:

Faghanizadeh V, Arbab Soleimani N, Khosravi A, Forghani Fard M.M. Investigating the effects of *Lactobacillus plantarum* strain ATCC 8014 on gene expression of NF-κB, TLR-4, and BCL-2 in oral rat cancer induced by 4-Nitroquioline 1-Oxide. *Jorjani Biomedicine Journal*. 2022; 10(4):12-20.