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Lactic acid bacteria as probiotic candidate and their application

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ABSTRACT

The different functional characteristic of microorganism play very important role in the improvement of our health. Lactic acid bacteria belong to Firmicuts and are play very important rule in food and infectious medicine. The lactic acid bacteria have many important applications such as lactic acid bacteria are essential in cheese flavor. Lactic acid bacteria are non sporulating, immobilized, catalase negative and have optimum temperature for growth in the range of 20-45^{0}C. This review particularly focused on the production of different antimicrobial compounds such as bacteriocins from lactic acid bacteria. The various types of bacteriocins are used as a barrier against the disease causing and food spoilage microorganisms.

Key words: LAB, Probiotic bacteria, Antibiotic resistance, Bacteriocins

1. INTRODUCTION

In recent years there is a great interest develop in the field of probiotic research and also the verification and characterization of health benefit related to the use of probiotic (1). The market of probiotic product continuously increases (2). The probiotic can be used to increase gastrointestinal microbiota and also for the treatment of cancer, allergies and infection of urogenital (3). However, there is great confusion upon the correct meaning of word "probiotic". The word probiotic is derived from Greek words "*pro*" and "*biotos*". In Greek the word "*pro*" means for and "*biotos*" meaning life but in Latin's meaning of "*pro*" is for. So the entomology of word "probiotic" is hybrid meaning "for life" (4). However, different scientists work and try to make a complete, comprehensive definition for word probiotics. In 1965 the Stillwell and Lilly use the term probiotic (5). They can define the probiotic as any compound produce by protozoan to enhance health and growth of other species. The different substance produces by bacterium *Colpidium campylum* can be used to enhance the 50% growth of protozoa *Tetrahymena pyriformis* (5). The GS Sperti in 1971 use the term probiotic for extract of tissues to enhance the growth and development of different microbes (6). In 1974, the RB Parker is the first person to use the probiotics in modern meanings. The RB Parker define the probiotic are different microbes and substances that enhance the growth of intestinal micro-flora. The Parker and Netherwood said that the probiotics enhance the health of animal by competitive exclusion when supply with feed. The probiotic bacteria enhance the health of host by suppressing the growth of harmful microbes and enhancing the growth of beneficial microbes (7). The introduction of probiotic bacteria change the micro flora of gastrointestinal tract and these changes can be evaluated by the use of different molecular and culturing techniques. The probiotic bacteria can enhance the nutrient uptake and digestion process within the body of host (8). The Fuller formulates the new definition of probiotic. According to Fuller the probiotic are the live microbial feeds that upon ingestion give health benefit to host by improving the balance of micro flora of intestine. According to this definition the probiotic must be viable in nature (9). Along with the Fuller, some other scientist also tries to make the complete definition of probiotics. In 1997 the Havenaar said that the probiotic are the mixed or pure culture of viable microbes confirms health benefit to the host by enhancing the

indigenous microbial population (10). According to that definition the probiotic must be contain live microorganism and also have the ability to improve the health of animal and man by enhancing the micro flora of intestine (11). Salminen said that the probiotic are the culture of microbes that can enhance the health by improving the nutrition of host. The probiotic can be defined based on their mechanism of action, selection criteria, viability and the non-viability. Instead of whole microbial cell, some parts of cell are considered as the probiotic. However the metabolites are not generally are not included in the current definition (12).According to Schaafsma the probiotic are the microbial cell which when supply in sufficient number confirm health benefit to host (13). The probiotic is an adjunct of live microbial cell and it has the ability to exert some beneficial effect on health of host by modifying the host microbial community, by improving the quality of specialized environment, by enhancing the host immune toward pathogen and by improving the nutritional values (14). The performance of different species enhance by the use of probiotic. The pure probiotic culture is less effective as compared to the mix probiotic culture (15). Irianto and Austin in 2002 said that the probiotic is a complete or a component of a microorganism that exert some beneficial effect on the health of host when supply in sufficient quantity. The probiotic can be used to control the different disease of host. Different species of Algae, Yeast, Gram-positive bacteria and the Gram-negative bacteria are used as a probiotic. The mode of action of these probiotic are poorly understood (16). With the passage of time there are large number of probiotic definition were proposed, creating a lots of confusion about the probiotic concept. In the present days a complete and most suitable definition of probiotic was proposed by the World Health Organization (WHO) and Food and Agriculture Organization (FAO). According to that definition the probiotic are living microorganism which when supply in sufficient quantity confer health benefit on host organism (17).

2. ELIE METCHNIKOFF AND THE MODERN CONCEPT OF PROBIOTIC

The modern concept of probiotic was introduced The Elie Metchnikoff to the world. By using the animal model, he studies the mechanism of phagocytosis. He also show the importance of phagocytosis in host defense against the microbial infection, immunity and he inflammation (18). He also shows that how the leucocytes destroy the diseases causing bacteria within the body. He was awarded the Nobel Prize in 1980 in medicine science. He is popular in Bulgaria due to their effort on boost of host health. According to his study lactic acid bacteria consumption enhance the host health. He also publish a book under the name of "The prolongation of life; Optimistic Studies". According to his thinking or observation, the population of gut micro biota depends upon the composition and quality of intake food. So by the modification of food the harmful bacteria in living organism can be replaced by beneficial bacteria (19). The Metchnikoff's also said that the Lactobacilli eradicate the toxins produced by pathogenic bacteria in body. In 1912 the Metchnikoff's manufactured the first bacterial drug "Lactobacillin" in St. Petersburg (19).

3. MICROBIOTA OF GASTROINTESTINAL TRACT

The Gastrointestinal tract (GIT) is a pathway of food. The food is taking by the mouth and then it is process large intestine, small intestine and stomach. During this processing of food, the useful material is taken out and the residue is entering in to colon. From colon this material is leaves the body. Each parts of the GIT is designated to perform the specific function and also the each parts has its own specific environment. That why each parts of GIT harbor specific population of microbes. These microbes are important in health of host (20) . The GIT of human provide specific environment and according to that environment, it contain the different species of three domains of life such as the *Archaea*, the *Bacteria*, and the *Eukarya*. The gut microbiota is the mixture of indigenous and transient bacteria. The gut microbiota constitutes an example of one of the highest cellular densities in natural ecosystems, reaching 1011 to 1012 cells/mL of luminal content. The biodiversity found in the human gut microbiota is on the low side. It has been found that this ecosystem is dominated by a relatively small number of bacterial taxa, in particular *Bacteroidetes* and *Firmicutes* in adults whereas Actinobacteria, Proteobacteria, and Verrucomicrobia are frequent but generally minor constituents (20). Bacterial colonization of the GIT has been traditionally assumed to occur immediately after delivery however there are reports indicating that this may begin earlier as bacteria have been detected in meconium, umbilical cord, and the amniotic fluid (21). However the whole process is affected by different condition such as the hygiene condition, diets and the maternal microbiota (22). All most 1800 microbial species are reported in GIT of human. Less bacteria are present in stomach but it can increase as move toward the colon of GIT. There are also variations in the predominant bacterial species not only along the length of the GIT but also across its thickness from the lumen to the epithelium (23).

4. EXOPOLYSACCHARIDES AND LACTIC ACID BACTERIA

The lactic acid bacteria produced exopolysaccharide although the physiological role of these exopolysaccharide has not been clearly understood. The exopolysaccharide acts as the reducing syneresis, increasing thickness and stability and as the natural bio thickener. These exopolysaccharides also have some important beneficial effect on human health. Due to the new dairy growing consumer demand, the need for better understanding the properties of existing polysaccharides and the searching of

strains which produced new exopolysaccharides have been increase. The GMO organism capable of producing high-level exopolysaccharides or biopolymers is still very limited series of science fiction. Therefore, to explore the natural environment from the wild strains of lactic acid bacteria biodiversity, currently the most appropriate search method required EPS- phenotype (24). The production of polysaccharides globally extent between lactic acid bacteria. Microbial storage polysaccharides such as glycogen can be synthesized in the cytoplasm; the structural polysaccharides of the cell wall such as lipoteichoic acid and peptidoglycan of Gram-positive bacteria, and lipopolysaccharide attached in the outer membrane of Gram-negative bacteria. In addition, certain bacteria can secrete polysaccharide layer on its surface, which organized with some glycoprotein, grouped under the general term "glycocalyx". These extracellular polymers include capsular polysaccharides to form an adhesive layer or capsule covalently attached to the cell surface and extracellular polysaccharide forming a layer of silt weak binding to the cell surface or secreted into the environment (25). Several microorganisms EPS used in industry, due to its physical and chemical properties similar to those of plants (starch, cellulose and pectin) and seaweed polysaccharides (carrageenan and alginate). In the industry of food, the lactic acid bacteria EPS and other bacteria are used as gelling agents, emulsifiers, stabilizers, solidifying to modify the product rheology and texture (26). The lactic acid bacteria produced the exopolysaccharide during milk fermentation that why these are used as food additive. For this cause, the use of EPS producing bacteria as naturally derived food bio thickeners has received abundant consideration in current years (27). It is believed that exopolysaccharride produced by LAB has a certain beneficial effect on human health for example prebiotic outcome, anticancer stimulates and immunomodulating effect (28, 29). The EPS produced by lactic acid bacteria are belong to the genera *Pediococcus, Leuconostoc, Lactococcus, Lactobacillus,* and *Streptococcus.* The bifidobacteria strains are also produced these EPS (30). Dependent on their chemical structure, the homopolysaccharides produced by lactic acid bacteria are grouping in to homopolysaccharides, containing only one kind of monosaccharide and heteropolysaccharides containing altered monosaccharide. The complete collection of a variety of techniques have been used to study in EPS, but a method of analysis has not been performed to date (31).

5. BACTERIOCINS FROM LACTIC ACID BACTERIA

The Gram negative and Gram-positive bacteria produced substances having proteins structure called bacteriocins (Table 1). The bacteriocins and proteins are not similar. The majors differences between the bacteriocins and antibiotics is that the antibiotic is wide spectrum and bacteriocins is narrow spectrum. Although the bacteriocins are primary metabolites and antibiotics are secondary metabolites (32). Some lactic acid bacteria associated with meat (LAB) is a major producer of natural bacteriocins. Bacteriocins are antagonistic protein substances, very important in the control of decomposition and pathogenic microorganisms. The use of lactic acid bacteria as protective culture increases the shelf life and microbial stability of fresh meat. The use of *CRL705 Lactobacillus curvatus* in meat as a protective culture effectively inhibit *Listeria innocua* and *Brochothrix thermosphacta* and contamination of indigenous lactic acid bacteria, has reserved its suppression effect at low temperatures and have insignificant effect at pH value for meat (33). Microbial populations prevailing characteristic of the development of meat and meat products, initially present in the raw material or cross-contamination of microorganisms. Intrinsic and extrinsic factors controlling microbial growth, determine the kind of bacteria in meat and number. Meat is a good substrate for bacterial growth; therefore, if you do not use control method, it is easily converted into breakage. The use of low-temperature during cooling passage is a first barrier to microbial development (34-36). Recently, intervention techniques to reduce pathogens meat, which has received significant consideration, has been proven effective. LAB in food use as preservative due to the ability to produce antimicrobial metabolites, include bacteriocins and organic acids. Acid assembly from carbohydrate catabolism is a common feature in the laboratory lactic acid bacteria, but all LAB do not manufactured antimicrobial peptides during growth phases. In the meantime many bacteriocins have been sequestered in the previous three periods, all LAB have common phenotype to produce these aggressive substances. Bacteriocins manufactured by LAB are a set of peptides and proteins heterogeneous. These bacteriocins can be divided in to three main categories, class I, class II and class III bacteriocins (37). The class I bacteriocins are also known as lantibiotics and it contain the unsaturated amino acid such as the dehydroalanine, 2-amino isobutyric acid along with the some polycyclic amino acid such as the lanthionine, methyllanthionine. According to their structure, they are further classified in to two types, type-A and type-B. The type-A have the molecular mass in the range of 2 to 4 kDa. Generally, it consists on the flexible, amphipatic, positively charge and elongated molecules. The type-B has molecular mass in the range of 2 to 3 kDa. It has globular structure and they have no charge or have negative charge (38, 39). The class II bacteriocins have round about 10 kDa molecular mass and are heat stable consisting on membrane active peptides. They are further subdivided in to two subclasses, subclass IIa and subclass IIb. The subclass IIa is pediocin like bacteriocins and it have an Tyr-Gly-Asn-Gly-Val-Xaa-Cys consensus sequence at N-terminal. The subclass IIb consist on two separate peptide and in order to show the antimicrobial activity these two peptides must work synergistically (40, 41). The bacteriocins are primary synthesize in the ribosome in the form of inactive prepeptide. These inactive

prepeptide can be converted in to the active peptide by the translational modification (39). Lactococcal bacteriocins are manufactured by numerous sorts of lactic acid bacteria sequestered from vegetables products, meat and dairy products (37, 42). Under satisfactory circumstances, nisin stops the growth of wide range of Gram-positive microorganisms. It has been widely characteristized, it molecular structure and mechanism of action has been determined (43). While the only marketable developed lantibiotics is nisin. However, considerable effort can be done to develop other lantibiotics. Lacticin 3147, lantibiotics having two peptide formed by Lactococcus lactis subsp. lactis DPC3147 sequestered from cereals Ireland, it show killing effect on wide range of food spoilage and pathogenic bacteria (44). High thermal stability and a wide pH range lacticin3147 make it good-looking for use in the food industry. While most lactococcal bacteriocins isolated from dairy products and vegetables, numerous strains of *Lactococcus lactis* producing nisin secluded from fermented sausages, suggesting the possible use of lactococcal meat fermentation. Lactococcus lactis nisin producting isolated from Spain and Thai traditional fermented sausages excellently inhibit diligently associated LAB strains (45, 46). Different species of lactic acid bacteria such as Lactobacillus sakei, Pediococcus acidilactici and Pediococcus pentosaceus produced different organic acid and bacteriocins having antimicrobial activity against different pathogenic microorganisms (47). The probiotic lactic acid bacteria use in aquaculture should have an antimicrobial activity against wide range of fish pathogen (48). The different strains of LAB such as *Lactobacillus paracasei, Pedoicoccus acidilactici , Lactobacillus pentosus* and *Lactobacillus brevis* are isolated from the goat milk, yoghurt and butter milk. All of these isolated strains produced bacteriocins like compounds having inhibitory effect against various pathogens such as *Salmonella, Listeria* and *Staphylococcus*. Furthermore, all of these isolated strains have cholesterol-lowering ability. All of these result suggested the isolated strains have probiotic potential (49).

Table 1. Some important bacteriocins and their producers

Bacteriocins	Producer Organisms	References
Bifidin	*B. bifidum* NCDC 1452	(50)
Bifilong	*B. longum*	(50)
Bifilact Bb-12	*B. lactis* Bb-12	(51)
Bifidin-I	*B. infantis* BCRC 14602	(52)
Gassericin A	*L. gasseri*	(53)
Reutericin 6	*L. reuteri*	(54)
Lacticin Q	*Lc. Lactis*	(55)

6. ANTIBIOTIC RESISTANCE IN LACTIC ACID BACTERIA

Antimicrobial susceptibility analysis of lactic acid bacteria isolated from Irish beef and pork. The 37 species comprised enterococci, streptococci, lecunostoc, lactobacilli, lactococci and pediococci are isolated from Irish beef and pork by using the biochemical tests and rRNA sequences analysis. The resistance of these species against the antibiotics such as ampicillin, vancomycin, chloramphenicol, tetracycline and erythromycin can be check by using the E- test and MIC method. The genes that are responsible for the resistance against antibiotic were categorized by PCR analysis. In trial experiments, the transfer of these marker genes can be study by using the filter matched assay. Out of these 37 species the 33 species was resistance against one or more antibiotics. However, all species were resistance against ampicillin and chloramphenicol. Antibiotic resistance spread through food Chain is an important public health problem (56). The antibiotic susceptibility of various lactic acid bacterial stains isolated from the yoghurt against eleven set of antibiotic such as gentamycin, neomycin, streptomycin, kanamycin, lincomycin, hlortetracycline, tetracycline, chloramphenicol, roxithromycin, penicillin G and ampicillin cab be studies by using agar dilution method. The result of this study show that the thirty-three isolated strains of LAB show resistance against the eleven antibiotics. In another study the antibiotic resistance of lactic acid bacteria isolated from various fermented food against the antibiotics such as erythromycin, clindamycin, ampicillin, ciprofloxacin, tetracycline, kanamycin and chloramphenicol cab be checked. The result of this studies show that the antibiotic resistance is wide spread among the LAB isolated from fermented food (57, 58). There are 31 LAB strains were isolated from the cheese. From these there are 18 LAB strains were tested for their antibiotic resistance. The result show that the multiple resistance is observed against various antibiotics (59). The various strains of bifidobacteria and lactobacillus can be isolated from dairy products by using the multiplex PCR. By using the micro dilution method the resistance of these strains were checked against different antibiotics (60).

7. APPLICATION OF LAB IN FOOD

Lactic acid bacteria (LAB) has long been ignored in flesh fish (

Table 2), because the examination at high pH, low fraction of sugar, the high content of low molecular weight and low molecular nitrogen temperate waters beneficial rapid growth of psychrotolerant ocean pH-sensitive gram-negative bacteria such as *photobacterium*, *Shewanella* and *Pseudomonas*. In both modified atmosphere (MAP) and vacuum packaging seafood (VP) in the package usually enriched CO_2 growing Gram-negative aerobic bacteria group is effectively suppressed during storage and reaches the number ratio of LAB is higher than in the air, but always several log units lower than the oxidation-resistant carbon dioxide trimethylamine (TMA-O). Therefore, LAB is shellfish concern not aerobic or storage both in VP and MAP (61). The alive fish's muscle is disinfected. But, gut, gills, mucus and skin comprise significant number of bacteria, although the composition and quantity of bacteria varies by species of fish, temperature, salinity, dissolved oxygen, pollution, diet, depression level. Fish from temperate waters have microbiota generally compromised of Gram-negative bacteria such as *Photobacterium, Psychrobacter, Moraxella, Vibrio, Aeromonas, Acinetobacter, Pseudomonas, Bacillus, Lactobacillus, Corynebacterium*, whose growth is possible at 0 ^{0}C and optimum around 25^{0}C (62-65). While not the most common, but generally agreed between the normal intestinal flora of fish LAB occurs early and latter (66, 67). Lactobacillus, especially Lactobacillus, found in Atlantic salmon, cod, arctic char and Pollock (68). Even though most LAB is generally considered safe by the US Food and Drug Administration has announced its participation in fish disease. The lactic acid bacteria isolated from different origin secreted some metabolites having antifungal activity (69). The improvement of cheese flavors is very complex biochemical process. However the different strains of lactic acid bacteria particularly Lactococcus lactis can be used in the development of cheese flavors by the hydrolysis of casein proteins in milk (70). The lactic acid bacteria play very important role in the bioprocessing of animals food and feeds. The LAB are involved in the fermentation of various milk, grains, meats, vegetables and fruits (Figure 1) (71).

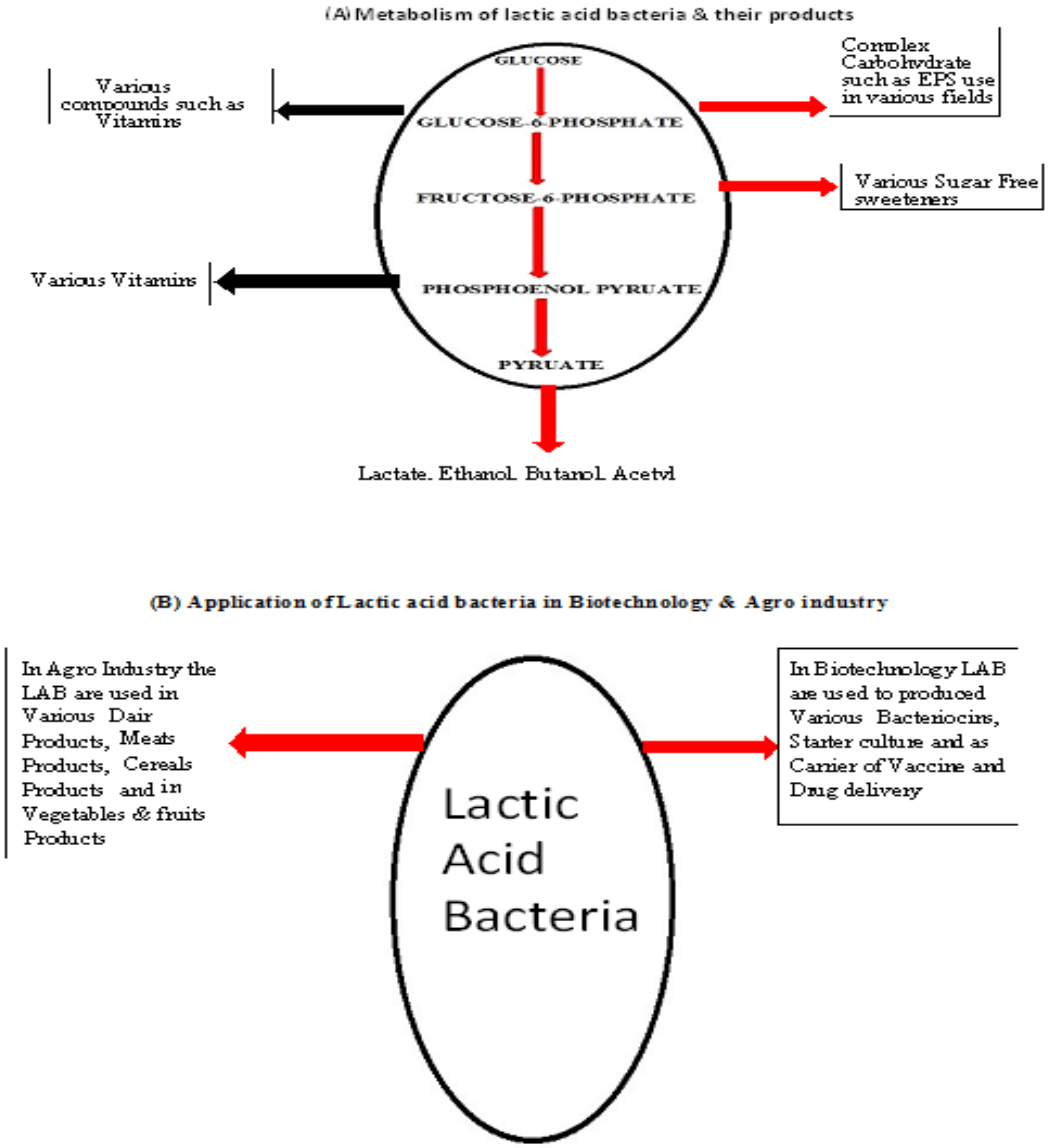

Figure 1. Various application of LAB

The lactic acid bacteria can be used as protective culture to extend the shelf life of meats products, foods products and seafood products (72).

Table 2. Some important LAB isolated from fish products

Species name	Sources	References
Carnobacterium divergens	Cold-smoked salmon	(73)
Carnobacterium piscicola/maltaromaticum	Gravad rainbow trout, Brine shrimp (preservatives), Cold-smoked salmon	(74)
Enterococcus sp	Cold-smoked rainbow trout	(75)
Enterococcus faecalis	Cold-smoked salmon	(76)
Enterococcus faecium	Traditionnel Himalayan salted or dried fish	(77)
Lactobacillus alimentarius	Cold-smoked salmon	(73)
Lactobacillus casei subsp. Tolerans, Lactobacillus coryneformis, Lactobacillus curvatus	Cold-smoked salmon Cold-smoked salmon Cold-smoked salmon	(76)
Weisella sp	Seafood salad	(78)
Weisella kandleri	Cold-smoked salmon	(76)
Weisella confuse	traditionnel Himalayan salted or dried fish	(76)
Vagoccocus sp	Seafood salad	(78)
Vagoccocus fluvialis/carniphilus	Cooked MAP shrimp (preservatives)	(79)

Streptococcus parauberis	Seafood salad	(78)
Lactococcus plantarum	Traditional Himalayan salted or dried fish	(79)
Leuconostoc carnosum	Cold-smoked salmon	(80)
Leuconostoc citreum	Cold-smoked rainbow trout	(80)
Leuconostoc mesenteroide	Cold-smoked salmon	(81)
Leuconostoc gelidum	Cold-smoked salmon	(81)
Leuconostoc pseudomesenteroides	Seafood salad	(78)
Pediococcus pentosaceus	Traditional Himalayan salted or dried fish	(79)
Pediococcus sp.	Seafood salad	(78)

8. LACTIC ACID BACTERIA AND PROTEOLYTIC SYSTEM

In bacteria, the proteolytic system is known to play a variety of functions, such as the key role of nourishment, poisonousness, protein turnover, regulatory actions and protein maturation and exports. The LAB, usually considered to be safe and extensively used in fermented foods, especially dairy industry in the event of record production, proteolytic system association with nitrogen nutrition. Although the bacillus is generally consider more proteolytic than LAB. Nitrogen importance of proteolytic system of lactic acid bacteria (LAB) can be illuminate by two main causes. The first step of LAB is related to inability of synthesis of various amino acid of which is considered to be auxotroph (82).

9. LAB AS THERAPEUTIC AGENT

The Irritable bowel syndrome is a genetic disease and not well understood. It is a common gastric disorder. The main symptoms of this disease are flatulence, abdominal distension, bloating, abdominal Ming and regular abdominal pain (83). The gastrointestinal tract has sterile environment that why there is no microbial ecology in GIT tract. The microbial community developed after the birth in GIT tract (84, 85). Many studies have taken advantage of this fact by using germ free animals clarify the role of intestinal microflora in health and disease, and assigned to the intestinal commensal bacteria collective protection, structure and metabolism (86). These bacteria reside in the intestinal epithelial cells and gut-associated lymphoid tissue (GALT), which is crucial for intestinal homeostasis, essential for health, on the contrary, for a delicate balance between disease risk factors (87, 88). This shows that the enteric bacteria and gastrointestinal function plays an important role in the regulation (including movement, secretion, blood spills, intestinal permeability, mucosal immunity and gut feeling) in the axis of brain gut (89). Some clinical trials have involved LAB include improvement of abdominal pain / discomfort, as one of its endpoints (primary or secondary) with 34 test evaluation report at least some of the benefits over placebo sound. In laboratory use, perhaps the most compelling evidence comes from trials involving bifidobacterium *infantis* 35624, where a single center and multicenter studies have demonstrated efficacy to reduce abdominal pain / discomfort compared to placebo (90, 91).

10. MODULATION OF IMMUNE SYSTEM

The Probiotic bacteria can modulate the immune system of host by improving innate immunity and modulating inflammations caused by some harmful organisms with the help of receptor-regulated pathway. The probiotic bacteria produced the anti-inflammatory cytokines to enhance the immune system of host (92).

11. REGULATION OF INTESTINAL HOMEOSTASIS

The probiotic bacteria can regulate the homeostasis of intestinal epithelium cells. They can regulate the homeostasis process by promoting some protective reactions, improvement of barrier function and intestinal epithelial cell survival (92).

12. CONCLUSION

Today, consumers are very worried about the correlation between health and food. The food additives are considered unnatural and unsafe. However, the addition of various food additives in daily food is very necessary. The lactic acid bacteria have received attention due to its application in food industry. Lactic Acid Bacteria (LAB) are major constituents of the human intestinal micro flora. These have been considered as the major microbial group having probiotic potential that is able to exert a wide range of beneficial health promoting effects that include inhibition of pathogen growth and production of antimicrobials and vitamins. In food industry, LAB has received considerable attention due to their probiotic activities. Lactic acid bacteria have increasing commercial interest in dairy products and baby formula diet, as the awareness of different benefits associated with human gastrointestinal tract health and prevention of diseases like diarrhea. Therefore, there is increased focus on research for health promoting food development containing probiotics. The probiotic strain's ability to resist unfavorable physiological conditions of the gastrointestinal tract (GIT) depends on various factors like tolerance to bile secretion and lysozyme resistance. The mucin Adhesion and biofilm study of probiotic microorganism to the intestinal mucosa is considered important for many of the observed probiotic health effects. Adhesion is regarded a prerequisite for colonization in the intestinal tract, antagonistic activity against entero-pathogens, modulation of the immune system and for increased healing of the damaged gastric mucosa of the host animal.

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CONFLICT OF INTEREST

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