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Assessment of Cytotoxic Effect of Para-probiotic Yogurts on Cancer Cell Lines

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Cancer Cell, Cytotoxic effect, Lactobacillus, Para-probiotic yogurt

ABSTRACT:

Background and Aim: Dairy products enhanced with para-probiotics and postbiotics offer a more convenient and streamlined option for industrial transportation and commercialization compared to probiotic products. Various sections of Lactobacillus, including whole cells, thermally inactivated cells, cell wall, peptidoglycan, and cytoplasmic sections, have demonstrated antiproliferative effects against human cancer cell lines. This study investigated the impact of para-probiotic yogurt on cancer cells. If proven effective, it has the potential to be utilized in the treatment of this form of cancer as well as other types of cancer.

Material and Methods: Following the culturing and amplification of the cancer cell line, an investigation was conducted to assess the impact of supernatant toxicity. The cells were then subjected to varying doses of para-probiotic supernatant, with the MTT assay being performed 72 hours later.

Results: The experiment involved testing the effects of various dilutions of para-probiotic yogurt supernatant on different types of cancer cells (MDA-MB-231, SK-BR-3, and SW-480). The results showed that YBpB had a significant inhibitory effect on cell viability in all three categories of cancer cells. Notably, it had the strongest impact on SK-BR-3 cells ($p \leq 0.05$). The concentration of the extract had a significant impact on the cytotoxicity effects, with higher concentrations resulting in increased growth inhibition. The highest percentage of growth inhibition was observed at concentrations of 1/4, 1/3, and 1/2, with statistical significance at $p = 0.05$ and $p = 0.01$, respectively.

Conclusion: It is recommended to consider the use of para-probiotic yogurt supernatant as an effective substance in the treatment of cancer. Future research could investigate the potential use of its substances in cancer treatment.

Introduction

Today, probiotic bacteria are widely recognized as a beneficial addition to one's health routine. Nevertheless, there are certain restrictions on their usage. One set of issues is connected to the sustainability of these problems. Not all individuals, regardless of age or physical condition, experience the same positive effects when prescribed live probiotic bacteria. Live bacteria can have a detrimental effect on patients with weakened immune systems and pose a potential risk. Extensive research has been conducted on the various therapeutic effects of probiotic LAB, including their potential anticancer and anti-tumor properties.[1,2] These bacteria prevent the occurrence of cancer by lowering the pH and inhibiting the growth of microbiota that produce carcinogens. The level of procarcinogenic enzymes can be

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reduced, cell proliferation can be enhanced by inhibiting normal cell apoptosis and promoting cell differentiation and cytoprotective activities, inflammation-induced cell apoptosis can be suppressed, innate immunity can be enhanced, various gut homeostasis can be promoted, and antioxidant activity can be displayed.[3-6]

Currently, there is a wide range of probiotic products available that include *Lactobacillus acidophilus* and *Bifidobacterium bifidum*.[7]When choosing probiotic bacteria, certain criteria must be considered, with the most crucial one being their association with the GRAS group. These bacteria have been extensively studied and their positive impact on the health of living organisms has been well-documented. This group exhibits remarkable resistance to gastric juice, including its acidity, salts, bile salts, and digestive enzymes. Additionally, they possess the unique ability to adhere to epithelial cells and display high retention activity against harmful bacteria. Not only are they resistant to antibiotics and phage bacteria, but they also possess desirable technological properties to produce microbial cultures, food, and pharmaceutical products. These properties make them ideal for various applications, including freeze-dried or lyophilized forms, severe freezing, and spray-drying. [8]

These non-viable probiotic bacterial cells are commonly referred to as "Para-probiotics" or "Probiotic ghost cells." Just like live probiotic cells, the deceased cells of probiotic bacteria have been found to elicit various biological responses in the hosts. While the precise way in which they work is still not fully understood, it is believed that they offer health benefits by strengthening the immune system through their cell wall and other cellular components. Additionally, they may help inhibit pathogens by adhering to the intestinal walls.[9] In addition, the effects caused by these living and deceased bacteria may also be attributed to the chemical compounds released in the liquid surrounding the cells, either by the living bacteria themselves or after the cells have broken down.[10]

The probiotic bacteria release secretory metabolites that are commonly referred to as "postbiotics" or "metabiotics." These metabolites have been found to have positive effects on the gastrointestinal tract of the host. Certain metabolites produced by probiotic bacteria, such as organic acids, bacteriocin, and H_2O_2, play a crucial role in reducing the viability of colorectal cancer cells and promoting apoptosis. These metabolites achieve this by influencing various signaling pathways.

Discussion has taken place regarding the advantages of incorporating para-probiotics and postbiotics as functional compounds in dairy products, along with the examination of analytical techniques that could be employed for quality control purposes.[11] Supplementing dairy products with para-probiotics and postbiotics offers a more convenient and efficient option for industrial transportation and commercialization compared to probiotic products. They have minimal contact with matrix compounds or food ingredients, which helps to extend their shelf life.

Several techniques have been employed to deactivate microorganisms in order to create para-probiotics. Heat treatment is widely used to deactivate microorganisms. Out of all these methods, pasteurization and sterilization take precedence.[12] Various factors influence how heat treatment affects microorganisms, including the type of cells, whether they are in vegetative or spore form, the conditions of the culture medium, the growth stage, water activity, and the method of heating. There are various methods available for inactivation, such as ultrasound, high pressure, ultraviolet, ionizing radiation, electric field heating, ohmic, supercritical CO_2, dehydration, and substrate pH change. Para-probiotics have been found to have numerous positive effects on health. These include inhibiting the growth of harmful pathogens, balancing the composition of intestinal microbes, promoting healing of intestinal injuries, providing relief from diarrhea, reducing inflammation, alleviating lactose intolerance, lowering cholesterol levels, mitigating respiratory disease, improving liver health in individuals who consume alcohol, preventing the progression of cancer, treating atopic dermatitis, preventing tooth decay, and offering treatment for colitis.[13]

Various sections of *Lactobacillus*, including whole cells, thermally inactivated cells, cell wall,

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peptidoglycan, and cytoplasmic sections, have been found to exhibit antiproliferative effects against human cancer cell lines. This study investigated the impact of para-probiotic yogurt on cancer cells, with potential implications for its use in treating not only this specific type of cancer but also other forms of cancer.

Material and Methods

Cell culture

For this study, a variety of cancer cells were obtained from a reputable institute in India and used for in-vitro culture. This included the SK-BR-3 cell lines. This cell line originated from a pleural effusion. The cultivation began in EMEM with 10% FBS and has since transitioned to McCoys 5a with 10-15% FBS. The current culture medium consists of McCoys 5a and 10% FBS. The cell line was adjusted to RPMI + 10% FBS in NCBI, while the preservation medium consisted of FBS + 10% DMSO. Information on MDA-MB-231 cell lines and culture medium: This cell line is cultured using ATCC formulated Leibovitz's L-15 medium and supplemented with fetal bovine serum to a final concentration of 10%. The cell line was cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS) according to the protocol provided by the National Center for Biotechnology Information (NCBI). Preservation medium: FBS+ 5% DMSO. Then we have the SW 480 cell lines, specifically the Human-Colon type. These cells were cultured in Leibovitz's L-15 medium with 10% FBS. The cell line was modified to RPMI 1640 with 10% FBS in NCBI, where the preservation medium consisted of FBS with 10% DMSO. Afterward, the cells were placed in an incubator set at 37◦ C with 90% humidity and 5% CO2.

Cells treatments

One method commonly employed to assess the harmful impact of different substances on various cell lines, including both cancerous and non-cancerous cells, is the MTT test. The cell viability was assessed using the MTT test. When the (MTT) is introduced into the culture medium, it undergoes a transformation into formazan dye thanks to the dehydrogenase activity of the cells. The level of dehydrogenase in cells of a particular type remains consistent, so the quantity of formazan generated is directly related to the cell count. To summarize, the cells were carefully placed in 96 wells of cellular platelets. After 72 hours of adding the extract to the cells, the supernatant was removed and the cells were mixed with a 0.5 mg/ml (MTT) solution in PBS at 4 ◦C. With 100 µl of Dulbecco's Modified Eagle's Medium (DMEM), the absorption of light at 750 nm was measured using an ELISA reader. The intensity of color or the number of living cells have a direct correlation.[13]

Preparation of Para-probiotics

Store freeze-dried and pure cultures of *B.animalis subsp. lactis* BB-12 and *L.acidophilus* ATCCSD 5221 at -18˚C. Heat-killed cells were prepared by subjecting these organisms to autoclaving at a temperature of 121◦C for 15 minutes in distilled water (20 mL) at a concentration of 107 CFU/L. Following this, they were cooled in an ice bath. After the thermal treatment, no colonies were found when the viable count of the heat-killed bacteria was determined through cultivation.

Para-probiotic yogurts production

Three yogurt treatments were made by using reconstituted skim milk powder. Skim milk samples with a milk solid non-fat content of 0.13% were inoculated with a starter culture and para-probiotics. The initial counts of *L. acidophilus* ATCCSD 5221 and *B. animalis subsp. lactis* BB-12 were 107 CFU/mL. The containers of reconstituted milk were heated to a temperature of 85◦C for a duration of30 minutes. Afterward, they were carefully cooled in an ice bath until they reached a precise temperature of 43◦C ± 0.01. The starter culture and para-probiotics were added to the milk after it had cooled. The fermentation process was carried out at a temperature of 42 °C until the pH level decreased from 6.5±0.02 to 4.5±0.02. The fermentation process was closely monitored by measuring biochemical parameters such as pH, redox potential (RP), titratable acidity (TA), and incubation time at 30-minute intervals. After the fermentation process was finished, the

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para-probioticyogurts were carefully cooled and kept at a refrigeration temperature of 4ºC for duration of 28 days.

Preparation of supernatant

The yogurt samples were centrifuged to obtain the supernatant. The study conducted by Parvarei MM et al.12 The solution obtained was separated and utilized in the experiment.

Statistical analysis

The data was compiled and enteredintoa spreadsheet computer program before being exported to the data editor page of SPSS version 15 (SPSS Inc., Chicago, Illinois, USA). Quantitative variables were reported using measures such as means and standard deviations or median and interquartile range, depending on their distribution. The qualitative variables were displayed as counts and percentages. Confidence level and level of significance were set at 95% and 5% respectively for all tests.

Results

Over the past few decades, there has been a significant focus on finding effective treatments for breast cancer. There are several treatment options available for cancer, such as chemotherapy, radiation therapy, hormone therapy, and surgery. Chemotherapy is often considered the most effective treatment for metastatic tumors. However, the side effects of these drugs and the challenges posed by the patient's immune system can significantly limit its use as a treatment method.

Lactobacilli para-probiotics and the postbiotics they produce consist of a diverse array of molecules, such as surface proteins, cell wall polysaccharides, peptidoglycans, secreted proteins, bacteriocins, and organic acids. Peptidoglycan is a specific component found in the cell wall of Gram-positive bacteria. This layer is composed of amino acids and sugar. The sugar component is made up of various residues of ß (1, 4) linked N-acetylglucosamine and N-acetylmuramic acid. Peptidoglycan serves as a framework for repairing the cell envelope, potentially involving proteins and teichoic acids. The teichoic acids found in *L. acidophilus* make up over 50% of the weight of the cell wall. Their structure can vary depending on factors such as the strain level of growth, the pH of the medium, the carbon source, and the presence of phosphate. There are various forms of it, including teichoic acids (TA), teichuronic acids (TUA), lipoteichoic acid (LTA), and lipoglycans. The LTA of L. acidophilus contains glycolipid and polyglycerophosphate. The growth and physiology of *L. acidophilus* are closely linked to LTA. Probiotic ruptured cells can have various positive effects on the host, including immunomodulatory, anti-tumor, antimicrobial, and barrier-preservation effects.[15,16] For this study, we examined the effects of three para-probiotic yogurt supernatants on the growth of three different cancer cell lines. By measuring the absorption of light based on the concentration of the supernatant and comparing it with the rate of cell proliferation, the results of (MTT) dye measurement were obtained. A pattern was drawn to illustrate the findings. To determine the impact of the extract on living cells, we utilized a formula to calculate the percentage of affected cells in comparison to those that were not exposed to the extract.

According to the findings of this laboratory study, the extract had no impact on control cells. However, it did demonstrate a cytotoxic effect on cancer cells, with the intensity of the effect varying based on the dosage and duration of exposure to the extract. The pH values, acidity, and redox potential of the supernatants of the three types of para-probiotic yogurts are presented in Table 2. During the incubation period, the treatments showed a significant decrease in pH, along with an increase in acidity and redox potential. The pH values varied from 0.0059 to 0.0054, while the acidity levels ranged from 0.22 to 0.26. In addition, the redox potential values varied between 0.32 and 0.36. Table 2 shows the impact of para-probiotic yogurt supernatants on cell line growth under various biochemical conditions, as determined by the MTT assay. YBp reported the smallest rise in acidity. The inactivated cells of *L. acidophilus* had a significant impact on increasing the rate of acidification and redox potential. Therefore, the treatments that experienced the most acidification

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had the highest redox potential as a result of the production of organic acids during fermentation. YLp-B exhibits a higher buffering capacity compared to other treatments. Having a higher buffering capacity can result in a slower decrease in pH and encourage starter bacteria to acidify at a faster rate. [17]

YBp-B was found to have a significant impact on cell viability in three different cell lines, with the greatest effect observed in SK-BR-3 ($p \leq 0.05$). There was a notable distinction at the 1% level when comparing the control sample to the various extract concentrations in all three cell lines after 72 hours. Cell line studies demonstrated the effectiveness of para-probiotics. In addition, YLP-B was found to effectively suppress the growth of both SKBRT3 and SW480 cell lines. The supernatant had a negative impact on cell viability in the MDA-MD231 cell line, with decreasing viability observed at concentrations of 1/4, 1/3, and 1/2. YLP-B exhibited a higher potency on breast cell lines compared to the colon cell line. The largest inhibitory effect was observed at SW480 1/4, 1/3, and 1/2 cell line concentrations, indicating a significant Y(LB) p-B effect. It may have a negative impact on the cell viability of MDA-MD231 and SK-BR-3.

Table 1: Using culture combinations for producing yogurts

Discription of culture component
YLp-B = S. thermophilus + L. delbrueckii ssp. bulgaricus + killed cells of L. acidophilus–before fermentation
YBp-B = S. thermophilus + L. delbrueckii ssp. bulgaricus + killed cells of B. animalis subsp. lactis BB-12–before fermentation
Y(LB)p-B = S. thermophilus + L. delbrueckii ssp. bulgaricus +killed cells of B. animalis subsp. lactis BB-12and L. acidophilus (paraprobiotic) –before fermentation

Table 2: The pH drop, acidity increase and redox potential increase rates in treatments

Treatments	mpH-DR (pH/day 1)	mA-IR (◦D/day 1	mRP-IR (mV/day 1)
YLp-B	0.0050 ± 0.06^{a}	0.22 ± 0.07^{a}	0.35 ± 0.04^{a}
YBp-B	0.0059 ± 0.05^{b}	0.21 ± 0.02^{c}	0.32 ± 0.01^{b}
Y(LB)p-B	0.0058 ± 0.05^{b}	0.23 ± 0.04^{b}	0.35 ± 0.02^{b}

mpH-DR = mean pH drop rate, mA-IR = mean acidity increase rate, mRP-IR = mean redox potential increase rate

Discussion

There have been numerous studies indicating the potential health benefits of *Lactobacillus* strains, including their potential role in cancer prevention. The study examined the anticancer activities of 21 *Lactobacillus* isolates through in vitro techniques. Our findings reveal that isolate Y8 exhibits probiotic properties and demonstrates potential anti-cancer effects.[18,19]

Prescribing probiotics or incorporating probiotic foods into your diet is a highly effective strategy for maintaining a healthy balance of intestinal microbiota. Probiotic food is a type of functional food that has positive impacts on human health. The fermentation of bovine colostrum was carried out using a two-step method, utilizing primer cultures on *C. lipolytica* and kefir seeds. Extracts of fermented products have been found to possess potent ACE inhibitory capacity and antioxidant activity. Furthermore, they demonstrated activity that is not harmful to cells and has the potential to promote cell growth.[20] Yogurt is an ideal choice for incorporating probiotics because of its pleasing taste and texture, as well as its ability to provide important nutrients. During fermentation, the production of amino acids and peptides gives rise to beneficial compounds. Yogurt contains probiotic bacteria that produce bioactive compounds with

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various health benefits, such as antimicrobial, hypolipidemic, antioxidant, antihypertensive, and immune-modulatory properties. *Saccharomyces boulardii* is a type of probiotic yeast that is well-known for its ability to alleviate symptoms of diarrhea. Goat's milk yogurts fortified with *Pistacia atlantica* resin extract, either alone or in combination with *Saccharomyces boulardii*, showed promising results in promoting the growth of LAB, preserving the beneficial phytochemicals in the resin, and enhancing the overall taste and sensory qualities. [21]

Postbiotics are the byproducts produced by probiotic bacteria as they go about their metabolic processes. These metabolites help maintain a healthy balance in the gut by reducing the activity of certain enzymes that can convert harmful substances into cancer-causing agents.[22] In addition to these, postbiotics contain short chain fatty acids (SCF) that have been found to stimulate chemo-preventive enzymes such as glutathione S transferase and Glutathione transferase pi.[23] Our studies revealed the cytotoxic effect of the CFS samples on the colon cancer cell lines, similar to what health journalists have reported.

Our findings indicate that the supernatant YBp-B may possess inhibitory properties against colorectal and breast cell lines. Research conducted on inactivated probiotics of *Bacillus amyloliquefaciens* FPTB16 and *Bacillus subtilis* FPTB13, at varying concentrations, in Catla fish, has shown that these probiotics have a positive effect on the cellular immune response of head kidney leukocytes. In certain cases, the stimulatory effect of the inactivated probiotics was found to be even better than that of the viable cells. This was particularly evident when thermal treatment was applied to the probiotics at the lowest concentration dose.[5] However; the current study discovered that the impact varies based on the dosage. Nevertheless, we demonstrated that the impact varies based on the dosage. The inactivated probiotic of *L. casei*[01] demonstrated hypoglycemic activity both in vitro by inhibiting α-glucosidase and α-amylase, and in a clinical trial by reducing postprandial glucose levels and maintaining glycemic response parameters, similar to the probiotic whey drink.[24,25]

Understanding how bacteria adhere to the intestinal mucosa involves studying the adhesion capacity of probiotics to the hydrophobic phase of the solvent. This helps prevent pathogens from sticking to the intestine and causing contamination in the digestive system.[26] Previous studies have demonstrated that probiotic bacteria typically display acceptant cell surface hydrophobicity, similar to the findings of the Y8 strain. These studies have also established a clear link between cell surface hydrophobicity and bacterial adhesion ability. Based on our findings, the variation in surface protein production plays a crucial role in determining the diverse levels of cell surface hydrophobicity in different *Lactobacillus* strains. It is crucial to consider the ability of probiotics to uptake cholesterol when introducing and selecting them. This is particularly true for strains like Y8 and C14, which have shown high efficacy in this regard. Research indicates that the hypocholesterolemic effects of probiotics may be attributed to their ability to either absorb cholesterol or bind it to the surface of bacterial cells. Probiotics play a crucial role in reducing cholesterol levels through various mechanisms. These include the conversion of cholesterol to coprostanol by the reductase enzyme, the incorporation of cholesterol in the cell wall, and the disruption of cholesterol micelle formation in the intestine by de-conjugated bile salts.[27,28] It is still unclear how probiotic metabolites effectively hinder and deter the growth of cancer cells. They utilize potent proteins to decrease the presence of harmful and cancer-causing fecal enzymes, including -glycosidase, -glucuronidase, IO hydratase-dehydrogenase, nitroreductase, nitrate/nitrite reductase, and azoreductase.[29]

Multiple studies have highlighted the potential of probiotics in reducing the risk of cancer.[30]The supernatant from 30-day matured cow's milk cedar cheese exhibited significant growth inhibition in colorectal cancer cells at concentrations of 400 and 500 µg/ml over a period of 150 days. It also effectively suppressed cell growth in the G0/G1 phase. It also triggered programmed cell death in the HCT-116 colon cancer cell line. Research[31] has

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indicated that probiotic conjugated linolenic acid can have a cytotoxic effect on the MDAMB2 cell line by downregulating NFκB. A recent study has revealed that *Lactococcus lactis* KC24, derived from kimchi, exhibits promising anti-cancer properties against various types of carcinoma cells, including gastric, colon, breast, and lung carcinoma. Recent studies[32,33] have shown that *Lactobacillus* cells and supernatants may have a beneficial impact on inhibiting the growth of the HT-29 human colorectal cancer cell line, as indicated by an increase in lactate dehydrogenase levels. Considering the global impact of cancer as a leading cause of death, it is crucial to prioritize research aimed at discovering innovative methods of treatment and prevention for the betterment of public health. According to recent studies, Prato Matrix dairy cheese has been found to have a superior nutritional composition and greater anti-hyperglycemic activity when tested in vitro. Our studies have consistently aligned with existing research, demonstrating the ability of these para-probiotics to effectively hinder the growth of colon and breast cancer cells.

Conclusion

The results of this study indicated that para-probiotic yogurt supernatant with anti-cancer effect over time and subtility on cancer cells (MDA-Md, SK-BR-3, and SW 480) can inhibit the growth of these cells. Gradually and over time at higher doses, it could further inhibit the growth of rat cancer cells.

References

[1] Sharma M, Shukla G. Metabiotics: one step ahead of probiotics; an insight into mechanisms involved in anticancerous effect in colorectal cancer. Front Microbiol. 2016;7:1940.

[2] Kahouli I, Tomaro-Duchesneau C, Prakash S. Probiotics in colorectal cancer (CRC) with emphasis on mechanisms of action and current perspectives. J Med Microbiol. 2013;62(Part 8):1107-1123.

[3] Donaldson MS. Nutrition and cancer: A review of the evidence for an anti-cancer diet. Nutr J. 2004;20:1-21.

[4] Lin PW, Nasr TR, Berardinelli AJ, Kumar A, Neish AS. The probiotic Lactobacillus GG may augment intestinal host defense by regulating apoptosis and promoting cytoprotective responses in the developing murine gut. Pediatr Res. 2008;64(5):511–516.

[5] Tiptiri-Kourpeti A, Spyridopoulou K, Santarmaki V, Aindelis G, Tompoulidou e, Lamprianidou EE, et al. Lactobacillus casei exerts anti-proliferative effects accompanied by apoptotic cell death and up-regulation of TRAIL in colon carcinoma cells. PloS one 2016;11(2):e0147960.

[6] Tiptiri-Kourpeti A, Spyridopoulou K, Santarmaki V, Aindelis G, Tompoulidou e, Lamprianidou EE, et al. Lactobacillus casei exerts anti-proliferative effects accompanied by apoptotic cell death and up-regulation of TRAIL in colon carcinoma cells. PloS one 2016;11(2):e0147960.

[7] Kristo E, Biliaderis CG, Tzanetakis N. Modelling of rheological, microbiological and acidification properties of a fermented milk product containing a probiotic strain of Lactobacillus paracasei. Int Dairy J. 2003;13(7):517–28.

[8] Nag A, Waterland M, Singh H. Effect on cell membrane structural integrity of xylitolcoated probiotics when stabilised with milk solids-A FTIR study. Int J Dairy Technol. 2021;74:128–38. doi:10.1111/1471- 0307.12738.

[9] Murosaki S, Muroyama K, Yamamoto Y, Yoshikai Y. Antitumor effect of heat-killedLactobacillus plantarumL-137 through restoration of impaired interleukin-12 production in tumor-bearing mice. Cancer Immunol Immunother. 2000;49(3):157-164.

[10] Fotiadis CI, Stoidis CN, Spyropoulos BG, Zografos ED. Role of probiotics, prebiotics and synbiotics in chemoprevention for colorectal cancer. World J Gastroenterol. 2008;14(42):6453- 6457. Available from: URL: http://www.wjgnet. com/1007-9327/14/6453.asp DOI: http://dx.doi.org/10.3748/ wjg.14.6453.

[11] Barrosa CP, Grom LC, Laís C, Grom JT, Guimarães CF, Balhazar RS, et al. Paraprobiotic obtained by ohmic heating added in whey-grape juice drink is effective to control postprandial glycemia in healthy adults. J Food Res Int. 2020;140:109905. doi:10.1016/j.foodres.2020.109905.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1240-1248 | ISSN:2251-6727

[12] Parvarei MM, Fazeli MR, Mortazavian AM, Nezhad SS, Mortazavi SA, Golabchifar AA, et al. Comparative effects of probiotic and paraprobiotic addition on microbiological, biochemical and physical properties of yogurt. J Food Res Int. 2021;140:110030. doi:10.1016/j.foodres.2020.110030.

[13] Shigwedha N, Sichel L, Jia L, Zhang L. Probiotical cell fragments (PCFs) as "Novel Nutraceutical Ingredients". J Biosci Med. 2014;2(3):43–55.

[14] Moheghi N, Afshari JT, Brook A, Brook A. The Cytotoxic Effect of Zingiber Afficinale in Breast Cancer (MCF7) Cell Line. Intern Med Today. 2011;17(3):28–34.

[15] Teame T, Wang A, Xie M, Zhang Z, Yang Y, Ding Q, et al. Paraprobiotics and Postbiotics of Probiotic Lactobacilli, Their Positive Effects on the Host and Action Mechanisms: A Review. Front Nutr. 2020;7:570344.

[16] Vollmer W, Blanot D, de Pedro M. Peptidoglycan structure and architecture. FEMS Microbiol Rev. 2008;32(2):149–67.

[17] Martin F, Cachon R, Pernin K, DeConinck J, Gervais P, Guichard E, et al. Effect of oxidoreduction potential on aroma biosynthesis by lactic acid bacteria in nonfat yogurt. J Dairy Sci. 2011;94(2):614–22.

[18] R.K. V J, et al., Putative probiotic Lactobacillus spp. from porcine gastrointestinal tract inhibit transmissible gastroenteritis coronavirus and enteric bacterial pathogens, Trop. Anim. Health Prod. 42 (8) (2010) 1855–1860.

[19] J.-S. Lee, M.-J. Chung, J.-G. Seo, In vitro evaluation of antimicrobial activity of lactic acid bacteria against Clostridium difficile, Toxicol. Res. 29 (2) (2013) 99–106.

[20] Gaspar-Pintiliescu A, Oancea A, Cotariet M, Vasile AM, Bhrim GE, Saposhnikov S. Oana Craciunescu and Elena Iulia Oprita (2020) Angiotensin-converting enzyme inhibition, antioxidant activity and cytotoxicity of bioactive peptides from fermented bovine colostrum. Int J Dairy Technol. 2020;73:108–16.

[21] Hadjimbi E, Botsaris G, Goulas V, Alxandri E, Gekas V, Gerothanassis IP. Vassilis Gekas and Ioannis Gerothanassis. Functional stability of goats' milk yoghurt supplemented with Pistacia atlantica resin extracts and Saccharomyces boulardii. Int J Dairy Technol. 2020;73(1):134– 43.

[22] Verma A, Shukla G. Administration of prebiotic inulin suppresses 1, 2 dimethylhydrazine dihydrochloride induced procarcinogenic biomarkers fecal enzymes and preneoplastic lesions in early colon carcinogenesis in Sprague Dawley rats. J Funct Foods. 2013;5(2):991-996. doi: 10.1016/j. jff.2013.02.006.

[23] Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, de Vos WM, et al., Demonstration of safety of Probiotics – a review. Int J Food Microbiol. 1998;44(1-2):93-106.

[24] Cuevas-González AM, Liceaga JE, Aguilar-Toalá JE. Postbiotics and Paraprobiotics: From concepts to applications. Food Res Int. 2020;136:109502. doi:10.1016/j.foodres.2020.109502.

[25] Barros CP, Guimarães JT, Esmerino EA, Duarte M, Silva MC, Silva R, et al. Paraprobiotics and postbiotics: concepts and potential applications in dairy products. Curr Opin Food Sci. 2020;32:1–8.

[26] B. Haghshenas, et al., Anti-proliferative effects of Enterococcus strains isolated from fermented dairy products on different cancer cell lines, J. Funct.Foods 11 (2014) 363–374.

[27]] F. Kouhi, et al., Potential probiotic and safety characterisation of Enterococcus bacteria isolated from indigenous fermented Motal cheese, Int. Dairy J. (2021), 105247

[28]] Y. Nami, et al., Hypocholesterolaemic activity of a novel autochthonous potential probiotic Lactobacillus plantarum YS5 isolated from yogurt, LWT 111 (2019) 876–882.

[29]] K.M. Tuohy, et al., Using probiotics and prebiotics to improve gut health, Drug Discov. Today 8 (15) (2003) 692–700.

[30] Bassaganya-Riera J, Viladomiu M, Pedragosa M, DeSimone C, Hontecillas R. Immunoregulatory mechanisms underlying prevention of colitis-associated colorectal cancer by probiotic bacteria, Probiotic bacteria: a promising tool in cancer prevention and therapy). PLoS One. 2012;7(4):e34676.

[31] Rafiq S, Gulzar N, Huma N, Hussanin I, Murtaza MS. Evaluation of anti-proliferative activity of Cheddar cheeses using colon adenocarcinoma

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1240-1248 | ISSN:2251-6727

(HCT-116) cell line. Int J Dairy Technol. 2020;73:255–60.

[32] Lee NK, Han KJ, Son SH, Eom SJ, Lee SK, Paik HD. Multifunctional effect of probiotic Lactococcus lactis KC24 isolated from kimchi. Multifunctional effect of probiotic Lactococcus lactis KC24 isolated from kimchi. LWT - Food Sci Technol. 2015;64(2):1036–41.

[33] Chen ZY, Hsieh YM, Huang CC, Tsai CC. Inhibitory Effects of Probiotic Lactobacillus on the Growth of Human Colonic Carcinoma Cell Line HT-29. Molecules. 2017;22(1):107.