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## RESEARCH ARTICLE

### The Influence of *Lactobacillus Acidophilus* on MUC1, GAL-3, IL-1 $\beta$ and IL-17 Gene Expression in BALB/c Mice Stomach

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#### Abstract:

#### Background and Objective:

*Lactobacillus acidophilus* has been widely used for the management of gastrointestinal carcinoma owing to its immunomodulation effect; however, the role of *L. acidophilus* and its specific mechanism of action in the stomach is not fully comprehended. The present study evaluated the expression profile of MUC-1, GAL-3, IL-1 $\beta$ , and IL-17 in the *L. acidophilus* treated mice stomach.

#### Methods:

The study was conducted utilizing three groups of mice, 6 mice for each group, administered with different doses of *L. acidophilus* and a control group treated with normal saline. The results were analyzed with the Mann-Whitney Test.

#### Results:

The results demonstrated that *L. acidophilus* elevated IL-1 $\beta$  insignificantly and inhibited the expression of IL-17. The MUC-1 expression is influenced by *L. acidophilus* and inversely proportional to GAL-3 expression.

#### Conclusion:

*Lactobacillus acidophilus* plays a prominent role against inflammatory responses and has a potential in the treatment of gastrointestinal cancer.

**Keywords:** *Lactobacillus acidophilus*, Gastrointestinal carcinoma, Immunomodulation, Muc-1, Gal-3, IL-1 $\beta$ , IL-17.

#### Article History

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## 1. INTRODUCTION

Probiotics are beneficial microorganisms that, when administered in adequate doses, provide a health benefit to humans [1]. Probiotics modulate the gastrointestinal tract by boosting the immune response and have a positive impact on gastrointestinal microflora [2]. Probiotics are known for their potential immunomodulatory properties and are often used for the therapeutic management of cancer [3].

*Lactobacillus* spp. are gram-positive, rod or coccobacilli shaped, non-spore forming and catalase-negative [4]. The bacteria adhere to the mucosal layer and epithelial surface of

the upper gastrointestinal tract [4]. The *in-vitro* and *in-vivo* analyses revealed that *Lactobacillus* spp. reduced the concentration of pro-inflammatory cytokines [3]. *Lactobacillus* spp. inhibit mutagenic compounds, which are associated with carcinogenesis and harmful enzymes [3]. *Lactobacillus* spp. interact with the epithelial cells through several mechanistic pathways that could modulate the host immune system, improve barrier integrity, and stimulate anti-inflammatory immune response [5]. An *in-vivo* study using the rat model demonstrated that *L. acidophilus* downregulated not only tumor incidence and tumor volume but also tumor multiplicity [3]. However, the specific mechanism of action of *L. acidophilus* has not been completely elucidated.

Gastric cancer is the second leading cause of cancer-related death because of its high incidence, poor prognosis, and high

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mortality rate, particularly in developing countries [6]. Environmental condition plays an essential role in the manifestation of gastric cancer [6]. The development of gastric carcinoma is closely associated with chronic inflammation [7]. Dietary factors and *H. pylori* infection act collectively and are believed to cause gastric carcinogenesis [6, 8]. *H. pylori* induce chronic inflammation that contributes to the development of carcinogenesis [9, 10].

MUC1 is membrane-bound mucin expressed on gastric mucosa and is known to limit pathogen infection and colonization [11]. MUC1 plays a role in counter regulate gastric inflammatory response to the pathogenic invasion of microbes [11]. MUC1 regulates inflammation during infection by modulating the NLRP3 inflammasome complex and as a negative regulator of TLR signaling. Gastric MUC1 forms a protective barrier that restricts the acute and chronic inflammation caused by colonization of *H. pylori* thereby impeding the infection [12].

Galectin-3 (Gal-3) is a  $\beta$ -galactoside-binding-lectin protein that is widely expressed in epithelial mucosa [13]. It is known to have pro-inflammatory activity and is involved in the recruitment of the immune cell [13]. Galectin-3 contributes to the adhesion of bacterial and tumor cells, inflammation, wound healing process, and immunity [14, 15]. Galectin-3, which can recognize PAMP (pathogen-associated molecular patterns) allows this glycan-binding protein to recognize pathogenic microorganisms and induces pro-inflammatory activity [16]. An increased concentration of Gal-3 expression is associated with the occurrence of gastric adenocarcinoma and other malignancies [17]. Little is known about Gal-3 interaction with commensal bacteria [13].

Interleukin-17 is a pro-inflammatory cytokine and is responsible for immune cell recruitment that helps in microbial elimination during infection. IL-17 stimulates cytokine production that induced inflammation response [18]. A previous study revealed that a high level of IL-17 is associated with tumor progression [19]. Therefore, it is believed that IL-17 plays a major role in the progression of gastric carcinogenesis.

The aim of the present study was to determine the MUC1, Gal-3, IL-1 $\beta$ , and IL-17 gene expression in the *L. acidophilus* treated mice stomach.

## 2. MATERIALS AND METHODS

### 2.1. Bacterial Strain and Culture Conditions

*L. acidophilus* (ATCC 4356) was purchased from Microbiologics™ Fisher Scientific, USA and is abbreviated as LA. Bacterial cultivation was carried out using MRS broth (Oxoid, CMO359) at 37 °C in microaerophilic condition comprising of 5% O<sub>2</sub>, 10% CO<sub>2</sub>, 85% N<sub>2</sub> (Campygen CN0035). The confirmation test to *Lactobacillus acidophilus* is determined with gram stain, carbohydrate fermentation, indol test and catalase test. Further, it was incubated for 24 h on MRS Agar and was harvested in 0.85% Na Cl. Three doses were prepared with a different bacterial density which was determined through a spectrophotometer (Thermo Spectrophotometer) at 600 nm and confirmed with plate count method.

The bacterial concentrations in the low dose, moderate dose, and high dose were approximately 10<sup>6</sup> CFU/mL (OD 0.3), 10<sup>7</sup> CFU/mL (OD 0.6), and 10<sup>8</sup> CFU/mL (OD 1.0), respectively. For long-term storage, *L. acidophilus* is maintained in MRS broth containing 20% glycerol at -80 °C.

### 2.2. Animal Study

24 BALB/c male mice were used in the study who were 7 weeks old, obtained from PT BIOFARMA, Bandung, Indonesia. Mice were kept in normal conditions of temperature and light and had access to food and water *ad libitum*. After seven days of the adaptation period, mice were divided into four groups, 6 mice for each group, namely the control group administered with normal saline (LA0), low-dose LA group (LA1), moderate dose LA group (LA2), and high-dose LA group (LA3). Mice were administered for 14 days with 0.5 mL LA suspension by orogastric gavage. Normal saline was given to LA0 group. On the 15<sup>th</sup> day, all mice were anesthetized with ketamine and sacrificed. Gastric tissues were collected and stored in RNAlater® tubes (Sigma, R0901) at -80 °C.

### 2.3. RNA Isolation

RNA extraction was done by homogenizing the gastric tissue using TRIzol Reagent (Invitrogen, USA). The extract was then centrifuged at 14000  $\times$ g in a column tube following the manufacturer's protocol. The RNA pellet was collected and purified using phenol-chloroform extraction and ethanol precipitation and was further dissolved in TAE buffer pH 8.0. The quantification of RNA was done using Nanodrop spectrophotometer ND-100 (Thermo Scientific, USA).

### 2.4. Real-Time PCR (qRT - PCR)

One-step qRT-PCR kit (Kapa Biosystem, USA) was used to study the mRNA expression. Primers were designed using *forward* and *reverse* sequences, as shown in Table 1. Primer designs were determined by Integrated DNA Technologies (IDT) Reference Sequences on *MUC1* (IDT DNA Ref No 102171764-102171765), *GAL-3* (IDT DNA Ref No 102171768-102171769), *IL-1 $\beta$*  (IDT DNA Ref No 102171770-102171771), *IL-17* (IDT DNA Ref No 102171772-102171773) and GAPDH (IDT DNA Ref No 102389365-102389366). The qPCR reaction was preceded by reverse transcription at 42 °C for 5 min followed by enzyme inactivation of reverse transcriptase (RT) at 95°C for 3 min. The PCR process was repeated for 40 cycles. The PCR requires temperature cycling for different stages of the reaction to occur. The denaturation of the dsDNA was done at 95 °C for 30 s followed by annealing of the primer at 60 °C for 20 s and extension was carried out at 72°C for 20 s. The results were presented as Ct value. The average Ct value was calculated for the targeted genes and the internal control gene (GAPDH). The  $\Delta$ Ct was obtained from the difference in threshold cycles for targeted genes and GAPDH. Gene expression was concluded from 2<sup>- $\Delta$ Ct</sup> [20].

### 2.5. Statistical Analysis

The experimental data were documented as mean  $\pm$ SD. The data were statistically analyzed by Mann Whitney test. The differences were considered statistically significant at p < 0.05.

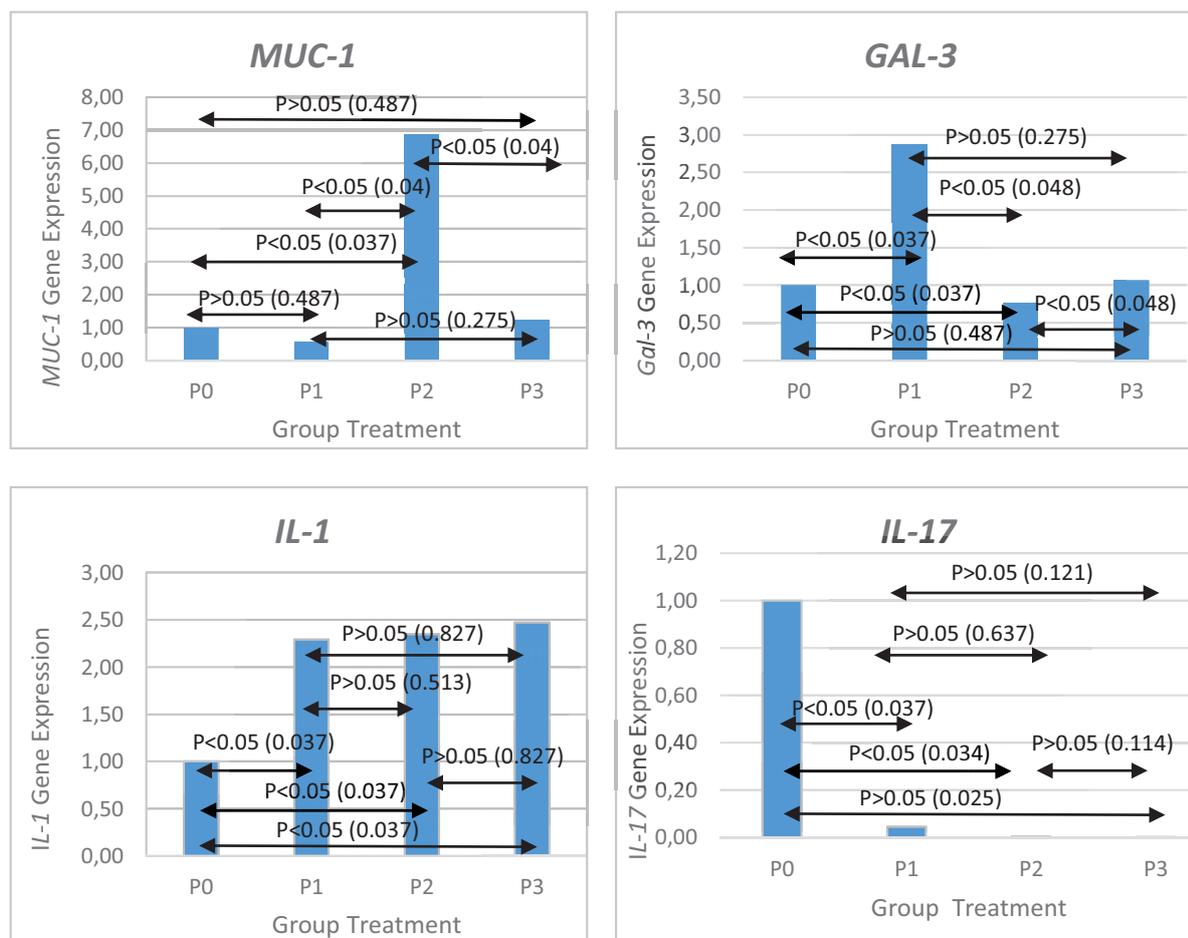
**Table 1. Primers designed for qPCR.**

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
MUC-1	CTT CCT GCT GCT ACT TCT AG	TGG TCG ATG CTA AGG AA
GAL-3	CGC ATG CTG ATC ACA AT	GTC CTG CTT CGT ACA CA
IL-1 $\beta$	CCA TTG ACC ATC TCT CTC TG	ACT CAG TCT CTT CTT CA
IL-17	GAG CTT CAT CTG TGT CTC TG	GAG GTT GAC CTT CAC ATT CT
GAPDH	TCA AGA TGG TGA AGC AG	ATG TAG GCC ATG AGG TCC AC

**3. RESULTS**

Administration of *L. acidophilus* at low moderate, and high concentration caused a 2-fold increase in IL-1 $\beta$  gene expression when compared with control (p <0.05), but the result did not show any significant difference. IL-1 gene expression were 2.29  $\pm$  1.12 for low concentration, 2.35  $\pm$  2,41 for moderate concentration and 2.51  $\pm$  1.27 at high concentration. Administration of *L. acidophilus* at low, moderate and high concentrations decreased IL-17 gene expression compared to the control group (p <0.05) but was not found significant. IL-17 gene expression were 0.04  $\pm$  0.05 for low concentration, 0.02  $\pm$  0.02 for moderate concentration and

0.00  $\pm$  0.0 for high concentration. MUC-1 gene expression after *L. acidophilus* administration revealed that it decreases at low concentrations (0.57  $\pm$  0.63 times), gets slightly increased at high concentrations (1.24  $\pm$  0.86 times), but an increase in the concentration of about 6.85  $\pm$  2.65 times was noticed at moderate concentrations (p <0.05). We noticed that Gal-3 gene expression increase at low concentration (2.87  $\pm$  1.28 times), decrease at a moderate concentration (0.77  $\pm$  0.14 times) and slightly increased at high concentration (1.07  $\pm$  0.04 times). The increased expression of mucin after administrating *L. acidophilus* at a certain concentration results in low Gal-3 expression and *vice versa*. Gene expression after administration of *L. acidophilus* is shown in Fig. (1).



**Fig. (1).** Gene expression of MUC-1,GAL-3,IL-1 $\beta$  and IL-17 after administration of *L.acidophilus*. P0 = control group, P1= Low concentration (10<sup>6</sup> CFU/ml), P2 = Moderate concentration (10<sup>7</sup> CFU/ml), P3 = High concentration (10<sup>8</sup> CFU/ml).

#### 4. DISCUSSION

The variation in *L. acidophilus* concentration was intended to determine whether the difference in the probiotic concentration causes different effects on the gene expression of *MUC-1*, *GAL-3*, *IL-1 $\beta$* , and *IL-17*. The probiotic concentration that gives the best effect is expected to be used as a reference dose for the management of diseases.

From the result of *IL-1 $\beta$*  gene expression, *L. acidophilus* administration caused a 2-fold increase in *IL-1 $\beta$*  gene expression when compared with control ( $p < 0.05$ ). Jiang *et al.* showed that *L. acidophilus* induced *IL-1* production [21]. In contrast to *L. acidophilus* and *L. sakei*, *L. johnsonii* did not induce *IL-1 $\beta$*  mRNA expression in studies with Caco2-cells [22]. We noticed that probiotic strains will induce different proinflammation cytokines. Induction of pro-inflammatory cytokines in probiotic administration is likely to occur because probiotics and commensal bacteria have PAMP (pathogen-associated molecular patterns) recognized by PRRs (pattern-recognition receptors) expressed in immune cells [23]. The administration of *L. acidophilus* increases bacterial concentration, followed by the increase of *IL-1 $\beta$*  gene expression ( $p > 0.05$ ). The absence of a significant difference between concentration could interpret that the administration of a higher concentration of *L. acidophilus* will not induce the inflammatory response that will damage the gastric mucosa.

Bacterial infection will increase *IL-17* levels and can be found in high levels in chronic inflammation. In this study, we found that *L. acidophilus* administration at low, moderate and high concentrations decreased *IL-17* gene expression compared to the control group ( $p < 0.05$ ). This may be due to the fact that *L. acidophilus* has the potential to inhibit *IL-17* activity. Therefore, *L. acidophilus* is suggested to play a role in gastric tissue damage that promotes gastric carcinogenesis. Chen *et al.* confirmed that proinflammatory cytokine *IL-17* in *L. acidophilus* administration may result in the downregulation of *IL-23*, *TGF $\beta$ 1* and *STAT3* [24]. Research conducted by Yang Wu *et al.* demonstrated that in *Histoplasma capsulatum* infection, *IL-23/17* axis in dendritic cells was negatively regulated by galectin-3.

Interleukin 17 is known to promote cytokine production to induce inflammation such as *IL-1*, *IL-6*, *TNF $\alpha$* , chemokines and CSFs infection [18, 25]. Based on this study, we also suspected that *IL-1 $\beta$*  expression was not associated with *IL-17*.

*MUC-1* gene expression after *L. acidophilus* administration revealed that it decreases at low concentrations (0.57 times), gets slightly increased at high concentrations (1.24 times), but an increase in the concentration of about 6.85 times was noticed at moderate concentrations ( $p < 0.05$ ). The difference in concentration of *L. acidophilus* seems to determine the expression of mucin in the stomach. The scientific community is still searching for possible mechanisms associated with probiotic density and mucin expression. Mucin in the gastrointestinal tract plays an important role in microbial attachment [26]. This layer forms the first physical barrier that provides protection to the inflammatory responses resulting from microbial infection, such as *Helicobacter pylori* [26]. In this study, we found that *L. acidophilus* induces *MUC-1*

expression in order to inhibit or reduce the adherence of pathogenic microbes to gastric epithelial cells. Decreased microbial pathogens adherence will reduce tissue damage due to activated proinflammation cytokines. *L. acidophilus* was known to play a role in regulating and modulating the protection action of intestinal epithelial tight junction in studies with Caco-2 cell [27]. Further research is needed to determine the specific mechanism of action of *L. acidophilus* in influencing the barrier function in the stomach.

In this study, it is revealed that the expression of *GAL-3* seems to be associated with the concentration of *L. acidophilus* and the *MUC-1* expression. The increased expression of mucin after administering *L. acidophilus* at a certain concentration results in low *Gal-3* expression and vice versa. We are still verifying whether there is a reciprocal relationship between *MUC-1* and *GAL-3*. Piyush *et al.* demonstrated that galectin-3 and *MUC-1* interactions cause *EGFR* dimerization and activation in epithelial cancer cells [28]. Research conducted by Merlin *et al.* showed that in pancreatic cancer cell *GAL-3* regulated the *MUC-1* transcription [29].

Unfortunately, because the material used in this study was gastric tissue, so it was not possible to carry out a cohort study in which the genes expression was determined prior to administration of *L. acidophilus* and compared their genes expression after treatment. By knowing the initial gene expression before treatment, the effect of *L. acidophilus* on the expression of these genes will be more clearly described.

#### CONCLUSION

*L. acidophilus* plays an important role in *MUC-1*, *GAL-3* and *IL-17* expression. Therefore, it may be suggested that *L. acidophilus* can be used in the prevention and treatment of tissue damage due to gastric inflammation. A further detailed study on *L. acidophilus* is needed to understand its specific mechanism of action and its role in stomach immunomodulation.

#### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Research Ethics Committee of Padjadjaran University, Indonesia with Approval No. 977/ UN6.KEP/EC/2019.

#### HUMAN AND ANIMAL RIGHTS

No humans were part of this study. This study is performed in accordance with the "Guide for the Care and Use of Laboratory Animals" eighth edition, 2011.

#### CONSENT FOR PUBLICATION

Not applicable.

#### AVAILABILITY OF DATA AND MATERIALS

The data supporting the finding of the article is available in the Zenodo Repository at zenodo.org, reference number: <https://doi.org/10.5281/zenodo.5006563>.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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## REFERENCES

- [1] Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A. Probiotic mechanisms of action. *Ann Nutr Metab* 2012; 61(2): 160-74. [http://dx.doi.org/10.1159/000342079] [PMID: 23037511]
- [2] Chen CC, Louie S, Shi HN, Walker WA, Gung C. Preinoculation with the probiotic *Lactobacillus acidophilus* early in life effectively inhibits murine *Citrobacter rodentium* colitis. *Pediatr Res* 2005; 58(6): 1185-91. [http://dx.doi.org/10.1203/01.pdr.0000183660.39116.83] [PMID: 16306191]
- [3] Górska A, Przystupski D, Niemczura MJ, Kulbacka J. Probiotic bacteria: A promising tool in cancer prevention and therapy. *Curr Microbiol* 2019; 76(8): 939-49. [http://dx.doi.org/10.1007/s00284-019-01679-8] [PMID: 30949803]
- [4] Varankovich NV, Nickerson MT, Korber DR. Probiotic-based strategies for therapeutic and prophylactic use against multiple gastrointestinal diseases. *Front Microbiol* 2015; 6(7): 685. [http://dx.doi.org/10.3389/fmicb.2015.00685] [PMID: 26236287]
- [5] Maldonado Galdeano C, Cazorla SI, Lemme Dumit JM, Vélez E, Perdígón G. Beneficial effects of probiotic consumption on the immune system. *Ann Nutr Metab* 2019; 74(2): 115-24. [http://dx.doi.org/10.1159/000496426] [PMID: 30673668]
- [6] Shi J, Qu YP, Hou P. Pathogenetic mechanisms in gastric cancer. *World J Gastroenterol* 2014; 20(38): 13804-19. [http://dx.doi.org/10.3748/wjg.v20.i38.13804] [PMID: 25320518]
- [7] Erik P, Lillehoj, Wei Guang, Hua Ding SJC and TGB. *Helicobacter pylori* and gastric inflammation: The role of MUC 1 Mucin. *Helicobacter* 2013; 2(3): 125-32. [http://dx.doi.org/10.3233/JPB-2012-00058]
- [8] Nagini S. Carcinoma of the stomach: A review of epidemiology, pathogenesis, molecular genetics and chemoprevention. *World J Gastrointest Oncol* 2012; 4(7): 156-69. [http://dx.doi.org/10.4251/wjgo.v4.i7.156] [PMID: 22844547]
- [9] Kim Sung Soo, Ruiz Victoria E, Carroll Jaqueline D. *Helicobacter pylori* in the pathogenesis of gastric cancer and gastric lymphoma I Department. *Helicobacter* 2012; 305(2): 228-38. [http://dx.doi.org/10.1016/j.canlet.2010.07.014]
- [10] Sekirov I, Russell S, Antunes L. Gut microbiota in health and disease. *Physiol Rev* 2010; 90(3): 859-904. [http://dx.doi.org/10.1152/physrev.00045.2009]
- [11] Dhar P, McAuley J. The role of the cell surface mucin MUC1 as a barrier to infection and regulator of inflammation. *Front Cell Infect Microbiol* 2019; 9(4): 117. [http://dx.doi.org/10.3389/fcimb.2019.00117] [PMID: 31069176]
- [12] McGuckin MA, Every AL, Skene CD, *et al.* Muc1 mucin limits both *Helicobacter pylori* colonization of the murine gastric mucosa and associated gastritis. *Gastroenterology* 2007; 133(4): 1210-8. [http://dx.doi.org/10.1053/j.gastro.2007.07.003] [PMID: 17919495]
- [13] Fowler M, Thomas RJ, Atherton J, Roberts IS, High NJ. Galectin-3 binds to *Helicobacter pylori* O-antigen: it is upregulated and rapidly secreted by gastric epithelial cells in response to *H. pylori* adhesion. *Cell Microbiol* 2006; 8(1): 44-54. [http://dx.doi.org/10.1111/j.1462-5822.2005.00599.x] [PMID: 16367865]
- [14] Kavanaugh D, Kane M, Joshi L, Hickey RM. Detection of galectin-3 interaction with commensal bacteria. *Appl Environ Microbiol* 2013; 79(11): 3507-10. [http://dx.doi.org/10.1128/AEM.03694-12] [PMID: 23524672]
- [15] Chen HY, Weng IC, Hong MH, Liu FT. Galectins as bacterial sensors in the host innate response. *Curr Opin Microbiol* 2014; 17: 75-81. [http://dx.doi.org/10.1016/j.mib.2013.11.006] [PMID: 24581696]
- [16] Vasta GR. Roles of galectins in infection. *Nat Rev Microbiol* 2009; 7(6): 424-38. [http://dx.doi.org/10.1038/nrmicro2146] [PMID: 19444247]
- [17] Subhash VV, Shi S, Ling M, Ho B. Extracellular galectin-3 counteracts adhesion and exhibits chemoattraction in *Helicobacter pylori* - infected gastric cancer cells. 2016; pp. 1360-6. [http://dx.doi.org/10.1099/mic.0.000322]
- [18] Abbas AK, Lichtman AH, Pillai S. *Cellular and Molecular Immunology Source*. 7th ed. Elsevier Saunders 2012.
- [19] Iida T, Iwahashi M, Katsuda M, *et al.* Tumor-infiltrating CD4+ Th17 cells produce IL-17 in tumor microenvironment and promote tumor progression in human gastric cancer. *Oncol Rep* 2011; 25(5): 1271-7. [http://dx.doi.org/10.3892/or.2011.1201] [PMID: 21369705]
- [20] Budiman, Zoraya Feranthy MNS. The existence of mRNAs and miRNAs expressions for maintaining cell survival networks associated with the human transparent and cataractous lens. *Source* 2015; 3(1): 1-8.
- [21] Jiang Y, Lü X, Man C, *et al.* *Lactobacillus acidophilus* induces cytokine and chemokine production via NF- $\kappa$ B and p38 mitogen-activated protein kinase signaling pathways in intestinal epithelial cells 2012; 603-8. [http://dx.doi.org/10.1128/CI.05617-11]
- [22] Haller D, Bode C, Hammes WP, Pfeifer AMA, Schi EJ, Blum S. Non-pathogenic bacteria elicit a di V erential cytokine response by intestinal epithelial cell / leucocyte co-cultures. 2000; pp. 79-87.
- [23] Azad M, Kalam A, Sarker M, Wan D. Immunomodulatory Effects of Probiotics on Cytokine Profiles. In: *BioMed research international*. 2018; 23: p. 2018. [http://dx.doi.org/10.1155/2018/8063647]
- [24] Chen L, Zou Y, Peng J, *et al.* *Lactobacillus acidophilus* Suppresses Colitis-Associated Activation of the IL-23 / Th17 Axis. 2015. [http://dx.doi.org/10.1155/2015/909514]
- [25] Onishi RM, Gaffen SL. Interleukin- 17 and its target genes: mechanisms of interleukin- 17 function in disease. 2010; pp. 311-21. [http://dx.doi.org/10.1111/j.1365-2567.2009.03240.x]
- [26] Quintana-hayashi MP, Padra M, Lind SK. Mucus-Pathogen Interactions in the Gastrointestinal Tract of Farmed Animals. 2018; pp. 1-21. [http://dx.doi.org/10.3390/microorganisms6020055]
- [27] Lépine AFP, de Wit N, Oosterink E, Wichers H, Mes J, de Vos P. *Lactobacillus acidophilus* attenuates *salmonella*-induced stress of epithelial cells by modulating tight-junction genes and cytokine responses. *Front Microbiol* 2018; 9(7): 1439. [http://dx.doi.org/10.3389/fmicb.2018.01439] [PMID: 30013538]
- [28] Piyush T, Chacko AR, Sindrewicz P, Hilkins J, Rhodes JM, Yu LG. Interaction of galectin-3 with MUC1 on cell surface promotes EGFR dimerization and activation in human epithelial cancer cells. *Cell Death Differ* 2017; 24(11): 1937-47. [http://dx.doi.org/10.1038/cdd.2017.119] [PMID: 28731466]
- [29] Monte D, Leteurte E, Van Seuningen I. Galectin-3 regulates MUC1 and EGFR cellular distribution and EGFR downstream pathways in pancreatic cancer cells. 2011; pp. 2514-25. [http://dx.doi.org/10.1038/nc.2010.631]