International Food Research Journal 29(2): 258 - 264 (April 2022)
Journal homepage: *http://www.ifrj.upm.edu.my*

Multi-strain probiotics combined with fruit-vegetable powders for regulating intestinal inflammation and intestinal epithelial barrier

[1]Lin, P., [2]Hsu, Y. C., [3]Lin, Y. K., [4]Lin, Y. H., [1]Lin, Y. H. and [1]*Chiang, C. F.

[1]*Research and Design Centre, TCI CO., Ltd., Taipei 114, Taiwan*
[2]*Research and Design Centre, TCI Gene Inc., Taipei 114, Taiwan*
[3]*Institute of Food Safety and Risk Management, National Taiwan Ocean University, Keelung City 202, Taiwan*
[4]*Global Business Centre, TCI CO., Ltd., Taipei 114, Taiwan*

Article history

Received:
16 October 2020
Received in revised form:
31 March 2021
Accepted:
30 July 2021

Keywords

fruit-vegetable powders, intestinal inflammation, probiotic, ZO-1

Abstract

The health-promoting effects of probiotics include maintenance of normal intestinal microbiota, increased nutritional value of foods, and immune system stimulation. Multi-strain probiotics have recently been proposed as health-enhancing foods and functional food ingredients. Fruit-vegetable powders (FVP), being a kind of prebiotic, are food supplements that are non-digestible by the host, but can improve the host's health by selectively stimulating the growth or activities of gastrointestinal tract bacteria. However, the intestinal efficacy of multi-strain probiotics combined with FVP remains unclear. Therefore, the purpose of the present work was to explore the effect of multi-strain probiotics combined with FVP on intestinal inflammation. Lipopolysaccharide (LPS) was used to treat RAW264.7, which was then co-cultured with Caco-2 cells to mimic the intestinal inflammatory environment. Caco-2 cells were incubated with various probiotics and FVP (0.125 and 0.25 mg/mL). The inflammatory cytokines from the medium were collected for ELISA analysis, and the ZO-1 expression in the Caco-2 cells was examined by fluorescence assay. Probiotics combined with FVP significantly decreased the inflammatory cytokines, IL-6, and TNF-α, and increased ZO-1 expression when compared with the LPS only group. Probiotics combined with FVP could decrease inflammatory cytokines, and protect the intestinal barrier from tight junction dysregulation.

Introduction

Probiotics are live microorganisms, most of which are bacteria, similar to the beneficial bacteria naturally occurring in the human gut (Wilkins and Sequoia, 2017). Once probiotics enter the human body, they produce microbial transformations in the intestinal flora, thus exerting various health-promoting properties including maintaining the intestinal barrier function, regulating the host immune system, and stimulating intestinal epithelial cell proliferation (Molska and Regula, 2019). There are many types of probiotics. Strains belonging to *Lactobacillus* and *Bifidobacterium* are the most widely used probiotic bacteria (Zhou *et al*., 2005).

Several studies showed that a combination of specific bacterial strains could act in optimal synergy for restoring intestinal balance. Recently, studies showed that combining two *Lactobacillus* could enhance and improve gastrointestinal metabolism (Prete *et al*., 2020). The combination of *L. delbrueckii* and *B. animalis* significantly reduced gastrointestinal inflammation (Li *et al*., 2019). *L. plantarum* with biologically active compounds could inhibit the occurrence of colorectal cancer (Sivamaruthi *et al*., 2020). In addition, commercial probiotic products which combined *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* are widely used to reduce inflammation, and form a biological protection barrier (Fan *et al*., 2006). Therefore, multi-strain probiotics could improve intestinal inflammation; but the effects of commercially available probiotic products are still limited.

Fruit-vegetable powders (FVP) are prebiotics which may be used as an alternative to probiotics, or supplementary support for probiotics (Markowiak and Slizewska, 2017; Shende and Datta, 2019). Prebiotics can stimulate the growth of intestinal bacteria, and modify the intestinal flora (Huang *et al*., 2019). One study indicated that using *Lactobacillus*

*Corresponding author.
Email: Jimmy.Chiang@tci-bio.com

DOI:
https://doi.org/10.47836/ifrj.29.2.04

combined with FVP increased the vitamins and carotenoids levels, and exerted no side effects on metabolic organs such as the liver or kidney (Zhai *et al*., 2013). Most probiotics products combined with prebiotics are presented in the form of dietary supplements or nutrition and health products (Davani-Davari *et al*., 2019), which often lead to flatulence and digestive tract discomfort. Therefore, we explored whether multi-strain probiotics combined with FVP could improve intestinal inflammation.

The present work used monocytes, RAW264.7, co-cultured with intestinal epithelial cell lines, Caco-2, to mimic the intestinal inflammatory environment. This was followed by multi-strain probiotics (*L. acidophilus*, *L. casei*, *L. lactis*, *B. longum*, *B. bifidum*, and *B. infantis*) combined with FVP to treat Caco-2 cells, and the intestinal inflammation and the epithelial barrier were then examined.

Materials and methods

Cell culture and reagents

Caco-2 intestinal epithelial cells and RAW264.7 monocytes were purchased from the American Type Culture Collection (ATCC). Both cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated FBS (FBS; Gibco BRL, Grand Island, NY, USA), 100 U/mL penicillin (Thermo, Wilmington, DE, USA), and 100 U/mL streptomycin (Thermo, Wilmington, DE, USA) under 5% CO_2 at 37°C. LPS (Cell Signalling, Danvers, MA, US) and probiotics combined with FVP (3:1) (*Lactobacillus* Enzyme Plus Powder, Hangzhou Yosto Cosmetics Co., Ltd, Hangzhou, China; TCI CO., Ltd., Taipei, Taiwan) were obtained for cell proliferation and *in vitro* studies.

Co-culture of RAW264.7 and Caco-2 cells

Caco-2 cells were seeded onto transwell insert plates (0.4 μm pore size; Corning Costar Corp., NY, USA) at 5×10^5 cells per well. The cell culture medium was changed every two to three days. RAW264.7 cells were seeded on the basolateral side of the transwell at 2×10^5 cells per well, and incubated for 24 h to facilitate complete adherence to the well. After 24 h, all media were replaced with serum-free DMEM. The "mock group" was the macrophages co-cultured with Caco-2 to analyse the inflammatory cytokine on Caco-2 cells. The "LPS group" was LPS stimulated macrophages first, and then co-cultured with Caco-2 cells to analyse the inflammatory cytokine on Caco-2 cells. The "probiotics combined with FVP group" was LPS stimulated macrophages first, and then co-cultured with Caco-2 cells, and then FVP-treated Caco-2 cells were used to analyse the inflammatory cytokine on Caco-2 cells.

Enzyme-linked immunosorbent assay (*ELISA) assay*

Following treatment for 24 h, the supernatants were collected, and the levels of human IL-6 and TNF-α were determined using an USCN ELISA kit (USCN, Wuhan, China) following the manufacturer's instructions. Each experiment was performed in triplicate, and repeated twice to assess the consistency of the results.

Fluorescence assay

Caco-2 cells were plated at a density of 5×10^4 cells/cm^2, and then fixed in 4% formaldehyde at room temperature for 30 min. Then, filters were permeabilised in a solution of 0.25% (w/v) Triton X-100 (Sigma-Aldrich) in PBS for 10 min, and then blocked with 5% bovine serum albumin for 1 h. After blocking, the cells were incubated with primary anti-ZO1 antibodies (ab96587) for 1 h, and then labelled with fluorescence-labelled antibodies (Thermo Fisher Scientific). The signal intensities of the acquired images were analysed using LSM software ZEN 2009.

Statistical analysis

All data were presented as means ± standard deviation (SD). Student *t*-test was used to compare the experimental results, with $p < 0.05$ considered as significant difference.

Results

Multi-strain probiotics combined with FVP decreased TNF-α cytokine

To examine whether probiotics combined with FVP could affect TNF-α cytokine expression in the intestinal environment, we used LPS (1 μg/mL) to treat RAW264.7 cells, and then co-cultured with Caco-2 cells to mimic the intestinal inflammatory environment. Caco-2 cells were incubated with various concentrations of probiotics combined with FVP (0.125 and 0.25 mg/mL) for 6 h, and then the

medium was collected for ELISA analysis. The ELISA assay showed that LPS could induce inflammatory cytokine such as tumour necrosis factor-alpha (TNF-α) (Figure 1). It was found that probiotics combined with FVP treatment significantly decreased TNF-α cytokine expression in a dose-dependent manner in the intestinal environment when compared with LPS only group (Figure 1).

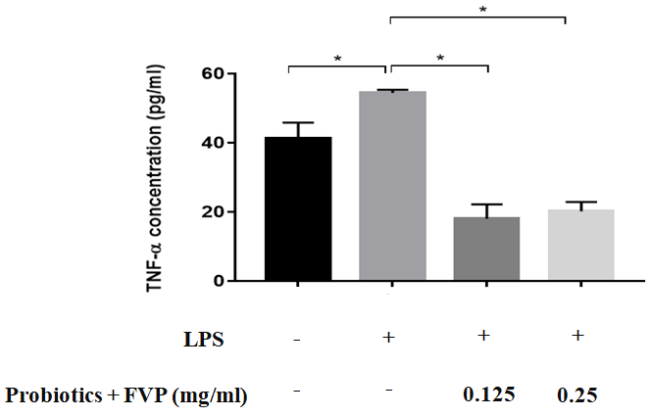

Figure 1. Multi-strain probiotics combined with FVP decreased TNF-α cytokine. LPS (10 µg/mL) was used to treat RAW264.7, and then co-cultured with Caco-2 cells to mimic the intestinal inflammatory environment. Caco-2 cells were incubated with probiotics and FVP (0.125 and 0.25 mg/mL) for 6 h. Incubations were terminated by media collection, and ELISA analysis was then performed. Values are means with error bars indicating S.D. of three experiments (n = 3). *significant difference at p < 0.05.

Multi-strain probiotics combined with FVP decreased IL-6 cytokine

Next, we examined whether probiotics combined with FVP could affect IL-6 cytokine expression. We used LPS to treat RAW264.7 cells, and then co-cultured with Caco-2 cells. Next, the Caco-2 cells were incubated with probiotics combined with FVP, and the medium was collected for ELISA analysis. The ELISA assay showed that LPS could induce inflammatory cytokine such as interleukin-6 (IL-6) (Figure 2). It was found that probiotics combined with FVP significantly decreased IL-6 cytokine expression in a dose-dependent manner when compared with the LPS only group (Figure 2).

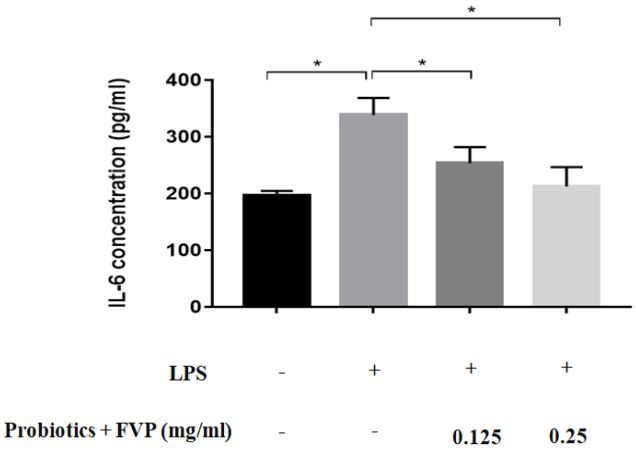

Figure 2. Multi-strain probiotics combined with FVP decreased IL-6 cytokine. LPS (10 µg/mL) was used to treat RAW264.7, and then co-cultured with Caco-2 cells to mimic the intestinal inflammatory environment. Caco-2 cells were incubated with probiotics and FVP (0.125 and 0.25 mg/mL) for 6 h. Incubations were terminated by media collection, and ELISA analysis was then performed. Values are means with error bars indicating S.D. of three experiments (n = 3). *significant difference at p < 0.05.

Multi-strain probiotics combined with FVP protected the intestinal barrier

The above results showed probiotics combined with FVP reduced intestinal inflammation. Intestinal barrier homeostasis is disrupted through tight junction protein dysregulation including microbial degradation, and exposure to proinflammatory cytokines. In addition, zonula occludens (ZO-1) are multi-domain scaffolding proteins required for the formation of the tight junction. ZO-1 is likely to play an important role in tight junction assembly. Further, we explored whether probiotics combined with FVP could protect the intestinal barrier. We used LPS to treat RAW264.7 cells, and then co-cultured with Caco-2 cells. After that, Caco-2 cells were incubated with various concentrations of probiotics combined with FVP, and then examined for fluorescence assay. Hoechst 33342 nucleic acid stain was used as a nuclear counterstain (blue colour), and ZO-1 was conjugated with red fluorescent protein (red colour). The fluorescence assay showed that ZO-1 expression decreased with LPS treatment alone (Figure 3). Moreover, we also found that ZO-1 expression increased when combining probiotics with FVP treatment (0.125 mg/mL) (Figure 3), thus suggesting

that probiotics combined with FVP could protect the intestinal barrier from tight junction dysregulation.

Discussion

The gastrointestinal tract is the most important digestive and immune organs of the human body (Pierre *et al*., 2016). There are more than 1,000 microbial groups in the gut, and these participate in the host's nutritional metabolism, excretion, transformation, and form a close symbiotic relationship between intestinal flora of the host (Pierre *et al*., 2016). Normal intestinal flora can maintain the integrity of the intestinal mucosal barrier while increasing host immunity (Chelakkot *et al*., 2018). A study has shown that abnormal intestinal flora were associated with metabolic diseases (Eid *et al*., 2017). Until now, the most common way to regulate intestinal flora was to use probiotics combined with prebiotics (Wosinska *et al*., 2019).

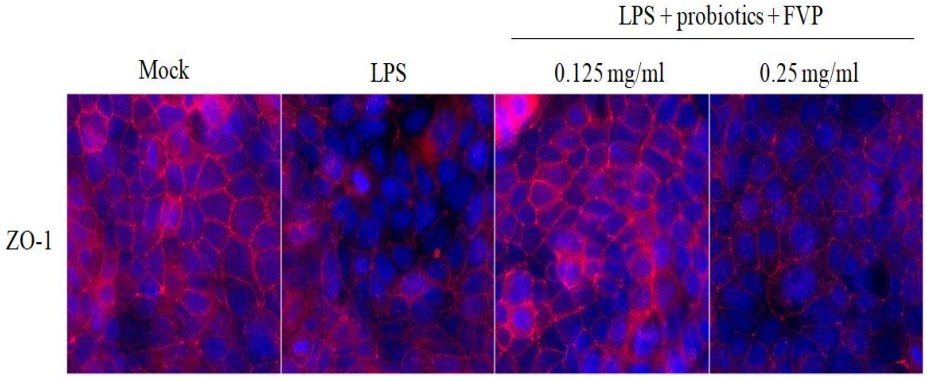

Figure 3. Multi-strain probiotics combined with FVP increased ZO-1 cytokine. LPS (10 μg/mL) was used to treat RAW264.7, and then co-cultured with Caco-2 cells to mimic the intestinal inflammatory environment. Caco-2 cells were incubated with probiotics and FVP (0.125 and 0.25 mg/mL) for 6 h. Caco-2 cells were then examined for fluorescence assay. Hoechst 33342 nucleic acid stain was used as nuclear counterstain (blue colour), and ZO-1 was conjugated with red fluorescent protein (red colour).

The present work used six strains of probiotics combined with FVP, namely *L. acidophilus*, *L. casei, L. lactis*, *B. longum*, *B. bifidum*, and *B. infantis*. *L. acidophilus* and *L. casei* could inhibit the severity of enteric infections, and modulate the immune system (Yan and Polk, 2011). *L. lactis* could reduce the pro-inflammatory cytokines of the intestinal epithelium, and inflammatory bowel disease (Yan and Polk, 2011). *B. longum* and *B. bifidum* could affect the Th1/Th2 balance by regulating inflammatory cytokines, possess anti-inflammatory properties, and increase immunity (Lopez *et al*., 2011). *B. infantis* could regulate the immune system of the intestinal mucosa of the host, thereby reducing intestinal irritability (Aragon *et al*., 2010). FVP are rich in vitamins, carotenoids, ascorbic acid, minerals, and dietary fibre. For example, dried blueberry is a rich source of total phenolics, fats, and dietary fibres (Skrovankova *et al*., 2015), and dried carrot and pumpkin have high β-carotene and crude fibre levels (Nyam *et al*., 2013; Nishijima *et al*., 2017). FVP contain different antioxidants, often as functional foods that could reduce the risk of various diseases such as cancers, diabetes, atherosclerosis, and ulcers (Lobo *et al*., 2010). Carrot powder could reduce lipid oxidation, and increase the defence capacity of antioxidants (Bub *et al*., 2000). Tomato powder could reduce lipid oxidation, and increase the anti-inflammatory adiponectin (Li *et al*., 2018). Cranberry powder could decrease IL-6 and oxidative stress (Kim *et al*., 2011). Therefore, probiotics combined with prebiotics have anti-inflammatory effects.

In addition to separate probiotic and prebiotic administrations, another viable option is to administer prebiotic and probiotic administration simultaneously, referred to as synbiotics (Pandey *et al*., 2015). The function of synbiotics can be complementary or synergistic (Pandey *et al*., 2015). The combined use of FVP and *L. casei* could enhance intestinal integrity, and reduce intestinal inflammation (Bai and Ouyang, 2006). Supplementation with prebiotics and probiotics could adjust the imbalanced gut microbiota to restore a healthy gut, thus significantly decreasing IL-6 and

TNF-α in diabetic patients (He and Shi, 2017). Studies have indicated that synbiotic combination of *L. paracasei* and maltodextrin could decrease *E. coli* colonisation (Markowiak and Slizewska, 2018). Studies have also shown that symbiotic treatment with *B. breve, L. casei,* and FVP could improve short bowel syndrome, and increase inflammatory response (Chapman *et al*., 2007). Our findings indicated that the six strains of probiotics combined with FVP could decrease inflammatory cytokines in an LPS-induced intestinal inflammatory environment. Thus, prebiotics combined with probiotics could improve intestinal inflammation.

In healthy individuals, epithelial paracellular permeability is controlled and regulated by the apical junctional complex, which consists of the tight junction and subjacent adherens junction (Lechuga and Ivanov, 2017). The tight junction complex is made up of several proteins such as occludin, claudin, and ZO-1. One study showed that ZO-1 was decreased in inflammatory bowel diseases (IBD) (Landy *et al*., 2016). Prebiotics combined with probiotics could protect the epithelial barrier in acute colitis by increasing tight junction protein expression (Mennigen *et al*., 2009). Our findings also indicated that multi-strain probiotics combined with FVP increased ZO-1 expression. The present work thus demonstrated the beneficial effects of this synbiotic formulation, with it having therapeutic and possibly preventive efficacy in human inflammatory diseases. A hallmark of human inflammatory intestinal disease is chronic recurring mucosal inflammation by infiltrating lymphocytes, leukocytes, and monocytes (Koch *et al*., 2010). The present work showed that multi-strain probiotics combined with FVP could regulate inflammatory cytokines. However, it was unclear if it directly affected the Caco-2 cells and then affected the inflammatory response on RAW264.7. Therefore, further research is warranted.

Conclusion

Multi-strain probiotics combined with FVP could decrease inflammatory cytokines, and protect the intestinal barrier from tight junction dysregulation. The present work thus provided a basis for the use of these supplements (multi-strain probiotics combined with FVP) in altering the gut microbiota and metabolism, which may support improved gut barrier function and inflammatory cytokines.

References

Aragon, G., Graham, D. B., Borum, M. and Doman, D. B. 2010. Probiotic therapy for irritable bowel syndrome. Journal of Gastroenterology and Hepatology 6(1): 39-44.

Bai, A. P. and Ouyang, Q. 2006. Probiotics and inflammatory bowel diseases. Postgraduate Medical Journal 82(968): 376-382.

Bub, A., Watzl, B., Abrahamse, L., Delincee, H., Adam, S., Wever, J., ... and Rechkemmer, G. 2000. Moderate intervention with carotenoid-rich vegetable products reduces lipid peroxidation in men. Journal of Nutrition 130(9): 2200-2206.

Chapman, T. M., Plosker, G. L. and Figgitt, D. P. 2007. Spotlight on VSL#3 probiotic mixture in chronic inflammatory bowel diseases. BioDrugs 21(1): 61-63.

Chelakkot, C., Ghim, J. and Ryu, S. H. 2018. Mechanisms regulating intestinal barrier integrity and its pathological implications. Experimental and Molecular Medicine 50(8): 1-9.

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J., ... and Ghasemi, Y. 2019. Prebiotics: definition, types, sources, mechanisms, and clinical applications. Foods 8(3): article no. 92.

Eid, H. M., Wright, M. L., Anil Kumar, N. V., Qawasmeh, A., Hassan, S. T. S., Mocan, A., ... and Haddad, P. S. 2017. Significance of microbiota in obesity and metabolic diseases and the modulatory potential by medicinal plant and food ingredients. Frontiers in Pharmacology 8: article no. 387.

Fan, Y. J., Chen, S. J., Yu, Y. C., Si, J. M. and Liu, B. 2006. A probiotic treatment containing *Lactobacillus*, *Bifidobacterium* and *Enterococcus* improves IBS symptoms in an open label trial. Journal of Zhejiang University Science B 7(12): 987-991.

He, M. and Shi, B. 2017. Gut microbiota as a potential target of metabolic syndrome: the role of probiotics and prebiotics. Cell and Bioscience 7: article no. 54.

Huang, M. L., Huang, J. Y., Kao, C. Y. and Fang, T. J. 2019. Fermented soymilk and soy and cow milk mixture, supplemented with orange peel fiber or *Tremella flava* fermented powder as prebiotics for high exopolysaccharide-

producing *Lactobacillus pentosus* SLC 13. Journal of the Science of Food and Agriculture 99(9): 4373-4382.

Kim, M. J., Ohn, J., Kim, J. H. and Kwak, H. K. 2011. Effects of freeze-dried cranberry powder on serum lipids and inflammatory markers in lipopolysaccharide treated rats fed an atherogenic diet. Nutrition Research and Practice 5(5): 404-411.

Koch, S., Kucharzik, T., Heidemann, J., Nusrat, A. and Luegering, A. 2010. Investigating the role of proinflammatory CD16+ monocytes in the pathogenesis of inflammatory bowel disease. Clinical and Experimental Immunology 161(2): 332-341.

Landy, J., Ronde, E., English, N., Clark, S. K., Hart, A. L., Knight, S. C., ... and Al-Hassi, H. O. 2016. Tight junctions in inflammatory bowel diseases and inflammatory bowel disease associated colorectal cancer. World Journal of Gastroenterology 22(11): 3117-3126.

Lechuga, S. and Ivanov, A. I. 2017. Disruption of the epithelial barrier during intestinal inflammation: quest for new molecules and mechanisms. Biochimica et Biophysica Acta - Molecular Cell Research 1864(7): 1183-1194.

Li, C. C., Liu, C., Fu, M., Hu, K. Q., Aizawa, K., Takahashi, S., ... and Wang, X. D. 2018. Tomato powder inhibits hepatic steatosis and inflammation potentially through restoring SIRT1 activity and adiponectin function independent of carotenoid cleavage enzymes in mice. Molecular Nutrition and Food Research 62(8): article ID e1700738.

Li, S. C., Hsu, W. F., Chang, J. S. and Shih, C. K. 2019. Combination of *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* shows a stronger anti-inflammatory effect than individual strains in HT-29 cells. Nutrients 11(5): article no. 969.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. 2010. Free radicals, antioxidants and functional foods: impact on human health. Pharmacognosy Reviews 4(8): 118-126.

Lopez, P., Gonzalez-Rodriguez, I., Gueimonde, M., Margolles, A. and Suarez, A. 2011. Immune response to *Bifidobacterium bifidum* strains support Treg/Th17 plasticity. PLoS One 6(9): article ID e24776.

Markowiak, P. and Slizewska, K. 2017. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 9(9): article no. 1021.

Markowiak, P. and Slizewska, K. 2018. The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathogens 10: article no. 21.

Mennigen, R., Nolte, K., Rijcken, E., Utech, M., Loeffler, B., Senninger, N. and Bruewer, M. 2009. Probiotic mixture VSL#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. American Journal of Physiology-Gastrointestinal and Liver Physiology 296(5): G1140-1149.

Molska, M. and Regula, J. 2019. Potential mechanisms of probiotics action in the prevention and treatment of colorectal cancer. Nutrients 11(10): article no. 2453.

Nishijima, Y., Taniguchi, S., Ikeda, S., Yoshitani, K., Hamano, T., Tani, H., ... and Kume, S. 2017. Effects of beta-carotene-enriched dry carrots on beta-carotene status and colostral immunoglobulin in beta-carotene-deficient Japanese Black cows. Animal Science Journal 88(4): 653-658.

Nyam, K. L., Lau, M. and Tan, C. P. 2013. Fibre from pumpkin (*Cucurbita pepo* L.) seeds and rinds: physico-chemical properties, antioxidant capacity and application as bakery product ingredients. Malaysian Journal of Nutrition 19(1): 99-109.

Pandey, K. R., Naik, S. R. and Vakil, B. V. 2015. Probiotics, prebiotics and synbiotics - a review. Journal of Food Science and Technology 52(12): 7577-7587.

Pierre, J. F., Busch, R. A. and Kudsk, K. A. 2016. The gastrointestinal immune system: implications for the surgical patient. Current Problems in Surgery 53(1): 11-47.

Prete, R., Long, S. L., Gallardo, A. L., Gahan, C. G., Corsetti, A. and Joyce, S. A. 2020. Beneficial bile acid metabolism from *Lactobacillus plantarum* of food origin. Scientific Reports 10(1): article no. 1165.

Shende, D. and Datta, A. K. 2019. Refractance window drying of fruits and vegetables: a review. Journal of Agricultural and Food Chemistry 99(4): 1449-1456.

Sivamaruthi, B. S., Kesika, P. and Chaiyasut, C. 2020. The role of probiotics in colorectal cancer management. Evidence-Based

Complementary and Alternative Medicine 2020: article ID 3535982.

Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T. and Sochor, J. 2015. Bioactive compounds and antioxidant activity in different types of berries. International Journal of Molecular Sciences 16(10): 24673-24706.

Wilkins, T. and Sequoia, J. 2017. Probiotics for gastrointestinal conditions: a summary of the evidence. American Family Physician 96(3): 170-178.

Wosinska, L., Cotter, P. D., O'Sullivan, O. and Guinane, C. 2019. The potential impact of probiotics on the gut microbiome of athletes. Nutrients 11(10): article no. 2270.

Yan, F. and Polk, D. B. 2011. Probiotics and immune health. Current Opinion in Gastroenterology 27(6): 496-501.

Zhai, Q., Wang, G., Zhao, J., Liu, X., Tian, F., Zhang, H. and Chen, W. 2013. Protective effects of *Lactobacillus plantarum* CCFM8610 against acute cadmium toxicity in mice. Applied and Environmental Microbiology 79(5): 1508-1515.

Zhou, J. S., Pillidge, C. J., Gopal, P. K. and Gill, H. S. 2005. Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. International Journal of Food Microbiology 98(2): 211-217.