Effect of Lactobacillus on Biological properties: Anticancer, immunomodulatory properties and improvement of bone health.

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ABSTRACT

Lactic acid bacteria (LAB) such as Lactobacillus are important micro-organisms in a healthy human microbiotic environment. Lactobacillus strains are beneficial micro-organisms, isolated from the fermented dairy products are generally regarded as safe (GRAS) and which have been associated with several probiotic effects in both humans and animals. Recently, many research studies have focused on biological functions of Lactobacillus including antioxidant, anticancerous, antimicrobial, immunomodulatory and bone health. There are several interesting data on biological effect of fermented foods showing potential role of Lactobacillus strains. This review discuss about the studies which showed a positive effect of Lactobacillus strains on anticancer, immunomodulatory properties and improvement of bone health.

Keywords: Lactobacillus, Anticancer, Immunomodulatory, Bone Health.

INTRODUCTION

Lactic acid bacteria (LAB) are a wide range of genera which includes number of species, lactobacillus is one of the most important genera of LAB and contains more than 80 species. Lactobacilli are the diverse group of gram positive, non-spore forming, catalase negative rod bacteria, present in raw milk and dairy products such as cheeses, yoghurts and fermented milks. Lactobacilli are a significant part of the gut normal flora also. They are able to produce lactic acid as the main end-product of the fermentation of carbohydrates and these bacteria are used as starter cultures for fermentation, especially for dairy products. Lactobacilli are considered as generally recognized as safe (GRAS) organisms and can be safely used as probiotics for medical and veterinary applications [1]. Probiotics, as defined in a FAO/WHO (2002) report, are “live microorganisms which when administered in adequate amounts confer a health benefit on the host”. Probiotics are beneficial bacteria in that they favorably alter the intestinal microflora balance, inhibit the growth of harmful bacteria, promote good digestion, boost immune function and increase resistance to infection [2].

Several lactobacilli strains are currently used as therapeutics, claiming various health benefits by acting as probiotics. Lactobacillus has various physiological benefits include removal of carcinogens, lowering of cholesterol, immunostimulating and allergy lowering effect, synthesis and enhancing the bioavailability of nutrients, alleviation of lactose intolerance, improving bone health [3]. This review mainly focuses on anticancer, immunomodulatory properties and improvement of bone health by Lactobacillus.
Anticancer properties

In view of the role of diet in human diseases such as cancer, the potential of dietary bacteria to prevent colon cancers is clearly a matter of great interest. Recently, lactobacilli have been reported to possess certain anticancer properties. Most studies, however, have focused on the effects of lactobacilli with regard to the reduction of cancer cell viability or tumour size [4,5]. Oral administration of Lactobacillus rhamnosus GG was shown to lower the faecal concentration of β-glucuronidase in humans implying a decrease in the conversion of procarcinogens to carcinogens [6]. Fermented milk containing Lactobacillus acidophilus given together with fried meat patties significantly lowered the excretion of mutagenic substances compared to ordinary fermented milk with Lactococcus fed together with fried meat patties [7].

Some of the probiotic strains have been reported to influence hematological cancers also. Lactobacillus reuteri enhanced TNF-induced apoptosis in human chronic myeloid leukemia-derived cells by modulation of NF-κB and MAPK signaling and reduced proteins that mediated cell proliferation (cyclin D1 and COX-2) or inhibited apoptosis (Bcl-2, Bcl-xL) [8]. Chiu et al., 2010 [9] described bacterial soluble factors secreted by Lactobacillus casei and L. rhamnosus induced apoptosis of human monocytic leukemia-cell line (THP-1). Meta-analysis of the efficacy of lactobacillus supplementation in the prevention and treatment of radiation-induced diarrhea showed its beneficial effect in experimental animal studies. However the results of human clinical trials were not consistent and should be well preformed as the randomized placebo-controlled studies [10]. Milk fermented with Lactobacillus acidophilus LA-2 was demonstrated to suppress faecal mutagenicity in the human intestine. Studies on antimutagenic activity of milk fermented with mixed-cultures of various lactic acid bacteria and yeast, showed that the fermented milks produced with mixed cultures of lactic acid bacteria had a wider range of activity against mutagens than those produced with a single strain of lactic acid bacteria [11]. Similar results were found by Nandhini and Palaniswamy, 2013 [12] in which anticancer activity of goat milk hydrolysate fermented by Lactobacillus plantarum and Lactobacillus paracasei was studied.

Choi et al., [13] have evaluated the inhibitory effects of Lactobacillus (L. acidophilus 606 and L. casei ATCC 393, L. rhamnosus GG and L. brevis ATCC 8287) on various human cancer cell lines. Among the strains of Lactobacillus tested in this study, the HK cells of L. acidophilus 606 and L. casei ATCC 393 manifested the most profound inhibitory effects on cancer cell growth. The inhibition of cancer cell growth by Lactobacillus has also been reported in other studies [4]. The HK cells of L. acidophilus 606 may be safely used as natural cancer therapeutic agents. The anticancer activities of the peptidoglycans or membrane components of the cell walls of various LAB strains, including Lactobacillus, have also been assessed [4,14]. However, these insoluble materials are difficult to administer directly. In the study carried out by Choi et al., 2006, the soluble polysaccharide fraction of the HK cells of L. acidophilus was found to inhibit cancer cell proliferation. Moreover, these polysaccharides proved to be much less cytotoxic to normal cells than the whole HK cells of the same strain. Wang et al., 2014 [15] evaluated in vitro antitumor activity of c-EPS produced by L. plantarum and result demonstrated that c-EPS significantly inhibited the proliferation of HepG-2, BGC-823, especially HT-29 tumor cells. The results suggested that the c-EPS produced by L. plantarum 70810 might be suitable for use as functional foods and natural antitumor drugs.

Lactobacilli produce peptides that have biological functions on the body. These are called bioactive peptides. Cytomodulatory peptides are the bioactive peptides that inhibit cancer cell growth and stimulate the activity of immunocompetent cells and neonatal intestinal cells, respectively. The cytomodulatory effects of peptidic fractions from milk fermented with Lb. helveticus were studied in mice [16]. Additionally, cytomodulatory effects of milk fermented by five bacterial species, i.e., Bifidobacterium infantis, Bifidobacterium bifidum, Bifidobacterium animalis, Lb. acidophilus, and Lb. paracasei in a human breast cancer cell line were reported, which were found to be due to the presence of nonbacterial peptides and compounds generated by the bacteria from milk [17].

MacDonald et al.,1994 [18] developed a cell culture based assay to identify cytomodulatory peptides generated by hydrolysis of casein with the yoghurt culture starter strains, Lb. delbrueckii ssp. bulgaricus and Streptococcus thermophilus. Cytomodulatory peptides that influenced colon Caco-2 kinetics in vitro were identified [19]. In a mouse model, De LeBlanc et al. [20] demonstrated that 7 days of cyclical feeding with milk fermented with Lb. helveticus R389 resulted in a delay of tumor development, which was related principally to a decrease in the cytokine IL-6, normally implicated in the synthesis of estrogen in both normal and tumor-invaded breasts in mice, and an increase in the cytokine IL-10. The increase in IL-10 was not observed in a murine model for milk fermented with Lb. helveticus L89, a proteolytic-deficient variant of Lb. helveticus R389. The ability of milk fermented with probiotic bacteria to enhance immune responses as reflected in macrophage cytokine production was also assessed, and it was reported that substances independent of the presence of live bacterial cells could enhance immune...
milk confirmed the reduction of PTH followed by increased in serum calcium levels. The ionized serum calcium, measuring the serum Parathyroid hormone (PTH), ionized calcium (iCa), Ca, P, and carboxyterminal telopeptide of women with a mean age of 65 years and mean body mass index (BMI) of 26. The effects were assessed by type I collagen (ICTP) and urinary calcium (U-Ca) and creatinine (U-Crea). Effect of although it has no reported effect on osteoblast proliferation, it stimulates prostaglandin synthesis, which has been shown to stimulate osteoblastic differentiation and bone formation [31]. It has been suggested that stimulating osteoclastic bone resorption [30]. Bradykinin receptors are present in human osteoblast cell lines and, fermented milk whey affects bone formation through prostaglandins such as prostaglandin E2 (PGE2) [32], although

Lactobacillus has the potential effect on increasing bone mass density which was demonstrated by a study carried out on ovariotomized (OVX) rats and mice which can simulate postmenopausal conditions [23, 24, 25, 26]. Chiang and Pan, [25] used Lactobacillus paracasei and Lactobacillus plantarum in OVX mice. The results showed that OVX mice fed lactobacillus fermented milk had higher trabecular number. An another study on male rats, with Lactobacillus helveticus fermented milk increased bone mineral density (BMD) or bone mineral content (BMC) which were measured by using dual-energy X-ray absorptiometry [26].

A study carried out by Ghanem et al., [27] reported higher calcium absorption in growing rats and 35% higher bone weight among the Lactobacillus casei, Lactobacillus reuteri, and Lactobacillus gasseri fed group compared to the control group. Kim et al., [28] showed that decreased level of BMD in OVX rats was significantly improved by administrating Lactobacillus casei in fermented milk. Similarly, McCabe et al., [23] found L. reuteri showed a significant effect on bone health via decreasing tumor necrosis factor (TNF) levels and reducing bone resorption.

Greater concentration of phosphorus and tibia ash, lateral and medial wall thickness of the tibio tarsi was observed in the chicks fed with diet contained Bacillus licheniformis and Bacillus subtilis [29].

Lb. helveticus-fermented milk whey has been reported to contain bioactive components that increase osteoblastic bone formation in vitro. (Angiotensin II has been shown to affect bone by decreasing osteoblast differentiation and stimulating osteoclastic bone resorption [30]. Bradykinin receptors are present in human osteoblast cell lines and, although it has no reported effect on osteoblast proliferation, it stimulates prostaglandin synthesis, which has been shown to stimulate osteoblastic differentiation and bone formation [31]. It has been suggested that Lb. helveticus-fermented milk whey affects bone formation through prostaglandins such as prostaglandin E2 (PGE2) [32], although the mechanisms of action of such peptides on bone health are not fully elucidated.

A study carried out to see the effects of Lactobacillus helveticus fermented milk on bone in twenty postmenopausal women with a mean age of 65 years and mean body mass index (BMI) of 26. The effects were assessed by measuring the serum Parathyroid hormone (PTH), ionized calcium(iCa), Ca, P, and carboxyterminal telopeptide of type I collagen (ICTP) and urinary calcium (U-Ca) and creatinine (U-Crea). Effect of Lactobacillus from fermented milk confirmed the reduction of PTH followed by increased in serum calcium levels. The ionized serum calcium, total calcium, phosphate, and urinary calcium were higher in the group that consumed L. helveticus fermented milk compared to the control group. The results proved that postmenopausal women who suffered from low bone mass density are potential targets to consume probiotics for increasing mineral bioavailability including calcium and consequently increasing bone mass density [33].

Increasing BMD by Lactobacillus helveticus was also confirmed by an in vitro study and results of calcium accumulation measured in osteoblast cultures found that Lactobacillus helveticus from fermented milk whey can increase osteoblast activity by 1.3-1.4 times [30]. Ghanem et al., [27] showed that yoghurt with Lactobacillus when fed to rats, increased SCFA production and improved calcium absorption. Perez-Conesa et al.,[34] fed infant formulae containing Lactobacillus as probiotics to rats, and found that calcium and magnesium absorption was enhanced and bone calcium improved.

Narva et al.,[35] showed that Lactobacillus helveticus increased calcium absorption and improved bone mass in growing rats fed with fermented milk for 14 weeks. Lactobacillus rhamnosus was also found to have positive effect on mineral absorption and bone properties.
Lactobacillus are also found for the release of caseinophosphopeptides from milk and other dairy products which have the potential to enhance Ca2+ absorption in vivo, a number of reports involving animal studies have revealed positive effect of CPPs on Ca2+ absorption [36]. Lactobacillus helveticus produces the proline-containing peptides isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) which may induce greater availability of minerals. Increased calcium absorption by some probiotic bacteria like Lactobacillus salivarius (UCC118) was demonstrated in Caco-2 cells [37].

One of the potential effects of Lactobacillus on bone possibly occurs via synthesis of vitamins [38]. Vitamins like D, C, K, and folate are involved in metabolism of calcium and are necessarily for bone formation [39,40]. Moreover, bacteria produce short chain fatty acids which decrease PTH followed by an increase in mineral absorption via their solubilisation [41]. Bacteria produce phytase enzyme, which can release minerals depressed by phytate, resulting in increased availability of minerals including calcium [42]. Moreover, increasing the bioavailability of minerals also happens in some foods with estrogenic activity due to hydrolysis glycoside bonds of estrogenic food in the intestines by Lactobacillus [25].

A recent study showed intake of a probiotic reduces intestinal inflammation and increases bone mass density, meaning that microbes in the gut have a significant effect on bone health [43]. Lactobacillus reuteri reduces proinflammatory cytokine levels systemically, which leads to increased bone volume fraction; similarly, it reduces the expression of proinflammatory cytokine in Jejunum and ileum. Lactobacillus reuteri 6475 affects bone health either through increased calcium uptake by increasing calcium solubility or by reducing epithelial cell inflammation which facilitate the transport or through production and transformation of estrogen like compounds that affect the intestinal epithelium or circulate in the blood and directly affect the bone cells [23]. Previous studies on the effect of probiotics and bone have all confirmed that probiotics can increase BMD and BMC and help reduce osteoporosis by different ways.

Immunomodulatory
Lactobacilli can elicit innate and adaptive immune responses in the host via binding to pattern recognition receptors (PRR) expressed on immune cells and many other tissues including the intestinal epithelium. In recent years, there has been encouraging interest in determining the biological role of each bacterial component. Several factors have been identified in lactobacilli that influence the immune response including metabolites, enzymes, surface and secreted proteins and recently cell surface carbohydrates [44,45].

Exopolysaccharides (EPS) are potentially useful as additives to improve the texture and viscosity of natural fermented milk products and to prevent syneresis. It has also been suggested that some EPS may confer health benefits to the consumer [46]. Sabina et al., [47] showed EPS produced by L. johnsonii 142 (LJ142), Lactobacillus johnsonii 151 (LJ151), Lactobacillus animalis murinus 148 (LAM148), Lactobacillus reuteri 115 (LR115), and Lactobacillus casei 0912 (LC0912) possessed immunoreactive properties. The Lactic acid bacteria (LAB), Lactobacillus acidophilus sp. 5e2 and Lactobacillus helveticus sp. Rosyski both secrete exopolysaccharides (EPSs) into their surrounding environments during growth. A number of EPSs have previously been shown to exhibit immunomodulatory activity with professional immune cells, such as macrophages, but only limited studies have been reported of their interaction with intestinal epithelial cells. An investigation of the immunomodulatory potential of pure EPSs, isolated from cultures of Lactobacillus acidophilus sp. 5e2 and Lactobacillus helveticus sp. Rosyski, with the HT29-19A intestinal epithelial cell line are reported [48]. For the first time the structure of the EPS from Lactobacillus helveticus sp. Rosyski which is a hetropolysaccharide with a branched pentasaccharide repeat unit containing D-glucose, D-galactose and N-acetyl-D-mannosamine is described. In response to exposure to lactobacilli EPSs HT29-19A cells produce significantly increased levels of the proinflammatory cytokine IL-8.

Additionally, the EPSs differentially modulate the mRNA expression of Toll-like receptors. Finally, the pre-treatment of HT29-19A cells with the EPSs sensitises the cells to subsequent challenge with bacterial antigens. The results reported here suggest that EPSs could potentially play a role in intestinal homeostasis via a specific interaction with intestinal epithelial cells. L. rhamnosus reduced the production of proinflammatory cytokines (TNF-a) and interleukins (IL-6 and IL-12) by immature DCs as well as IL-12 and IL-18 by mature DCs. Moreover, the cytokine response may vary greatly in the presence of different probiotics. The mixture of eight different probiotic and LAB strains including L. acidophilus, L. delbrueckii sp. bulgaricus, L. casei, L. plantarum, B. longum, B. infantis, B. breve and S. thermophilus upregulated production of IL-10 and downregulated production of IL-12 by DCs derived from human blood and lamina propria. The pro-inflammatory effect was reduced by suppression of IL-12 production in the presence of probiotics, while maintaining high production of IL-10, which was regulated by...
bifidobacteria that upregulated IL-10 production. Furthermore, most of the strains suppressed IL-12 production [49,50].

One important class of human antimicrobial peptides is the family of defensins. Human β-defensin 2 (HBD-2) is a major inducible peptide which plays an important role in host defense and represents a link between innate and adaptive immune responses. This linkage is in part mediated through the recognition of conserved bacterial products or bacteria by Toll-like receptors (TLRs). Paolillo et al., [51] investigate the effects of Lactobacillus plantarum on intestinal epithelial cells and found that Caco-2 cells exposed to L. plantarum bacteria significantly induced HBD-2 mRNA expression and HBD-2 secretion in a dose- and time-dependent manner, compared to controls; in addition, when LPS was added to cells for 48 h, the interleukin (IL)-23 secretion and IL-23 mRNA expression increased; while it was reduced when LPS was cocultured with L. plantarum. The L. plantarum-induced increase in HBD-2 expression is inhibited by anti-TLR-2 neutralizing antibodies, in the same way the pre-treatment with the anti-TLR-2 antibody inhibited the production of IL-23 induced by LPS in Caco-2 cells. The results of study help to achieve a better understanding of how the intestinal epithelium participates in the innate immune response to commensal bacteria and pathogens in the gut.

Lymphocyte proliferation in response to mitogens is commonly examined when analyzing the efficacy of immunomodulatory agents, and lymphoproliferation assays have been used to evaluate the effects of probiotic bacteria on the immune function [52]. Bujalance et al., [53] found that L. plantarum, when given to normal mice, was without effect on the spleen cell proliferation in response to LPS, and slightly stimulated the proliferation in response to Con A. However, the suppressive effect of cyclophosphamide on the LPS-driven lymphoproliferation was attenuated by L. plantarum. The mechanism of this immune restoration remains unknown. A probiotic strain of L. plantarum was a potent inducer of cytokine release by mononuclear PBL from healthy human donors [54]. Based on its capacity to increase the production of interleukin-10 (IL-10) by macrophages and T cells from the intestinal mucosa, possible use of L. plantarum in the treatment of inflammatory bowel diseases has been considered [55]. But IL-10 is a pleiotropic molecule that exerts a variety of effects in addition to the inhibition of inflammatory reactions and suppression of T cell-mediated immune responses. IL-10 is able to promote proliferation of B cells and CD8+ T cells, and to antagonize the immunosuppressive effect of cyclosporine [56]. Therefore, the induction of IL-10 might be involved in the stimulatory effects of L. plantarum on the proliferative capacity of splenocytes from immunologically intact or compromised mice. In conclusion, a probiotic strain of L. plantarum, was able to exert immunomodulatory effects. Moreover, administration of L. plantarum improved some lymphocyte responses in immunocompromised hosts. However, more studies are also carried out to extend this conclusions to other probiotic strains and different immunosuppression models.

Wang et al., [57] isolated five Lactobacillus strains (Lactobacillus plantarum L2, L. plantarum L8, and L. plantarum L9) from the human intestinal tract. Lactobacillus rhamnosus GG (ATCC 53103)) were tested for their ability to adhere to Caco-2 and IEC-6 cell lines as in vitro models and to induce the secretion of pro- and anti-inflammatory cytokines by human peripheral blood mononuclear cells (PBMCs). Among the tested strains, Lactobacillus plantarum L2 was the most adhesive strain, of the added bacteria adhered to Caco-2 or IEC-6 cell cultures, respectively. Furthermore, L. plantarum L2 was also found to induce a considerable level of IL-10 from PBMCs, but low levels of all three pro-inflammatory cytokines TNF-α, IFN-γ and IL-12 (The potentially probiotic strain Lactobacillus plantarum BFE 1685 isolated from a child's faeces and the probiotic strain Lactobacillus rhamnosus GG were investigated for their capability to influence the innate immune response of HT29 intestinal epithelial cells towards Salmonella enterica serovar Typhimurium. Furthermore, their capacity to modulate toll-like receptor expression of HT29 cells was investigated at the mRNA and protein levels. Lactobacillus acidophilus Bar13, L. plantarum Bar10, Bifidobacterium longum Bar33 and B. lactis Bar30 were effective in displacing the enteropathogens Salmonella typhimurium and Escherichia coli H10407 from a Caco-2 cell layer. Moreover, L. acidophilus Bar13 and B. longum Bar33 have been assessed for their immunomodulatory activity on IL-8 production by HT29 cells. Both strains showed the potential to protect enterocytes from an acute inflammatory response. These probiotic strains are potential candidates for the development of new functional foods helpful in counteracting enteropathogen infections.

CONCLUSION

The peptides generated by milk fermentation with Lactobacillus strains may contribute with a variety of bioactive compounds to a positive effect on human health. Moreover, they could also be considered for the development of
functional foods and simultaneously enhance the shelf-life of the food products. The powerful proteolytic system of these strains opens up future opportunities to develop novel functional foods with potential health promoting properties.

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