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EFFICACY AND POTENTIAL OF LACTIC ACID BACTERIA MODULATING HUMAN HEALTH

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ABSTRACT

Lactic acid bacteria are safe microorganisms that are capable of providing healthy conditions to human beings and animals. They might be effective in exerting beneficial effects on human host in terms of maintenance of homeostasis and promotion of health. Some studies suggest that fermented food products of the bacteria provide better digestibility, nutrient assimilation and effects on constipation. The anti-microbial, anti-allergic, anti-inflammatory and anti-viral activity against urogenital and respiratory infections expresses the ability of the microbe to resist pathogens while immunomodulatory, lactose intolerance, hypocholesterolemic, anti-cancerous, prevention from dairrhoea, tooth decay, stomach ulcers and various other gastrointestinal problems results in an approach towards improved health conditions. The metabolites from the microbe; bacteriocins are very common in food industry with their ability to preserve food products for longer times. Modern science also suggests the use of lactic acid bacteria as live vaccines. There is a need to reconcile the applications and tremendous approach of lactic acid bacteria, which will provide a broader view of its potential in health modulation.

KEY WORDS: ***Lactic acid bacteria, Health benefits, anti-microbial, immunomodulation, anti-cancerous, probiotics and bacteriocins.***

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INTRODUCTION

A human body intestine comprises of billions of live bacteria of which some are beneficial and some are hazardous to human health. These living bacteria require a microbiological balance environment in the intestine for healthy and energetic body. Many factors like diet, stress conditions, use of drugs etc can impact on balance within the different populations in gut microbiota, resulting in a higher number of detrimental species like Clostridia and Bacteroides species. Therefore, the oral consumption of probiotic microorganisms is required to produce a protective effect on the gut flora. Thus, this results the term "probiotic" that is becoming an important part of many individual's healthy lifestyle. The most commonly used probiotics are lactic acid excretors like lactobacilli and bifidobacteria which are usually added to fermenting milk, or given in lyophilised forms. These fermented food products may contain one or more several species of probiotic bacteria which are considered to be health benefiting bacteria [1, 2].

Lactic acid bacteria (LAB) are regarded as a major group of probiotics [3, 4]. The term LAB gradually accepted in the beginning of the 20th century, but previously this bacterial group creates confusion with names like: milk sourcing and lactic acid producing [5]. These LAB are industrially important organisms recognised for their fermentative ability and comprise of an ecologically diverse group of microorganisms united by formation of lactic acid as the primary metabolite of sugar metabolism [6]. These bacteria are basically Gram-positive, non-spore forming cocci, cocci-bacilli or rods, non-rispiring, catalase-negative bacteria that are devoid of cytochromes and are of non-aerobic habitat but are aero-tolerant, fastidious, acid tolerant and strictly fermentative; lactic acid is the major end-product of sugar fermentation by homo-lactic fermentation [7]. They are chemo-organotrophic and grow in complex media and generally are non-pathogenic to man and animals. The LAB consist of several genera, which include *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Lactosphaera, Leuconostoc*, *Melissococcus, Oenococcus*, *Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella* [8,9]. Based on similarities in physiology, metabolism and nutritional needs, these genera are grouped together.

LAB were first isolated from milk, [6,10] occurring naturally in soil, water, manure and sewage [9] and since have been found in such foods and fermented products like meat, milk products, vegetables, beverages and bakery products [11,12]. LAB can work as spoilage organisms in foods such as meat, fish and beverages [11]. They are now commercially available in live form or in a heat-inactivaed form in pharmacological preparations while bifidobacteria are used specially for children to restore immune defence. Bifidobacteria are active in bile acid deconjugation, allows synthesis of vitamins and catabolism of dietary carbohydrates [13]. LAB produces acetaldehyde, hydrogen peroxide, diacetyl, carbon dioxide, polysaccharides and bacteriocins during fermentation [14,15,16,17], of which, some may act as antimicrobials.

LAB supplements are becoming more and more popular all over the world. These supplements contain live food microbial ingredients that are found to be very beneficial to human health on ingestion and show a wide range of human health modulating properties **(Fig. 1)**. Some LAB secretes exocellular carbohydrate polymers named exopolysaccharides (EPS). A large diversity of EPS from LAB strains exists regarding their chemical characteristics, yield, technological and functional properties [18]. These EPS have been claimed to participate in various process leading to health modulation like immunomodulatory, cholesterol-lowering and anti-ulcer activities [19].LAB has been cited to be the part of human [20,21, 22] and animals micro biota [23,24,25]. However, some of these bacteria are part of the oral flora that can cause dental carries [26,27]. It's also constituted as an integral part of the healthy gastrointestinal

(GI) microecology and is involved in the host metabolism. Various scientific results and their implications especially the nutritional, medicinal and therapeutic part will be reviewed and discussed in the further of the review.

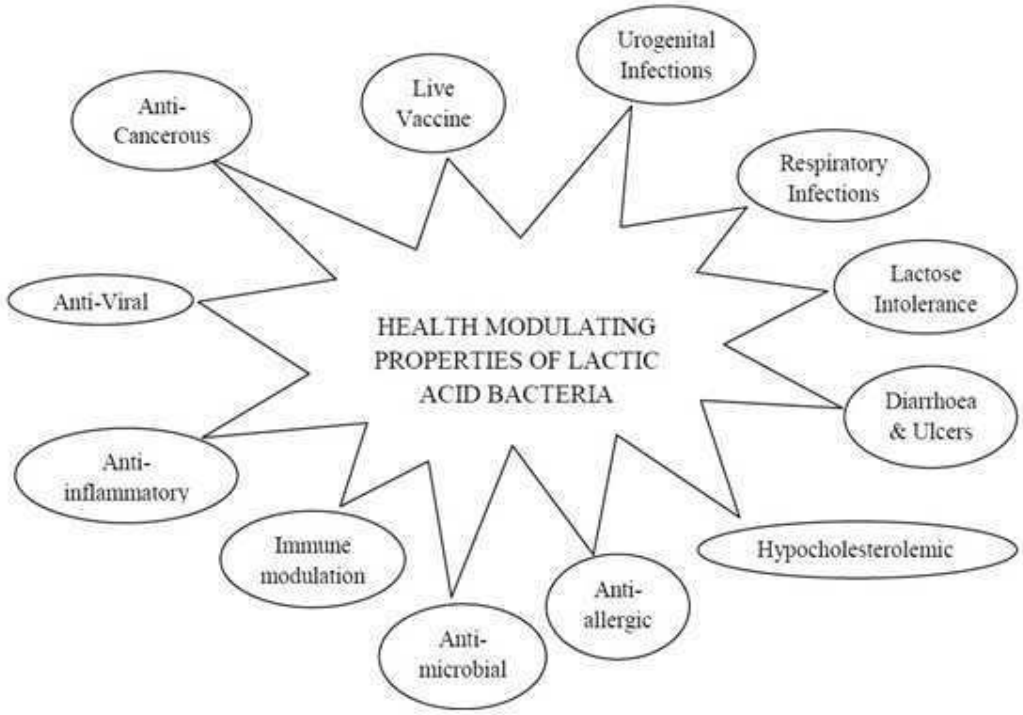

Figure 1
Health modulating properties of Lactic acid bacteria

ANTIMICROBIAL ACTIVITY

Lactic acid bacteria provide numerous benefits to mankind by producing metabolites that inhibit the growth of pathogenic and non-pathogenic microorganisms [28]. They produce metabolites by fermenting some food materials which serves food industry in providing better shelf life through anti-microbial activities [29]. Among different genera of LAB; Lactobacilli produce various organic acids like lactic acid, acetic acid and propionic acid exhibiting anti-microbial activity [30,31]. *Lactobacillus lactis* and *Streptococcus thermophilus*, inhibit food spoilage and pathogenic bacteria and preserve the nutritive qualities of raw food material for a longer shelf life [12, 32]. Lactobacillus plantarum isolated from soy milk also strong anti-bacterial activity against E.coli and other pathogenic bacteria [33]. Carbon dioxide that is another bi-product of lactic acid bacterial hetero-fermentation can cause dysfunction in the permeability of lipid bilayer. It can also inhibit the growth of many food spoilage microbes; especially Gram-negative psychrotrophic bacteria. Diacetyl was found to be active against gram-negative bacteria, yeasts, moulds etc. Diacetyl is thought to react with the arginine binding protein of gram-negative bacteria and thereby interfering the utilization of arginine.

(1) Bacteriocins: A novel compound of LAB

Bacteriocins are proteinaceous substances and exhibit bactericidal activity against the closely related organisms [28]. In other words, bacteriocins are defined as protein antibiotics that work against the same or closely related species by adsorption to receptors on the target cells. Four classes of bacteriocins produced by LAB: (I) lantibiotics, (II) small

hydrophobic heat-stable peptides or *Listeria*-active bacteriocins (< 13000D), (III) large heat-labile proteins (>30000D) and (IV) complex bacteriocins, which are proteins with lipid and/or carbohydrate [34]. Among the four classes, lantibiotics are the most documented and industrially exploited [12]. Antimicrobial peptides occupy an inhibition spectrum narrower than that of antibiotics [35,36].

Lantibiotics are basically very small (<5 kDa) peptides containing unusual amino acids lanthionine, dehydroalanine, a-dehydroalanine and dehydrobutirine. According to their chemical structures and mode of action, they are subdivided into type A and type B lantibiotics [37]. Type A lantibiotics are elongated amphiphilic lantibiotics, like nisin, with a net positive charge, which are active through the formation of pores in bacterial membranes, leading to the dissipation of membrane potential. Type B lantibiotics are smaller globular peptides, like mersacidin, which have negative or no net charge, and act through the inhibition of specific enzymes [37,38].

Bacteriocin shows anti-microbial activity by inhibiting the growth of spores and also by modulating various enzyme activities. Most bacteriocins interact with anionic lipids that are abundantly present in the membranes, and consequently initiate the formation of pores in the membranes of susceptible cells [39]. The lantibiotic nisin naturally produced by *Lactococcus lactis* ssp. *lactis* is commercially available as food additive [3]. The nisin variants A and Z, differ by one amino acid [40]. In addition, a new nisin variant, nisin Q, has been isolated from a *L. lactis* strain found in river water in Japan. Nisin Q differs in four amino acids as a mature peptide and in two amino acids of the leader sequence [41]. All forms of nisin are antimicrobially active against gram-positive bacteria, such as LAB, *Listeria* sp., *Micrococcus* sp. and sporeforming bacteria like *Bacillus* sp. and *Clostridium* sp. [34,41.] The electric transmembrane potential is strongly reduced in the presence of nisin and lipid II [43]. Furthermore, nisin inactivates endospores by preventing post-germination swelling and subsequent spore outgrowth. Nisin applied as a food preservative to extend the shelf life of a product [12, 43]. Bacteria have self-protective mechanisms limiting the bacteriocin production, as in the case of nisin-producing *Lactococcus lactis* [44]. The bacteriocin production is highest at the end of the exponential and early stationary phase and reduction is caused by proteolytic degradation of the bacteriocins.

IMMUNOMODULATORY ACTIVITIES

Studies on the relationship between nutritional food and immune-modulation have been increased due to based on the hypothesis that consumption of some foods creates a barrier for immunological diseases [45]. Gut microflora participates in immune exclusion. It prevents other bacteria from adhering by competing for nutrients and places of adhesion, it produces anti-bacterial agents, and it stimulates the production of specific antibodies [46]**.** There are many reports on the immunomodulatory activities of LAB [47,48, 49]. Probiotics can influence the microflora composition by increasing the number of Lactobacilli and other anaerobes [50]. Dietary supply of probiotic bacteria stimulates IgA immune response [51] and the transport of target antigens through Peyer's patches [52].

LAB produces some extracellular polysaccharides (EPS) associated with rheology, mouthfeel and texture of fermented milk products. These extracellular polysaccharides from *Lactobacillus bulgaricus* purified from the supernatant have certain immuomodulatory activities shown in case of mice [53]. So, the yoghurt containing immunostimulative EPS would have an immunomodulatory effect on human body [53]. A halophilic lactic acid bacterium, *Tetragenococcus halophilus*, was found to possess an immunomodulatory activity that promotes T helper type 1 (Th1) immunity in addition to its important roles in soy sauce brewing [54]. *Oenococcus oeni* and *Pediococcus parvulus* also found to have immune-stimulatory activities as the strains found to stimulate cytokines released by peripheral blood mononuclear cells [54].

ANTI-VIRAL ACTIVITIES

A lactic acid bacterium is found effective against the 'Transmissible Gastroenteritis Coronavirus (TGEV) and Rotavirus RF strain (RV)' [55]. A selected number of strains of LAB were used in the study along with the CLAB cell line from pig. However, the co-incubation of the CLAB cell line with specific lactic acid bacteria strains resulted in increased survival percentages, from 40% to 80%.

Various literature and clinical studies have confirmed the beneficial and alleviating effects of probiotic bacteria. *Lactobacillus rhamnosus* had the strongest influence in reducing prevalence, severity and duration of diarrhea and was therefore chosen for combination treatment with immunoglobulins [56,57]. Also probiotics have been reported on their antiviral activities on animal [58]. *L. acidophilus* NCFM has a significant immune-stimulating effect on the intestinal and systemic HRV-specific T and B cell immune responses induced by the AttHRV vaccine and is safe in neonates; therefore it may have the potential to be used as an adjuvant for rotavirus vaccines [59].

ANTI-INFLAMMATORY

The genus Lactobacillus includes a restricted set of intestine-indigenous species from a pool of more than 100 *Lactobacillus* species and studies suggest that some strains of the species have potent anti-inflammatory effects [60]. The strains have the capability to down regulate the production of TNF-α by macrophage cell lines and successfully suppressed inflammation in mouse colitis models. Human-derived *Lactobacillus reuteri* strains have been identified with potent human TNF-α-inhibitory effects on lipopolysaccharide (LPS)-activated human myeloid cell lines and primary monocyte-derived macrophages from children with Crohn's disease [60]. Some LAB has been shown to regulate mucosal immune responses by modulating the production and liberation of regulatory agents such as cytokines by the host.

Some of these cytokines, such as anti-inflammatory interleukin-10 (IL-10), modulate the inflammatory immune responses, thus immunomodulation is a mechanism by which LAB can prevent certain inflammatory bowel diseases (IBD) [61]. Extracts from soymilk fermented with LAB and *bifidobacteria* showed the inhibitory effect on LPS-induced pro-inflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β and prostaglandin E_2 (PGE_2) produced by RAW 264.7 macrophages [62].

ANTI-ALLERGIC

Lactic acid bacterial fermentation also increases the anti-allergic effects of *Ixeris dentata***.** *Lactobacillus acidophilus* increases its inhibition of degranulation in RBL-2H3 cells induced by the IgE-antigen complex and improves ID-mediated inhibition of IgE-induced allergic diseases such as rhinitis and asthma [63]. There are many more studies targeting on the anti-allergic activities of LAB [64,65,66]. The application of probiotics to prevent allergic reactions became more prominent with the double-blind, randomized, placebo-controlled trial showing that *L.rhamnosus* GG given to pregnant women for 4 weeks prior to delivery and then to newborn children is at high risk of allergy for 6 months and caused a significant reduction in early atopic disease [67]. Furthermore clinical studies with *L. rhamnosus* GG and *B.lactis* BB-12 appear to have been useful in infants allergic to cow's milk.

HYPOCHOLESTEROLEMIC EFFECTS

Hyperlipidemia is a leading death cause in many countries of the world with the risk of cardiovascular diseases. A number of scientific studies suggest that lactic acid bacterial fermented food shows hypolipidimic effects by reducing the cholesterol biosynthesis and decreasing low-density lipoproteins [68]. The hypocholesterolemic effects of LAB have now become an area of great interest and controversy for many scientists. Some strains of *L. acidophilus* can take up cholesterol in the presence of bile; these reports suggest that LAB can reduce cholesterol level up to 50% in presence of

bile salt [69,70]. Some LAB could adjust blood lipid and lower cholesterol, which can also prevent some of the diseases by stimulating antioxidant enzymes [71,72].

These strains have the ability to tolerate both acid and bile concentrations typically found in the upper gastrointestinal tract of humans. In a study, milk fermented with LAB was fed to rats and it was observed that they reduce the serum total cholesterol and LDL cholesterol levels after 12 days of feeding [73]. Hypocholesterolemic effect of *Lc. lactis subsp. lactis* was attributed to its ability to suppress the reabsorption of bile acids into the enterohepatic circulation and to modulate the excretion of bile acids in feces of hypercholesterolemic rats [73].

ACTIONS AGAINST ULCERS

LAB also shows prominent actions against ulcers especially against stomach ulcers. Lactobacillus *acidophilus* was found active against *Helicobacter pylori,* which is common in causing infection and ulcers in stomach. A small study of patients with ulcers showed that *Bifidobacterium bifidum* promoted healing of gastric ulcers in 50% of the patients and eradication of *H. pylori* from the mucous membranes in 30% of the patients [74]. The anti-ulcer effects of bifidobacteria, lactobacilli and streptococci were also examined using the acetic acid-induced gastric ulcer and ethanol-induced erosion models in rats [75]. LAB was administrated orally and anti ulcer effects were confirmed not only with the organism but also with their polysaccharide fractions. In particular, polysaccharides in which the rhamnose content exceeded 60% were more effective in healing gastric ulcers [76].

RESPIRATORY INFECTIONS

Acute lower respiratory tract infections are a persistent public health problem. They cause a greater burden of diseases worldwide, than human immunodeficiency virus infections, malarias, cancers, or heart attacks [77]. LAB has been used for the preparation of various probiotic foods with the capability to modulate respiratory immunity, which will stimulate the adoption of resistance against respiratory infections. Studies suggest that LAB with immunomodulatory properties is able to stimulate the innate immunity system; on the other hand LAB can be used as adjuvant in order to increase the innate and specific immunity against respiratory infections. Researchers across the world also suggest that there is a possibility of using LAB as vaccines against pneumococcal infections and is an important tool for medical application [77].

UROGENITAL INFECTIONS

Urogenital infections include the infections that affect the bladder, kidneys, vagina, urethra, periurethra, and cervix. *L. rhamnosus* was found to be the best strains isolated from dairy, poultry, health food, and human sources, and has properties of adhesion to squamous and transitional uroepithelial cells, competitive exclusion of pathogens and production of inhibitors for uropathogen growth.

Hydrogen peroxide producing strains of LAB are believed to be important in vaginal colonization [78]. One small study showed that women with recurrent vaginal candidiasis who ate 8 oz. daily of a yogurt containing *L acidophilus* had fewer occurrences of vaginal candidiasis than during the control period in which they ate no yogourt [79]. The evidences are growing day by day of LAB strains present in the healthy urogenital tract protects the host against infections by pathogenic microorganisms.

PREVENTION FROM DIARRHOEA

Intestinal microflora maintains a barrier against the development of pathogenic bacteria's in the digestive tract of humans and it is mandatory of establishing oral tolerance to food antigens. LAB may be useful in preventing and shortening the duration of several types of diarrhea. It is believed that lactic acid bacterium competes with the pathogenic bacteria in terms of nutrition and space in the intestines shortening its chances of survivability. Undergoing a strong metabolism process LAB produces various metabolites like

acidophilin and bulgarican that inhibits the growth of other bacteria preventing and shortening the duration of diarrhoea. In recent study carried out in India, probiotics feeding in children resulted in effective prevention against diarrhoea [80].

ANTI-GASTRIC ACTIVITY

Gastritis is a common disorder in which discontinuity of the gastric mucosa is observed. It is caused by various factors like excess alcohol and infection through intensive consumption of anti-inflammatory drugs with *Helicobacter pylori* or sometimes through stress [81]. The first therapeutic effect of the fermented milk with the polymer producing strain of *S. thermophilus* CRL 1190 on chronic gastritis was induced by associated chronic gastritis ASA in mice [82]. Also, ASA affects various mucosal defense lines such as bicarbonate secretion, mucus synthesis, and decreases mucosal blood flow [83]. The fermented milk with the EPS-producing strain *S. thermophilus* CRL 1190 and/or its exopolysaccharides (EPS) constitute a potential natural alternative for the prevention and treatment of ASA-associated gastric damage. It was able to generate immune response in mice and increased the thickness of the gastric mucus gel layer [84]. Studies suggest that recombinant lactic acid bacteria are the excellent candidates for the production of various bio therapeutic proteins and also their delivery to specific places of requirement within the gastrointestinal tract [84].

LIVE VACCINE

The most promising new application for LAB are their use as live delivery vectors for antigenic or therapeutic protein delivery to mucosal surfaces. Such engineered LAB are able to elicit both mucosal and systemic immune responses [85]. The delivery of vaccine in the body through mucosal routes is much more beneficial as compared to direct systemic inoculation. But human mucosal surface is a site in which the host encounters a large variety of microorganisms initiating infections. LAB have proved to be an effective delivery vehicle for functional proteins to mucosal tissues. Oral administration of *Lactococcus lactis* has been shown to induce antigen-specific oral tolerance (OT) to secreted recombinant antigens [86].

Non-pathogenic food grade bacteria, LAB are being tested for their efficacy as live antigen carriers [87]. The advantages of using live bacterial vaccines include their mimicry of a natural infection, intrinsic adjuvant properties and their possibility to be administered orally. Derivatives of pathogenic and non-pathogenic food related bacteria are currently being evaluated as live vaccines [88]. The first evidence that recombinant commensal bacterium can be used as a live vaccine vector was obtained with *S. gordonii* [89]. There are two major approaches being followed to achieve efficient mucosal delivery of antigens [90,91]. A variety of synthetic (non-living) delivery systems, in which purified antigens are entrapped in microspheres, liposomes, nanoparticules, or ISCOMS, are presently being investigated. An attractive alternative delivery system consists of use of live virals or bacterial vectors for the production of replicative particulate antigens *in vivo*. This technology, which alleviates the drawbacks of subunit vaccine development, was first described in the early 1980s [92]**.** In addition to the difficulties often encountered in the construction of stable attenuated mutants of pathogenic organisms, attenuated pathogens may retain a residual virulence level that renders them unsuitable for the vaccination of partially immunocompetent individuals such as infants, the elderly or immune compromised patients [93].

Most of the LAB is quite acid resistant and various strains are able to effectively survive passages through stomach. Today LAB has created a vaccine vehicle system depending upon their immunization routes and models of antigens they carry. It is found that the LAB has a low innate antigenicity even though several strains clearly exhibit immunoadjuvant properties [94]. There is no example of any problems in literature using bacterial vaccines in case of human but a possibility always exist. The ideal genetically modified microorganism (GMM) for use in

humans should therefore contain the minimal amount of foreign DNA and must not include an antibiotic resistance marker [95].

LACTOSE INTOLERANCE

Lactose intolerance is the insufficient ability to digest lactose. A significant quantity of lactose is present in the milk of mammals that gets degraded in the human digestive tract by intestinal lactase (β galactosidase) to glucose and galactose [96]. The reduction of lactase in the body causes various digestive and intestinal disorders like distension, diarrhoea, vomiting, etc. Most people with lactose intolerance can tolerate some amount of lactose in their diet. Thus a reduction of lactose in dairy products is the method of choice. However, lactose is often present in processed food and instant food, making it difficult to keep a lactose-reduced diet. It is assumed that various microorganisms have the ability to decrease the lactose amount in the intestine and improves the nutritional properties. It is a well-known fact that the presence of LAB in the body improves lactose digestion [97]. Also the slower transit time of yogurt provides more time for residual intestinal lactase and the bacteria to digest the lactose.

CONTROVERSIAL ANTI-CANCEROUS PROPERTY

The most interesting and controversial property of LAB is an anti-cancerous property, as the mechanisms by which probiotic bacteria may inhibit colon cancer are still poorly understood. In spite of various controversies, LAB have been shown to affect intestinal barrier by interfering the metabolic activity of tumor cells preventing and treating a variety of cancers [98,99]. Some researchers suggest that short-lived metabolite mixtures isolated from milk that was fermented with strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are more effective in deactivating etiologic risk factors of colon carcinogenesis [99]. LAB plays a very important role in retarding colon carcinogenesis by possibly influencing metabolic, immunologic and protective functions in the colon**.** It was studied that increase in the ingestion of LAB prevent carcinogen induced preneoplastic lesions and tumors [100].

LAB deactivate genotoxic carcinogens which show prevention against mutations by preventing DNA damage. The various *in vitro* effects of LAB on the viability of cancerous cells along with the anti oxidant activity were studied and observed that the local lactobacilli strains of Taiwan have strong anti-cancerous and anti-oxidant activities [101]. Some specific cellular components in LAB seem to induce very strong adjuvant effects like cytokine pathway augmentation, activation of the reticulo-endothelial system, modulation of cell-mediated immune responses, interleukins regulatory mechanism and on various tumor and necrosis inducing factors [103]. Studies on the effect of probiotic consumption on cancer appear promising, recently *in vitro* and *in vivo* studies have indicated that probiotic bacteria might reduce the risk, incidence and number of tumours of the colon, liver and bladder [102].

CONCLUSION

Lactic acid bacteria (LAB) constitute a heterogeneous group of bacteria that play a crucial role in the health of humans and animals. Due to their economic importance for the food industry and their health-related implications as probiotics, the genetics, physiology and metabolism of LAB have been under rigorous investigation over the past decades. During food processing and storage, LAB resides under adverse environmental conditions designed to be bacteriostatic or bactericidal for food spoilage microorganisms and food borne pathogens. In addition, during consumption, the key feature of probiotic strains is their aptitude to survive through the harsh environment of the gastrointestinal tract of the host so as to reach and colonize the intestine and exerts their health-promoting effects like lowering the cholesterol levels, protection from various infections, better digestibility and reduction in gastrointestinal problems. Apart from this, it can also improve lactose digestion, prevent

and shorten the duration of diarrhoea and enhance the immune system function. The demand of consumers to use natural methods for health maintenance rather than long-term chemotherapeutic agents (i.e. antibiotics), linked to their expectation that food becomes a source of prolonged well-being, supports the speculation that the probiotic market will expand rapidly. Much of this growth will also depend on the reliability of claims that these products can bare. Therefore, the legislator will have to provide clear rules and regulations that will depend on measurable biomarkers and criteria based on scientific evidence. With advancements in technologies and further refinements and developments in new techniques, research in this area will continue to provide novel bio-therapeutics and therapeutic targets as well as novel probiotic strains for the treatment and prevention of infectious diseases.

REFERENCES

1. Salminen S, Nybom S, Meriluoto J, Collado MC, Vesterlund S, El-Nezami H, Interaction of probiotics and pathogens-benefits to human health. Curr Opin Biotechnol, 21 (2): 157–167, (2010).
2. Tuohy KM, Probert HM, Smejkal CW, Gibson GR, Review: Using probiotics and probiotics to improve gut health. Drug Discov Today, 8 (15): 692−700, (2003).
3. Khalid K, An overview of lactic acid bacteria. Int J Biosci, 1 (3): 1–13, (2011).
4. Schrezenmeir J, de Vrese M, Probiotics, prebiotics, and synbiotics – approaching a definition. Am J Clin Nutr, 73 (2): 361S−364S, (2001).
5. Carol AR, Leon MTD, Horizontal gene transfer amongst probiotic lactic acid bacteria and other intestinal microbiota: what are the possibilities? A review. Arch Microbiol, 193 (3): 157−168, (2010).
6. Carr FJ, Hill D, Maida N, The lactic acid bacteria: A literature survey. Crit. Rev. Microbiol, 28 (4): 281−370, (2002).
7. Derek AA, Joost VDB, Inge MKM, Pronk JT, van Maris AJ, Anaerobic homolactate fermentation with *Saccharomyces cerevisiae* results in depletion of ATP and impaired metabolic activity. FEMS Yeast Res, 9 (3): 349–357, (2009).
8. Jin YL, Ai HL, Cheng J, Yang WM, First description of a novel Weissella species as an opportunistic pathogen for rainbow trout Oncorhynchus mykiss (Walbaum) in China. Vet Microbiol, 136 (3-4): 314-320, (2009).
9. Holzapfel WH, Haberer P, Geisen R, Bjorkroth J, Schillinger U, Taxonomy and important features of probiotic microorganisms in food nutrition. Am J Clin Nutr, 73: 365S−373S, (2001).
10. Ali AA, Isolation and identification of lactic acid bacteria from raw cow milk in Khartoum State, Sudan. Int J Dairy Sci, 6: 66-71, (2011).
11. Liu SQ, Review article: Practical implications of lactate and pyruvate metabolism by lactic acid bacteria in food and beverage fermentations. Int J Food Microbiol, 83 (2): 115−131, (2003).
12. O'Sullivan L, Ross RP, Hill C, Review: Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. Biochimie, 84 (5-6): 593−604, (2002).
13. Deguchi Y, Morishita T, Mutai M, Comparative studies on synthesis of water soluble vitamins among human species of bifidobacteria. Agric Biol Chem, 49: 13–29, (1985).
14. Khay EIO, Idaomar M, Castro LMP, Bernárdez PF, Senhaji NS, Abrini J, Antimicrobial activities of the bacteriocins like substances produced by lactic acid bacteria isolated from Moroccan dromedary milk. Afr J Biotechnol, 10 (51): 10447–10455, (2011).
15. Saranya S, Hemashenpagam, Antagonistic activity and antibiotic sensitivity of Lactic acid bacteria from

fermented dairy products. Adv Appl Sci Res, 2 (4): 528–534, (2011).
16. Olaoye OA, Onilude AA, Quantitative estimation of antimicrobials produced by Lactic Acid Bacteria isolated from Nigerian beef. Int Food Res J, 18 (3): 1155–1161, (2011).
17. Rodriguez E, Arques JL, Rodriguez R, Nunez M, Medina M, Reuterin production by lactobacilli isolated from pig faeces and evaluation of probiotic traits. Lett Appl Microbiol, 37 (3): 259−263, (2003).
18. Hassan AN, Awad S, Application of exopolysaccharide-producing cultures in reduced-fat Cheddar cheese: cryo-scanning electron microscopy observations. J Dairy Sci, 88 (12): 4214–4220, (2005).
19. Welman AD, Maddox IS, Exopolysaccharides from lactic acid bacteria: perspectives and challenges. Trends Biotechnol, 21 (6): 269–274, (2003).
20. Prakash S, Tomaro-Duchesneau C, Saha S, Cantor A, The Gut Microbiota and Human Health with an Emphasis on the Use of Microencapsulated Bacterial Cells. J Biomed Biotech, doi:10.1155/2011/981214, (2011).
21. Bernardeau M, Vernoux JP, Henri-Dubernet S, Guequen M, Safety assessment of dairy microorganisms: the *Lactobacillus* genus. Int J Food Microbiol, 126 (3): 278–285, (2008).
22. Martin RS, Langa C, Reviriego E, Jimínez E, Marín ML, Xaus J, Fernández L, Rodríguez JM, Human milk is a source of lactic acid bacteria for the infant gut. J. Pediatr. 143 (6):754−758, (2003).
23. Bederska-Lojewska D, Pieszka M, Modulating gastrointestinal Microflora of pigs through nutrition using feed additives. Ann Anim Sci, 11 (3): 333–355, (2011).
24. O‘ Donnell MM, Forde BM, Neville B, Ross PR, O’ Toole PW, Carbohydrate catabolic flexibility in the mammalian intestinal commensal *Lactobacillus ruminis* revealed by fermentation studies aligned to genome annotations. Microb Cell Fact, 10: S1–S12, (2011).
25. Devirgiliis C, Barile S, Perozzi G, Antibiotic resistance determinants in the interplay between food and gut microbiota. Genes Nutr, 6: 275–284, (2011).
26. Fernandez R, Sridhar M, Sridhar N, Effect of Lactic Acid Bacteria Administered Orally on Growth Performance of *Penaeus indicus* (H. Milne Edwards) Juveniles. Res J Microbiol, 6 (5): 466–479, (2011).
27. Sbordone L, Bortolaia C, Oral microbial biofilms and plaque-related diseases: microbial communities and their role in the shift from oral health to disease. Clin Oral Investig, 7 (4):181−188, (2003).
28. Prameela K, Mohan CM, Smita PV, Kumar SS, Sreelatha R, Hemalatha APJ, Partial purification and chatecterization of bacteriocin produced by Lactobacillus plantarum and its antibacterial activity. J Pharm Res, 4 (8): 2563–2565, (2011).
29. Coda R, Cassone A, Rizzello CG, Nionelli L, Cardinali G, Gobbeti M, Antifungal Activity of *Wickerhamomyces anomalus* and *Lactobacillus plantarum* during Sourdough Fermentation: Identification of Novel Compounds and Long-Term Effect during Storage of Wheat Bread. Appl Environ Microb, 77 (10): 3484–3492, (2011).
30. Oluwafemi F, Adetunji AF, Antimicrobial activities of lactic acid bacteria isolated from traditionally- fermented maize (ogi) against *Candida albicans.* J Appl Biosci, 41: 2820–283, (2011).
31. Arokiyamary A, Sivakumar PK, Antibacterial activity of Bacterocin producing *Lactobacillus* sp., isolated from traditional milk products. Curr Bot, 2 (3): 05–08, (2011).
32. Akpinar A, Yerlikaya O, Kili S, Antimicrobial activity and antibiotic resistance of *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* strains isolated from Turkish homemade yoghurts. Afr J Microbiol Res, 5 (6): 675-682, (2011).

33. Srinivasan P, Khan KA, Perumal UR, Kumar RV, Suganya K, Rajalakshmi M, *Invitro* Anti-bacterial activity of *Lactobacillus plantarum* isolated from soy milk. Int J Pharm Bio Sci, 3 (3) : 209-219, 2012.
34. Bilkova A, Sepova HK, Bilka F, Balazova A, Bacteriocins produced by lactic acid bacteria. Ceska Slov Farm, 60 (2): 65–72, (2011).
35. McAuliffe O, Ross RP, Hill C, Lantibiotics: structure, biosynthesis and mode of action. FEMS Microbiol Rev, 25 (3): 285−308, (2001).
36. Morency H, Mota-Meira M, LaPointe G, Comparison of the activity spectra against pathogens of bacterial strains producing a mutacin or a lantibiotic. Can J Microbiol 47 (4): 322−331, (2001).
37. Suskovic J, Kos B, Beganovic J, Srecko M, Blazenka K, Jasna B, Antimicrobial Activity of Lactic Acid Bacteria. Food Technol Biotechnol 48 (3): 296−307, (2010).
38. Cotter PD, Hill C, Ross RP, Bacterial lantibiotics: strategies to improve therapeutic potential. Curr Protein Pept Sci, 6 (1): 61−75, (2005).
39. Chen H, Hoover DG, Bacteriocins and their food applications. Compr Rev Food Sci, 2: 82−110, (2003).
40. Piper C, Hill C, Cotter PD, Ross PR, Bioengineering of a Nisin A-producing *Lactococcus lactis* to create isogenic strains producing the natural variants Nisin F, Q and Z Microbial Biotechnol, 4 (3): 375–382, (2011).
41. Zendo T, Fukao M, Ueda K, Higuchi T, Nakayama J, Sonomoto K, Identification of the lantibiotic nisin Q, a new natural nisin variant produced by *Lactococcus lactis* 61-14 isolated from a river in Japan. Biosci Biotechnol Biochem 67 (7): 1616−1619, (2003).
42. Wiedemann I, Benz R, Sahl HG, Lipid II-mediated pore formation by the peptide antibiotic nisin: a black lipid membrane study. J Bacteriol, 186 (10): 3259-3261, (2004).
43. Mythili RS, Sathiavelu A, Recovery of Bacteriocin (NISIN) from *Lactococcus lactis* and testing its ability to increase the shelf life of vegetables (Carrot and Beans). Res J Biol Sci, 5 (11): 727-730, (2010).
44. Phumkhachorn P, Rattanachaikunsopon P, Bacteriophage specific to nisin producing- *Lactococcus lactis subsp. lactis* TFF221, a starter culture inThai fermented food. Afr J Microbiol Res, 5 (10): 1203-1210, (2011).
45. Sandre C, Gleizes A, Forestier F, Gorges-Kergot R, Chilmonczyk S, Léonil J, Moreau MC, Labarre C, A peptide derived from bovine betacasein modulates functional properties of bone marrow-derived macrophages from germfree and human flora-associated mice. J Nutr, 131 (11): 2936−2942, (2001).
46. Premalatha M, Dhasarathan P, Probiotic Action of Lactobacillus Isolated from the Milk Sample against Some Human Pathogens. Res J Immunol, 4 (1): 31–37, (2011).
47. Wells J, Mucosal Vaccination and Therapy with Genetically Modified Lactic Acid Bacteria. Ann Rev Food Sci Technol, 2: 423–445, (2011).
48. Izumo T, Ida M, Maekawa T, Yuichi F, Yoshinori K, Yoshinobu K, Comparison of the Immunomodulatory effects of live and heat killed Lactobacillus pentosus S-PT84. J Health Sci, 57 (3): 304–310, (2011).
49. Foligne B, Deutsch SM, Breton J, Cousin FJ, Dewulf J, Samson M, Pot B, Jan G, Promising Immunomodulatory effects of selected strains of dairy propionibacteria as evidenced *in vitro and in vivo.* Appl Environ Microbiol, 76 (24): 8259**–**8264, (2010).
50. Salminen S, Bouley C, Boutron-Rusult MC, Cummings JH, Franck A, Gibson GR, Isolauri E, Moreau MC, Roberfroid M, Rowland I, Functional food science and gastrointestinal physiology and function. Brit J Nutr, 80 (S1): S147−5171, (1998).
51. Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H, Enhancement of the circulating antibody secreting cell

response in human diarrhoea by a human Lactobacillus strain. Pediatr Res, 32 (2): 141-144, (1992).
52. Isolauri E, Majamea H, Arvola T, Rantala I, Virtanen E, Arvilommi H, Lactobacillus strain GG reverses increases intestinal permeability induced by cow milk in suckling rats. Gastroenterology, 105 (6): 1643-1650, (1993).
53. Makino S, Ikegami S, Kano H, Sashihara T, Sugano H, Horiuchi H, Saito T, Oda M, Immunomodulatory effects of polysaccharides produced by Lactobacillus delbrueckii ssp. bulgaricus OLL1073R-1. J Dairy Sci, 89 (8): 2873−2881, (2006).
54. Masuda S, Yamaguchi H, Kurokawa T, Shirakami T, Tsuji RF, Nishimura I, Immunomodulatory effect of halophilic lactic acidbacterium *Tetragenococcus halophilus* Th221 from soy sauce moromi grown in high-salt medium. Int J Food Microbiol, 121 (3): 245−252, (2008).
55. Foligne B, Dewulf J, Breton J, Claisse O, Lonvaud-Funel A, Pot B, Probiotic properties of non-conventional lactic acid bacteria: immunomodulation by *Oenococcus oeni*. Int J Food Microbiol, 140 (2-3): 136–145, (2010).
56. Botic T, Klingberg TD, Weingartl H, Cencic A, A novel eukaryotic cell culture model to study antiviral activity of potential probiotic bacteria. Int J Food Microbiol, 115 (2): 227−234, (2007).
57. Isolauri E, Probiotics in human disease. Am J Clin Nutr 73 (6): 1142S−1146S, (2007).
58. Pant N, Marcotte H, Brussow H, Svensson L, Hammarström L, Effective prophylaxis against rotavirus diarrhea using a combination of Lactobacillus rhamnosus GG and antibodies. BMC Microbiol, 7: 86, (2007).
59. Zhang M, Wu X, Lee AJ, Jin W, Chang M, Wright A, Imaizumi T, Sun SC, Regulation of IkappaB kinase-related kinases and antiviral responses by tumor suppressor CYLD. J Biol Chem, 283 (27): 18621−18626, (2008).
60. Versalovic J, Iyer C, Ping Lin Y, Yanhong H, Walter D, Commensal-derived probiotics as anti-inflammatory agents. Microb Ecol Health Dis, 20 (8): 86−93, (2008).
61. Moreno de LeBlanc A, del Carmen S, Zurita-Turk M, Santos Rocha C, van de Guchte M, Azevedo V, Miyoshi A, Leblanc JG, Importance of IL-10Modulation by Probiotic Microorganisms in Gastrointestinal Inflammatory Diseases. ISRN Gastroenterol doi:10.5402/2011/892971, (2011).
62. Liao CL, Huang HY, Sheen LY, Chou CC, Anti-inflammatory activity of soymilk and fermented soymilk prepared with lactic acid bacteria and bifidobacterium. J Food Drug Anal, 18 (3): 202−207, (2010).
63. Park EK, Sung JH, Trinh HT, Bae EA, Yun HK, Hong SS, Kim DH, Lactic acid bacterial fermentation increases the antiallergic effects of Ixeris dentate. J Microb Biot, 18 (2): 308−313, (2008).
64. Kadooka K, Imahayashi A, Koiso A, Yamashita M, Teruya K, Matsumoto T, Hasegawa T, Morimatsu F, Katakura Y, Establishment of a novel method of screening anti-allergic lactic acid bacteria. Biosci Biotechnol Biochem, 75 (5): 1016–1018, (2011).
65. Peng S, Lin JY, Lin MY, Antiallergic Effect of Milk Fermented with Lactic Acid Bacteria in a Murine Animal Model. J Agric Food Chem, 55 (13): 5092–5096, (2007).
66. Ishida Y, Nakamura F, Kanzato H Sawada D, Hirata H, Nishimura A, Kajimoto O, Fujiwara S, Clinical effects of *Lactobacillus acidophilus* strain L-92 on perennial allergic rhinitis: a double-blind, placebocontrolled study. J Dairy Sci, 88 (2): 527−533, (2005).
67. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E, Probiotics in primary prevention of atopic disease: a randomized placebo-controlled trial. Lancet, 357 (9262): 1076-1079, (2001).

68. Gao D, Zhu G, Gao Z, Liu Z, Wang L, Guo W, Antioxidative and hypolipidemic effects of lactic acid bacteria from pickled Chinese cabbage. J Med Plants Res, 5 (8): 1439–1446, (2011).
69. Lavanya B, Sowmiya S, Balaji S, Muthuvelan B, Screening and Characterization of Lactic Acid Bacteria from Fermented Milk. British J Dairy Sci, 2 (1): 5–10, (2011).
70. Guslandi M, Giollo P, Testoni PA, A pilot trial of Saccharomyces boulardii in ulcerative colitis. Eur J Gastroenterol Hepatol, 15 (6): 697–698, (2003).
71. Jain S, Yadav H, Sinha PR, Antioxidant and cholesterol assimilation activities of selected lactobacilli and lactococci cultures. J Dairy Res, 76 (4): 385–391, (2009).
72. Koiche M, Dilmi BA, Selection of local extremophile lactic acid bacteria with high capacity to degrade lactose: Potential use to reduce intolerance to lactose *in vitro*. Afr J Biotechnol, 9 (11): 1635–1640, (2010).
73. Pato U, Surono IS, Koesnandar, Hosono A, Hypocholesterolemic effect of indigenous dadih lactic acid bacteria by deconjugation of bile salts. Asian-Aust J Anim Sci, 17 (12): 1741−1745, (2004).
74. Salminen S, Tanaka R, Annual review on cultured milks and probiotics. IDF Nutrition Newsletter, 4: 47−50, (1995).
75. Nagaoka M, Hashimoto S, Watanabe T, Yokokura T, Mori Y, Anti-ulcer effects of lactic acid bacteria and their cell wall polysaccharides. Biol Pharm Bull, 17 (): 1012−1017, (1994).
76. Mizgerd JP, Acute lower respiratory tract infection. N Engl J Med, 358 (7): 716−727, (2008).
77. Villena J, Leonor S. Oliveira M, Salva S, Alvarez S, Lactic acid bacteria in the prevention of pneumococcal respiratory infection: Future opportunities and challenges. Int. Immunopharmaco. doi:10.1016/j.intimp, (2011).
78. Eschenbach DA, Davick PR, Williams SJ, Klebanoff SJ, Young-Smith K, Critchlow CM, Hilmes KK, Prevalence of hydrogen peroxideproducing *Lactobacillus* species in normal women and women with bacterial vaginosis. J Clin Microbiol, 27 (2): 251−256, (1989).
79. Hilton E, Isenberg HD, Alperstein P, France K, Borenstein MT, Ingestion of yogurt containing *Lactobacillus acidophilus* as prophylaxis for candidal vaginitis. Ann Int Medicine, 116 (5): 353−357, (1992).
80. Hajela N, Nair GB, Ganguly NK, Are probiotics a feasible intervention for prevention of diarrhoea in the developing world? Gut Pathogens, 2:10, (2010).
81. Gamboa-Dominguez A, Ubbelohde T, Saqui-Salces M, Romano-Mazzoti L, Cervantes M, Dominguez-Fonseca C, de la Luz Estreber M, M.Ruiz-Palacois G, Salt and stress synergize H. pylori-induced gastric lesions, cell proliferation, and p21 expression in Mongolian gerbils. Dig Dis Sci, 52 (6): 1517−1526, (2007).
82. Rodriguez C, Medici M, Mozzi F, Font de Valdez G. Therapeutic effect of Streptococcus thermophilus CRL 1190-fermented milk on chronic gastritis**.** World J Gastroenterol 16 (13): 1622−1630, (2010).
83. Wallace JL, Nonsteroidal anti-inflammatory drugs and gastroenteropathy: the second hundred years. Gastroenterology, 112 (3): 1000−1016, (1997).
84. Daniel G, Roussel Y, Kleerebezem M, Pot B, Recombinant lactic acid bacteria as mucosal biotherapeutic agents. Trends Biotechnol, 29 (10): 499–508, (2011).
85. LeBlanc JG, de LeBlanc A de M, Perdigón G, Anderson M, Tatiana R, Luis BH, Philippe L, Fernando S, Vasco A, Anti-inflammatory properties of lactic acid bacteria: Current knowledge, applications and prospects. Anti-Inf Agents in Med Chem, 7 (3): 148−154, (2008).
86. Wells J, Mucosal Vaccination and Therapy with Genetically Modified Lactic

Acid Bacteria. Ann Rev Food Sci Technol, 2: 423–445, (2011).
87. Mercenier A, Müller-Alouf H, Grangette C, Lactic acid bacteria as live vaccines. Curr Issues Mol Biol, 2 (1): 17−25, (2000).
88. Detmer A, Glenting J, Live bacterial vaccines- a review and identification of potential hazards. Microbial Cell Fact, 5:23, (2006).
89. Medaglini D, Pozzi G, King TP, Mucosal and systemic immune responses to a recombinant protein expressed on the surface of the oral commensal bacterium *Streptococcus gordonii* after oral colonisation. Proc Natl Acad Sci USA, 92 (15): 6868−6872, (1995).
90. Lamm ME, Interaction of antigens and antibodies at mucosal surfaces. Ann Rev Microbiol 51: 311−340, (1997).
91. Walker RI, New strategies for using mucosal vaccination to achieve more effective immunisation. Vaccine, 12 (5): 387−400, (1994).
92. Formal SB, Baron LS, Kopecko DJ, Washington O, Powell C, Life CA, Construction of a potential bivalent vaccine strain: introduction of Shigella sonnei form I antigen genes into the galE Salmonella typhi Ty21a typhoid vaccine strain. Infect Immun, 34 (3): 746−750, (1981).
93. Tarahomjoo S, Development of vaccine delivery vehicles based on lactic acid bacteria. Mol Biotechnol, 51 (2): 183-199, 2012.
94. Pouwels PH, Leer RJ, Shaw M, Heijne den Bak-Glashouwer MJ, Tielen FD, Smit E, Martinez B, Jore J, Conway PL, Lactic acid bacteria as antigen delivery vehicles for oral immunization purposes. Int J Food Microbiol, 41 (2): 155−167, (1998).
95. Lee P, Biocontainment strategies for live lactic acid bacteria vaccine vectors. Bioeng Bugs, 1 (1): 75−77, (2010).
96. Fraissl L, Leitner R, Missbichler A, Novel formulation of neutral lactase improves digestion of dairy products in case of lactose intolerance. Clin Transl Allergy, 1:104, (2011).
97. Srividya AR, Vishnuvarthan VJ, Probiotic: a rational approach to use Probiotic as medicine IJPFR, 1 (1): 126–134, (2011).
98. Qi-Wei Y, Chun-Feng W, Gui-Lian Y, The Research Progress of Anti-tumor of Lactic Acid bacteria. Food Sci, 32 (9): 303–306, (2011).
99. Wollowski I, Rechkemmer G, Pool-Zobe BL, Protective role of probiotics and prebiotics in colon cancer. Am J Clin Nutr, 73 (2): 451S–455S, (2001).
100. Rowland IR, Rumney CJ, Coutts JT, Lievense LC, Effect of *Bifidobacterium longum* and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. Carcinogenesis, 19 (2): 281−285, (1998).
101. Liu CF, Pan TM, *In vitro* effects of lactic acid bacteria on cancer cell viability and antioxidant activity. J Food Drug Anal, 18 (2): 77-86, (2010).
102. Kumar M, Kumar A, Nagpal R, Mohania D, Behare P, Verma V, Kumar P, Poddar D, Aggarwal PK, Henry CJ, Jain S, Yadav H, Cancer-preventing attributes of probiotics: An update. Int J Food Sci Nutr, 61 (5): 473−496, (2010).