JJBS

Volume 18, Number 3,September 2025
ISSN 1995-6673
Pages 479 – 485

Jordan Journal of Biological Sciences

Effect of orally administered *Fusobacterium nucleatum*, *Bifidobacterium animalis*, and *Lactobacillus bulgaricus* on skin cancer development in a mouse model

Muhamad Ali K. Shakhatreh[1,*], Nadeen M. Alotaiby[1] , Diala Alshiyab[2] , Zaid Shakhatreh[3], Jacob H. Jacob[4], Mo'ath M. Alrjoub[5]

[1] *Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, Irbid, Jordan;* [2]*Department of Dermatology, King Abdullah University Hospital, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan;* [3]*Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan;* [4]*Department of Biological Sciences, Faculty of Science, Al al-Bayt University, Al-Mafraq, Jordan;* [5] *Department of Pathology and Microbiology, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan*

Received: December 28, 2024; Revised: February 15, 2025; Accepted: March 10, 2025

Abstract

Skin cancer is one of the most common cancers worldwide. While ultraviolet radiation is the main causative agent of skin cancer, there is some evidence that the microbiome, both of the skin and gastrointestinal tract, may negatively or positively influence its development depending on the abundance of certain bacterial species. The influence of administrating three bacterial species on the early development of skin cancer in mouse two-factor skin carcinogenesis model was evaluated in this study. The bacterial species include *Fusobacterium nucleatum*, which is associated with multiple cancers and skin conditions, *Bifidobacterium animalis*, which has beneficial properties as a probiotic, and *Lactobacillus bulgaricus*, one of the main bacteria used in yogurt production. Mice treated with *B. animalis* had mildly decreased signs of early carcinogenesis, while the mice treated with *L. bulgaricus* had a more severe outcome along with a significant change in cancer promoting cytokines in serum. Finally, administration of *F. nucleatum* significantly increased blood IL-10 concentration but did not significantly affect early cancer development in mice in the short term (12 weeks).

Keywords: microbiome, skin cancer, carcinogenesis, mice model.

1. Introduction

The bacteria of the human gut have been known to influence many diseases of the gastrointestinal tract (Glassner *et al*., 2020) and other body systems ((Helmink *et al*., 2019, Cenit *et al*., 2017). One of those effects is their modulation of the body's immune system through antigen presentation and the release of molecules such as cytokines (Wang *et al*., 2021, Polkowska-Pruszyńska *et al*., 2020). One of the biggest factors in skin cancer development and progression is decreased immunity; immunosuppression is associated with more aggressive and invasive cutaneous tumors. UVB radiation has a large immunosuppressive role in exposed skin. It can induce natural killer cells (Moodycliffe *et al*., 2000), T-regulatory cells, keratinocytes, and macrophages, all of which suppress anti-tumor immunity such as cytotoxic T-cells and helper T-cells by the secretion of cytokines such as interleukin-10. Some studies have shown that the oral administration of bacteria can affect the development of skin conditions such as eczema (Kim *et al*., 2010a) and atopic dermatitis (Sheikhi *et al*., 2017, Rusu *et al*., 2019), and protect against UV damage (Guéniche *et al*., 2006).

Some probiotics, which are beneficial bacteria used to enhance health, have shown anti-cancer activities (Kumar *et al*., 2010), mainly through immune modulation (Sao *et al*., 2017) and the release of anti-tumor compounds (Nakatsuji *et al*., 2018). Two of the most commonly used probiotic genera are *Lactobacillus* and *Bifidobacterium* (Sharma *et al*., 2021), which are key bacteria in the healthy gut microbiome (Turroni *et al*., 2014).

Lactobacillus delbrueckii subsp. *bulgaricus* (*L. bulgaricus*) is a Gram-positive pleomorphic bacterium. *L. bulgaricus* and *Streptococcus thermophilus* are the main fermenters responsible for fermenting milk into yogurt. *L. bulgaricus* has been used to treat constipation and other gastrointestinal disorders since the early 20th century. A study found *L. bulgaricus* may be able to modulate immunity in atopic dermatitis (Sheikhi *et al*., 2017). Another study showed that a probiotic mix containing *L. bulgaricus* and *Streptococcus thermophilus* may inhibit tumor growth (Guha *et al*., 2019, Neish 2017).

Bifidobacterium animalis subsp. *lactis* (*B. animalis*) is a Gram-positive anaerobic pleomorphic bacterium that is one of the most commonly used probiotic species. Studies found that healthy individuals tend to have a higher amount of *Bifidobacterium* in their guts than individuals with gastrointestinal diseases (Uusitupa *et al*., 2020). Some

* Corresponding author. e-mail: mkshakhatreh@just.edu.jo.

strains of *B. animalis* have shown several benefits as a probiotic; studies have found that it enhances NK cell and neutrophil function in elderly subjects (Miller *et al*., 2017), improves cholesterol and LDL-C concentration in type 2 diabetics when combined with *L. acidophilus* (Ejtahed *et al*., 2011), and reduces the incidence of atopic dermatitis in infants when given to mothers with *B. bifidum* and *L. acidophilus* during pregnancy and breastfeeding (Kim *et al*., 2010a). *B. animalis* also suppressed NF-κB activity in intestinal epithelial cells and suppressed the development of acute colitis and colitis-associated carcinogenesis (Kim *et al*., 2010b).

Fusobacterium nucleatum is part of the oral flora (Verma *et al*., 2018) and can be found in other microbial environments of the human body, particularly in tumor microenvironments. It is associated with colorectal (Shang and Liu, 2018), oral (McIlvanna *et al*., 2021), and other cancers. There is some evidence it may also act as an oncogenic bacteria in skin cancer (Mrázek *et al*., 2021).

In the current study, we studied the effects of oral administration of *Fusobacterium nucleatum* and the probiotics *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Bifidobacterium animalis* subsp. *lactis* on skin cancer development in an *in vivo* mouse carcinogenesis model.

2. Materials and Methods

2.1. Mouse model

Five groups of female Swiss albino mice were used to examine the effects of orally administered bacteria on skin cancer development *in vivo* (Table3). Experiments included two control groups: no-treatment control (NTC), which was only treated with vehicles to establish the healthy baseline for the mice (n=5), and cancer control (CC) that was not given any bacteria (n=5), *L. bulgaricus* group (n=8), *B. animalis* group (n=8), and *F. nucleatum* group (n=8). Four mice were excluded due to sudden death during the isolation period of the treatment. The final group counts were NTC=5, CC=4, *L. bulgaricus* =8, *B. animalis* =7, and *F. nucleatum* =6. All mice weighed 18 grams and were 10 weeks old at the beginning of the experiment. The dorsal area of the mice was shaved before treatment and once every 2 weeks to ensure direct application of the treatment to the skin.

2.2. Bacterial strains

Three types of bacteria were administered to the mouse subjects: Fusobacterium nucleatum subsp. nucleatum knorr ATCC 23726 (F. nucleatum), Bifidobacterium animalis subsp. lactis ATCC 27536 (B. animalis), and Lactobacillus delbruekii subsp. bulgaricus (L. bulgaricus) strain isolated from a commercially available yogurt mixture of lyophilized L. bulgaricus and Streptococcus thermophilus used in industrial yogurt production. The ATCC strains were revived according to the manufacturer's instructions. The yogurt mixture was revived by suspending the lyophilized powder in skim milk and incubating at 37°C for 2 hours, followed by culturing on blood agar for 24 hours. L. bulgaricus was isolated by subculturing an isolated L. bulgaricus colony.

Bacterial stocks of the strains were preserved at -80 °C, and a fresh subculture was grown from this stock weekly for use in treatment by suspending a loopful of stock in thioglycollate broth and incubating it at 37°C for 24 hours. The bacteria were suspended in normal saline to produce a 0.5 optical density (OD_{600nm}) suspension, approximately equal to 1.5 x 10^8 CFU/ml. Each mouse was given 0.2 ml of the suspension, except the no-treatment and cancer control groups which were given normal saline. The suspension was given via oral gavage using a 20-gauge curved gavage needle twice weekly.

2.3. Cancer induction

Pre-cancerous changes were induced using two-stage carcinogenesis as shown in (Tables 1 and 2, Figure 1). The initiation stage was done once using 50 µg of 7,12-dimethylbenz[a]-anthracene (DMBA; a mutagen that damages DNA) in 200 µL of acetone, and the promotion stage was done twice weekly for 12 weeks using 5 µg of 12-O-tetradecanoyl phorbol-13-acetate (TPA; promotes tumor growth) in 200 µL of acetone (Vähätupa *et al*., 2019). The no treatment control group was only given 200 µL of the vehicle (acetone) at the same time. Physical changes (hair loss and skin texture) were noted and rated using the rating system in (Table 3). The animals were weighed in the last week of treatment following the last dose of TPA and bacteria.

Table 1. Mouse carcinogenesis treatment schedule. DMBA (a carcinogen) was administered once, and TPA (a pro-inflammatory chemical) was administered twice each week.

Week 1	Week 2-13
50 µg DMBA once	5 µg TPA twice weekly

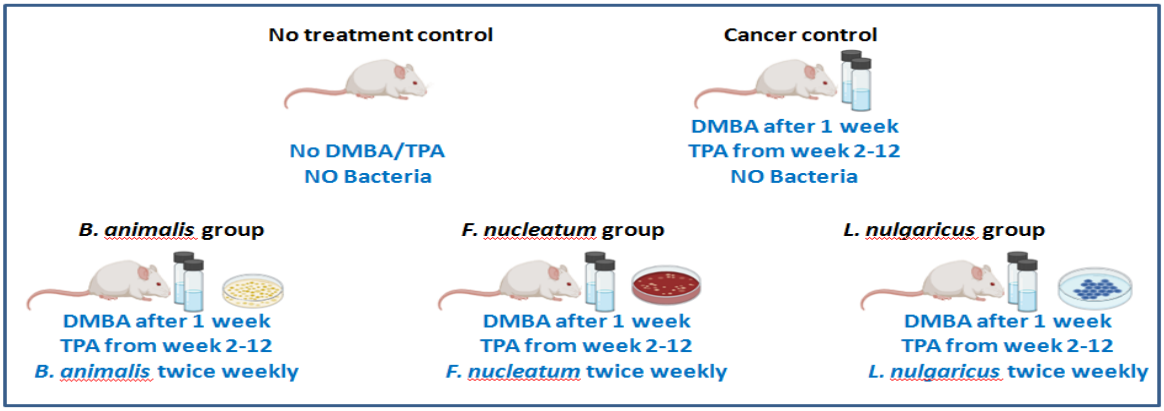

Figure 1. Explanation of the different treatments groups.

Table 2. Mouse treatment groups.

	No treatment control	Cancer control	*B. animalis*	*L. bulgaricus*	*F. nucleatum*
DMBA/TPA treatments	no	yes	yes	Yes	Yes
Bacterial strain administered	none	none	*Bifidobacterium animalis* subsp. *lactis* ATCC 27536	*Lactobacillus delbruekii* subsp. *bulgaricus*	*Fusobacterium nucleatum* subsp. *nucleatum* knorr ATCC 23726

Table 3. Physical grading of mouse skin changes guidelines.

	Degree of hair loss	Skin texture
0	0%	Smooth skin
1	<25%	Isolated areas of changed texture
2	25%-75%	Large areas of changed texture not visibly apparent
3	>75%	Visibly rough skin covering most of the treatment area
Physical score= hair loss score + skin texture score		

2.4. Sample collection and storage

Animals were euthanized a week after the last TPA administration. Blood was collected into silica gel blood tubes with no additives and centrifuged after clot formation. The serum was stored at -80 °C until analysis. The dorsal skin was cut and stored in Bouin's solution then washed with cold phosphate-buffered saline and placed in 70% alcohol at 4 °C until histological analysis.

2.5. Cytokine analysis

As the systemic effects of bacteria are often mediated through immune modulation, the concentrations of interleukin 6 (IL-6), interleukin 10 (IL-10), and tumor necrosis factor-alpha (TNFα) were measured in mouse serum using Mouse IL-6 ELISA Kit (ab222503), Mouse IL-10 ELISA Kit (ab255729), and Mouse TNF alpha ELISA Kit (ab46105) (Abcam PLC, UK) according to the manufacturer's instructions.

2.6. Histology

To examine the microscopic effect the carcinogenesis and bacterial treatment had on mouse skin, mouse tissue was formalin-fixed and embedded in paraffin wax blocks. The tissue was cut using a microtome and placed on slides and the slides were then stained with hematoxylin and eosin. The stained slides were examined by a pathologist for any pathological changes including mast cell numbers, keratinocytes layer thickness, and dysplastic changes.

2.7. Statistical analysis

IBM's SPSS statistics software was used to analyze the results and a P value lower than 0.05 using Mann–Whitney U test was considered significant. Mann-Whitney U test was used as the data was not normally distributed and the medians were calculated using only the positive samples.

3. Results

3.1. Physical changes of mouse groups

To study the *in vivo* effects of orally administered bacteria on skin cancer, five groups of mice were given a two-factor carcinogenesis treatment as well as each group was treated with *F. nucleatum*, one of two probiotics (*L. bulgaricus* or *B. animalis*), or no bacteria. Mice were ranked on a scale of 0-6 for both hair loss and skin texture changes (Figure 2). There was no significant difference in physical score between the bacteria groups and the cancer control group (Figure 3). There were also no significant weight changes between the groups (not shown).

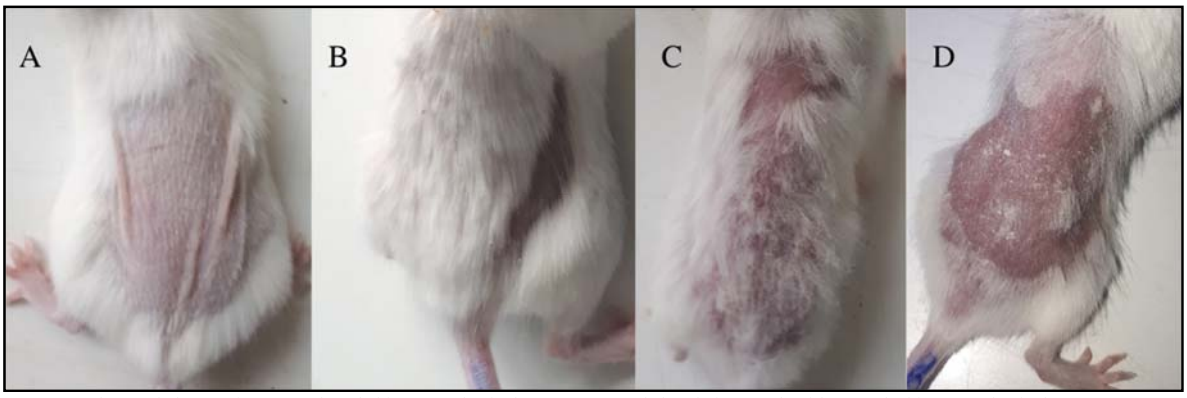

Figure 2. Observed changes in mouse dorsal skin. A: 0 physical score, even and slow hair growth with smooth skin. B: 2 physical score, with slightly irritated skin and a single area of complete hair loss. C: 4 physical score with several areas of hair loss and slightly rough skin. D: 6 physical score, near complete hair loss with rough, irritated skin.

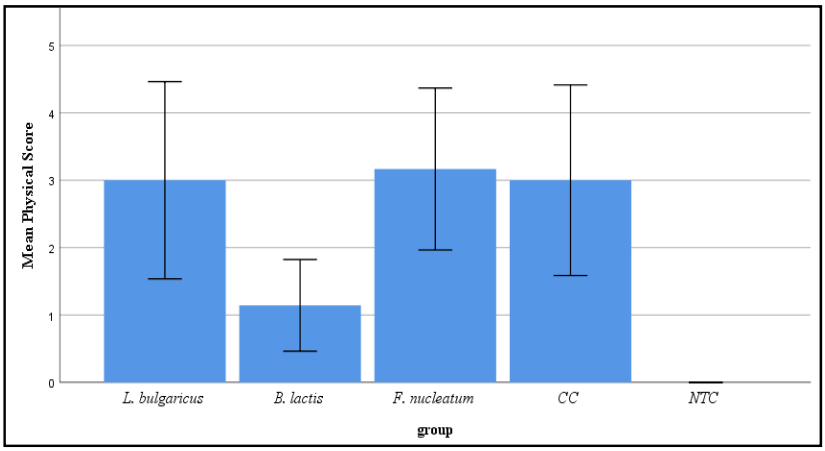

Figure 3. Mean physical score for mice. Mice were given a physical score of 0-6 depending on physical characteristics of treated skin (skin texture and hair loss) and showed no significant difference. Data represents Mean +/- SEM.CC: cancer control group. NTC: no treatment control.

3.2. L. bulgaricus and B. animalis groups had an increase in keratinocyte layer thickness

Following carcinogenesis treatment, mice were sacrificed and skin biopsies were taken from the treated skin and examined microscopically by a pathologist for changes between treatment groups. Keratinocytes layer thickness (as shown in Figure 4) was not significantly different *in L. bulgaricus*, *B. animalis*, or *F. nucleatum* groups in comparison with CC. *L. bulgaricus* and *B. animalis* groups had significantly higher keratinocytes layer thickness than the no treatment control group (p= 0.036, 0.009) (Figure 5).

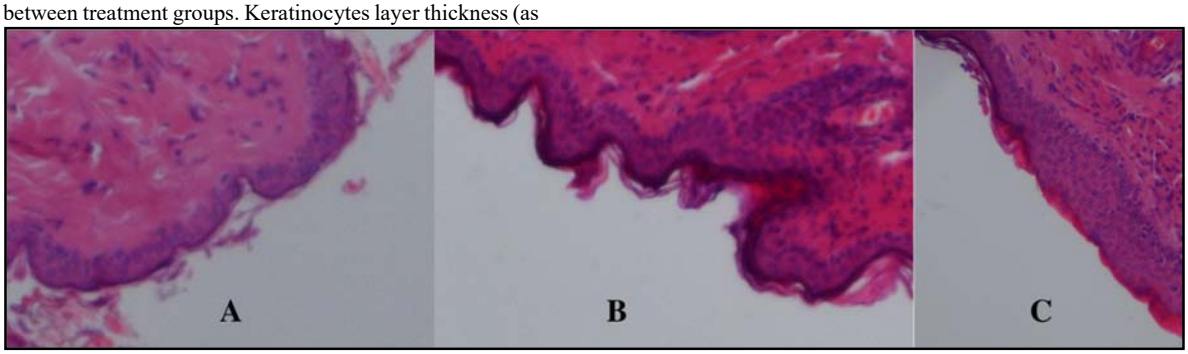

Figure 4. Microscopic examination of mouse skin. Mouse skin biopsies were examined at 100x magnification to determine characteristics such as keratinocyte layer thickness and mast cell invasion. A: 2-3 cell layers. B: 3-4 cell layers. C: focal keratinocytes proliferation.

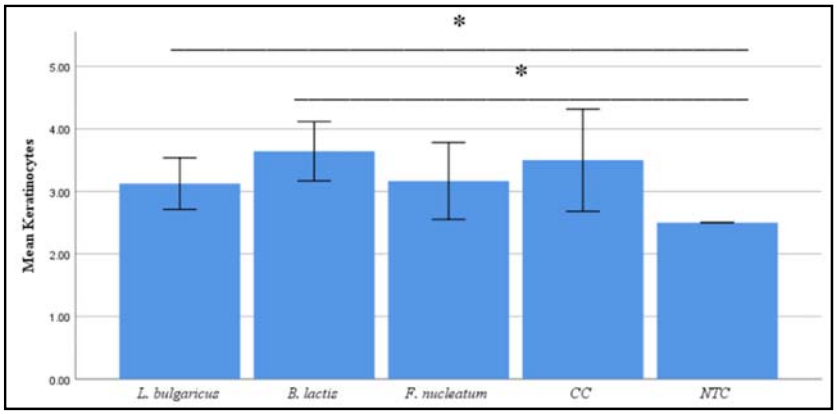

Figure 5. Mean number of keratinocyte layers in mouse skin. Microscopic examination of mouse skin biopsies showed no significant changes between groups given bacteria and the cancer control group. Data represents Mean +/- SEM. CC: cancer control group. NTC: no treatment control. *: p <0.05.

3.3. Mice treated with L. bulgaricus had more severe signs of dysplasia

Microscopic examination of mouse skin biopsies showed that, as detailed in (Table 4), mice treated with *B. animalis* had fewer focal papillomas than other groups. The *L. bulgaricus* group had the highest amount of mast cell invasion. One cancer control and two *L. bulgaricus* treated animals developed dysplasia.

Table 4: Mice subjects' results for the presence of focal apilloma, mast invasion, and dysplasia.

Subject	Focal papilloma	Degree of mast cell invasion	Dysplastic changes
NTC1	-	NONE	-
NTC2	-	RARE	-
NTC3	-	NONE	-
NTC4	-	RARE	-
NTC5	-	NONE	-
CC1	+	MILD	-
CC2	+	MILD	-
CC3	+	MILD	+
CC4	+	MILD	-
L1	+	MODERATE	+
L2	+	MILD	-
L3	-	MILD	-
L4	+	MODERATE	+
L5	+	MODERATE	-
L6	+	MODERATE	-
L7	+	MILD	-
L8	+	MILD	-
B1	-	MILD	-
B2	-	RARE	-
B3	-	MILD	-
B4	-	MILD	-
B5	-	MILD	-
B6	+	MILD	-
B7	+	MILD	-
F1	-	MILD	-
F2	-	MILD	-
F3	+	MILD	-
F4	+	MILD	-
F5	+	MILD	-
F6	+	MILD	-

NTC: no treatment control group, CC: cancer control group, L: *L. bulgaricus* group, B: *B. animalis* group, F: *F. nucleatum* gro

3.4. Mice treated with L. bulgaricus had significantly changed cytokine levels

Cancer-associated cytokines were examined by ELISA to study the systemic effects the bacterial treatment had on the mouse subjects. As shown in (Figure 6), the *F. nucleatum* group had significantly higher IL-10 serum levels than CC (p= 0.033) and no treatment control (p=0.008). The *L. bulgaricus* group had significantly higher IL-10 levels than no treatment control (p=0.026), significantly higher IL-6 levels (p= 0.017), and significantly lower TNFα levels (p=0.027) than cancer control.

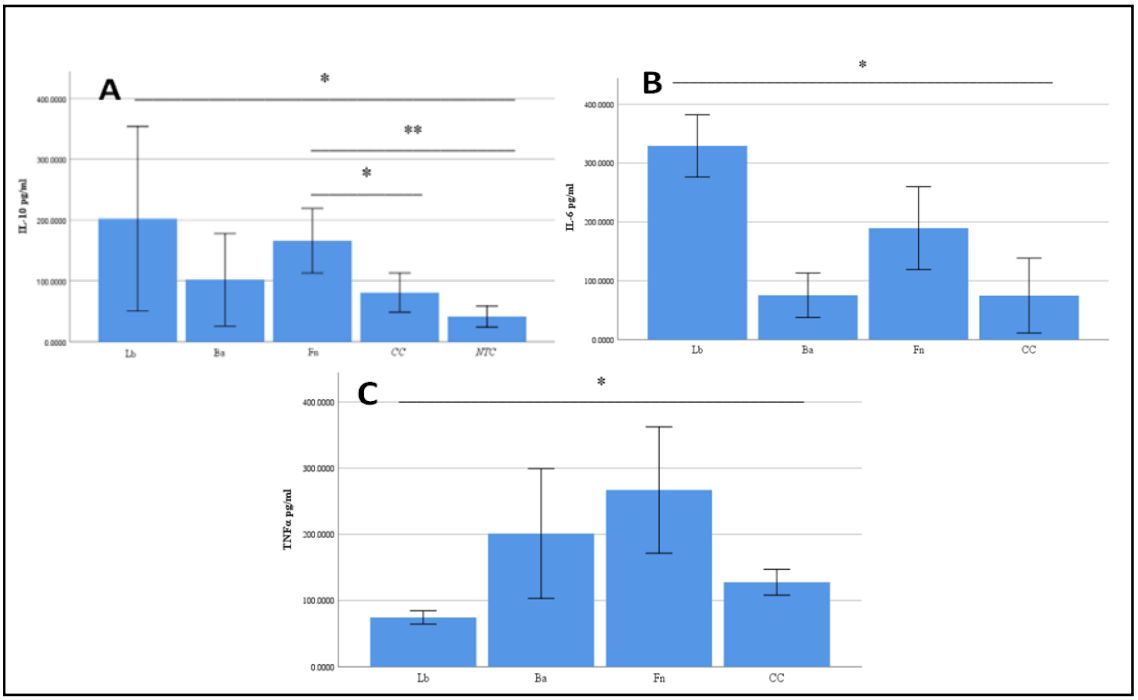

Figure 6. ELISA results for IL-10 (A) , IL-6 (B) , and TNFα (C). Cancer-associated cytokines were examined in mouse subjects' serum to determine the systemic effects of the bacteria administered. Data represents Mean +/- SEM. Lb*: L. bulgaricus* group. Ba: *B. animalis* group. Fn: *F. nucleatum* group. CC: cancer control group. NTC: no treatment control group. *: $p < 0.05$. **: $p < 0.01$.

4. Discussion

In the current study, mice were divided into 5 groups: the first is the No Treatment Control, which was only treated with vehicle (acetone) and no carcinogens or bacteria. This group was used to establish the healthy characteristics of the mice and determine if the changes observed were pathological or physiological. The second group was the Cancer Control (CC), where the cancer was induced but no bacteria were given. CC was used as a baseline to determine how carcinogenesis affected the mice. The 3 groups of interest were the ones treated with *F. nucleatum*, *B. animalis*, and *L. bulgaricus*. We focused on the effects the bacteria had on cancer initiation and not progression, and as such no macroscopic tumors were observed. Regarding the ELISA studies, the *F. nucleatum* group did not show significant changes from the CC group except for IL-10 levels in mouse serum. IL-10 is an anti-inflammatory cytokine that inhibits the activity of anti-tumor immunity.

It is possible in the long term that systematic effects of *F. nucleatum* would aid the progression of skin cancer. However, we observed no meaningful influence on initiation. Both probiotic groups, *B. animalis* and *L. bulgaricus*, had significantly higher non-papilloma keratinocytes thickness compared to the no treatment control group. Other probiotics, *L. rhamnosus* GG and *L. reuteri*, have been shown to stimulate keratinocyte proliferation in a wound-healing model (Mohammedsaeed *et al*., 2015). This suggests a possible beneficial effect for *B. animalis* and *L. bulgaricus* in wound healing. While *B. animalis* did not significantly change the levels of the 3 cytokines studied, it inhibited cancer initiation. This was evident on a microscopic scale with the *B. animalis* treated group showing much less mast cell invasion than other bacteria-treated groups. Furthermore, only 2 of the 7 mice in the *B. animalis* treated group developed papillomas which was less than that of the other bacteria-treated groups. A different strain of *B. animalis* was shown to inhibit NF-κB activity in intestinal epithelial cells (Kim *et al*., 2010b). This inhibition may be associated with cancer initiation.

Interestingly, the *L. bulgaricus* group had the worst outcome of all groups, with 2 mice showing dysplastic changes and a higher degree of mast cell invasion, as well significantly higher IL-6 levels and significantly lower TNFα serum levels. This result is consistent with a study of the effects of an *S. thermophilus* and *L. bulgaricus* yogurt culture on dextran sodium sulfate (DSS)-induced colitis. In this study, they observed that the culture increased IL-6 in the spleen. There was also an increase in T-regulatory cells in mesenchymal lymph nodes and peripheral blood in the yogurt culture treatment group (Wasilewska *et al*., 2019). As cancer prevention includes measures that can decrease the threat of developing cancer (Khabour *et al*., 2023), *L. bulgaricus* was proposed to be beneficial to the hosts with colitis and other diseases (Mahasneh *et al*., 2015). However, it was found in our study that later species may promote skin carcinogenesis.

5. Conclusions

Mice treated with *B. animalis* had mildly decreased signs of early carcinogenesis, while the mice treated with *L. bulgaricus* had a more severe outcome along with a significant change in cancer-promoting cytokines in serum. Administration of *F. nucleatum* significantly increased blood IL-10 concentration but did not show any significant effect on early cancer development in mice in the short term. However, we recommend exploring of the use of such probiotic bacteria in the presence of cancer-treatment drugs to evaluate their effect on cancer therapy.

References:

Cenit MC, Sanz Y, and Codoñer-Franch P. 2017. Influence of gut microbiota on neuropsychiatric disorders. *World J Gastroenterol.*, **23(30):** 5486–98.

Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, and Mofid V. 2011. Effect of probiotic yogurt containing *Lactobacillus acidophilus* and Bifidobacterium lactis on lipid profile in individuals with type 2 diabetes mellitus. *J Dairy Sci*., **94(7):** 3288–94.

Glassner KL, Abraham BP, and Quigley EMM. 2020. The microbiome and inflammatory bowel disease. *J Allergy Clin Immunol*., **145(1)**:16–27.

Guéniche A, Benyacoub J, Buetler TM, Smola H, and Blum S. 2006. Supplementation with oral probiotic bacteria maintains cutaneous immune homeostasis after UV exposure. *Eur J Dermatol*., **16(5):** 511–7.

Guha D, Banerjee A, Mukherjee R, Pradhan B, Peneva M, and Aleksandrov G. 2019. A probiotic formulation containing *Lactobacillus bulgaricus* DWT1 inhibits tumor growth by activating pro-inflammatory responses in macrophages. *J Funct Foods*., **56:**232–45.

Helmink BA, Khan MAW, Hermann A, Gopalakrishnan V, and Wargo JA. 2019. The microbiome, cancer, and cancer therapy. *Nat Med*., **25(3):** 377–88.

Khabour O, Alzoubi K, Alkofahi A, and Al-Awad R. 2023. Skin-Cancer Protective Effect of Ziziphus Spina-christi Leaf Extract: In vitro and in Vivo Models. *Jordan J Biol Sci*., **16(2)**: 243 – 248.

Kim JY, Kwon JH, Ahn SH, Lee S Il, Han YS, and Choi YO. 2010a. Effect of probiotic mix (Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus) in the primary prevention of eczema: A double-blind, randomized, placebo-controlled trial. *Pediatr Allergy Immunol*., **21**(2 PART 2).

Kim SW, Kim HM, Yang KM, Kim SA, Kim SK, and An MJ. 2010b. Bifidobacterium lactis inhibits NF-κB in intestinal epithelial cells and prevents acute colitis and colitis-associated colon cancer in mice. *Inflamm Bowel Dis*., **16(9):** 1514–25.

Kumar M, Kumar A, Nagpal R, Mohania D, Behare P, and Verma V. 2010. Cancer-preventing attributes of probiotics: An update. *Int J Food Sci Nutr*., **61(5):** 473–96.

Mahasneh A, Hamdan S, and Mahasneh S. 2015. Probiotic Properties of *Lactobacillus* Species Isolated from Local Traditional Fermented Products. *Jordan J Biol Sci*., **8(2)**: 81- 87.

Miller LE, Lehtoranta L, and Lehtinen MJ. 2017. The effect of bifidobacterium animalis ssp. lactis HN019 on cellular immune function in healthy elderly subjects: Systematic review and meta-analysis. *Nutrients*., **9(3)**.

Mohammedsaeed W, Cruickshank S, McBain AJ, and O'Neill CA. 2015. *Lactobacillus rhamnosus* GG Lysate Increases Re-Epithelialization of Keratinocyte Scratch Assays by Promoting Migration. *Sci Rep.*, **5**:1–11.

Moodycliffe AM, Nghiem D, Clydesdale G, and Ullrich SE. 2000. Immune suppression and skin cancer development: Regulation by NKT cells. *Nat Immunol*., **1(6)**:521–5.

Mrázek J, Mekadim C, Kučerová P, Švejstil R, Salmonová H, and Vlasáková J. 2018. Melanoma-related changes in skin microbiome. *Folia Microbiol (Praha)*., **64(3):** 435–42.

Nakatsuji T, Chen TH, Butcher AM, Trzoss LL, Nam SJ, and Shirakawa KT. 2018. A commensal strain of *Staphylococcus epidermidis* protects against skin neoplasia. *Sci Adv.*, **4(2)**.

Neish AS. 2017. Probiotics of the Acidophilus Group: *Lactobacillus acidophilus*, delbrueckii subsp. bulgaricus and johnsonii. The Microbiota in Gastrointestinal Pathophysiology. Elsevier Inc.

Polkowska-Pruszyńska B, Gerkowicz A, and Krasowska D. 2020. The gut microbiome alterations in allergic and inflammatory skin diseases – an update. *J Eur Acad Dermatology Venereol.*, **34(3):** 455–64.

Rusu E, Enache G, Cursaru R, Alexescu A, Radu R, and Onila O. 2019. Prebiotics and probiotics in atopic dermatitis (Review). *Exp Ther Med*., **2019:** 926–31.

Shang FM and Liu HL. 2018. Fusobacterium nucleatum and colorectal cancer: A review. *World J Gastrointest Oncol*., **10(3):** 71–81.

Sharma M, Wasan A, and Sharma RK. 2021. Recent developments in probiotics: An emphasis on Bifidobacterium. *Food Biosci*., **41(February):**100993.

Sheikhi A, Giti H, Heibor MR, Jafarzadeh A, Shakerian M, and Baharifar N. *Lactobacilus Delbrueckii* subsp. 2017. Bulgaricus Modulates the Secretion of Th1/Th2 and Treg Cell-Related Cytokines by PBMCs from Patients with Atopic Dermatitis. *Drug Res* (Stuttg)., **67(12):** 724–9.

Soa SSY, Wana MLY, and El-Nezami H. 2017. Probiotics-mediated suppression of cancer. *Curr Opin Oncol.*, **29(1):** 62–72.

Turroni F, Ventura M, Buttó LF, Duranti S, O'Toole PW, and Motherway MOC. 2014. Molecular dialogue between the human gut microbiota and the host: A Lactobacillus and Bifidobacterium perspective. *Cell Mol Life Sci*., **71(2):**183–203.

Uusitupa HM, Rasinkangas P, Lehtinen MJ, Mäkelä SM, Airaksinen K, Anglenius H, Ouwehand AC, and Maukonen J. 2020. Bifidobacterium animalis subsp. lactis 420 for Metabolic Health: Review of the Research. *Nutrients*., **12(4):**892.

Vähätupa M, Pemmari T, Junttila I, Pesu M, and Järvinen T.A. 2019. Chemical-Induced Skin Carcinogenesis Model Using Dimethylbenz[a]Anthracene and 12-O-Tetradecanoyl Phorbol-13-Acetate (DMBA-TPA). *J. Vis. Exp.*, **154**: e60445, doi:10.3791/60445.

Verma D, Garg PK, and Dubey AK. 2018. Insights into the human oral microbiome. *Arch Microbiol*., **200(4):** 525–40.

Wang Z, Zhong J, Meng X, Gao J, Li H, and Sun J. 2021. The gut microbiome-immune axis as a target for nutrition-mediated modulation of food allergy. *Trends Food Sci Technol*., **114**:116–32.

Wasilewska E, Zlotkowska D, and Wroblewska B. 2019. Yogurt starter cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* ameliorate symptoms and modulate the immune response in a mouse model of dextran sulfate sodium-induced colitis. *J Dairy Sci*., **102(1)**: 37–53.