Role of Lactobacilli as Probiotics in Human Health Benefits: Current Status and Future Prospects

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Abstract
The Lactic Acid Bacteria (LAB) has extensively been used in food industry for the production of varied fermented products such as yoghurt, cheese etc. These are also been used as probiotics for animal as well as human welfare in terms of health and well being. Also, The present review focuses on available literature, current market status and on the characteristics of Lactic Acid Bacteria with a special emphasis on the probiotic properties of the genus Lactobacillus. The industrial scale development of Lactobacillus as commercial products has benefitted the market in several facets.

Keywords: Lactic acid bacteria, Health benefits, Probiotics, Market scope

I. Introduction

The awareness amongst the health conscious individuals has proved to be very useful for the food sector, particularly in the production of probiotics. Probiotics are the living microorganisms that are found in the human gut. In 1989, the concept of probiotics was coined by Fuller as, “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. In scientific literature, the Lactic acid bacteria (LAB) is generally been reported as probiotics.

Probiotics play a very crucial role in proper functioning of our gastro intestinal system and forms an integral part of the human biological system. It helps in maintaining the healthy intestinal microbiota and thereby reducing the population of pathogenic bacteria in the intestine. The literature reports suggest that amongst Lactic acid bacillus, large no. of genera having beneficial effects on humans are constituted within the phylum Firmicutes. The genera constitutes, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus. Lactospheara, Leuconostoc, Melissococcus, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vogoccus and Weissella. The intake of LAB in recommended dosage has shown to exert beneficial effects on host such as antiinflammatory, immunomodulatory, anti-diabetic etc even the non-viable microorganisms or microbial cell extracts have also shown beneficial effects on host.

CHARACTERISTICS OF LACTIC ACID BACTERIA
The natural source for the indigenous microflora of lactic acid bacillus constitutes raw milk, yogurt etc. The first isolation of LAB has been reported from milk by Metchnikoff. Thereafter, these bacteria have been isolated from a variety of naturally fermented food products such as meat, milk products, vegetables, beverages and bakery products. Besides this, the occurrence of LAB has also been noted from water, soil, sewage, manure as well as it has also been identified in humans and animals. The LAB are categorised as ‘Generally Recognized as Safe’ (GRAS) organism, which are cocci or rod shaped, Gram positive, catalase negative and fastidious organisms. They have the ability to convert carbohydrates into energy and lactic acid via fermentation. The LAB cultures are extensively been used in the food industry as starter cultures and have shown immense potential in determining the texture, flavour and nutritional value of feed and food products.

Mostly, LAB cultures are known as aerotolerant anaerobes as the grow under anaerobic conditions. On the other hand, these can also grow in the presence of oxygen since they possess peroxidase enzymes which protect these cells against free radicals.

The lactic acid bacteria (LAB) are classified into two major groupson the basis of the product formed during glucose fermentation. The homofermentative bacteria ferment glucose to produce two moles of lactic.
acid generating a net of 2ATP per moles of glucose metabolized. In case of heterofermentative metabolism, 1 mole of glucose is metabolized to produce 1 mole of lactic acid, 1 mole of ethanol and 1 mole of CO₂. The LAB containing products possess aroma and flavour due to the small organic compounds produced by these bacteria. The lactic acid bacteria is widely used for the production of a variety of fermented food products such as cheese, chocolate, pickles, beer, sourdough bread etc. These bacteria lowers the pH of the food product thereby arresting the growth of harmful bacteria and preserving the nutritive value of food products which thereby increases their shelf life. Bacteriocins, the compound produced by LAB have the ability to penetrate the outer membrane of Gram negative bacteria and inhibit their growth. Apart from bacteriocins, the lactic acid bacteria produces compounds such as Nisin that have been approved by Food and Drug Administration (FDA) for use in the foodstuffs like the Nisin variants A and Z. The Nisins posses antimicrobial properties against spore forming disease causing Gram negative bacteria like Bacillus sp. and Clostridium sp. The possession of these antimicrobial substances such as bacteriocins, hydrogen peroxide, polysaccharides etc. led to the inhibition of the growth of pathogenic organisms. The bacteriocins are the protein molecules that possess anti-cholesterol and antitumor properties. Reports suggest that highest bacteriocin production in LAB occurs during end of the exponential and early stage stationary phase.

The antimicrobial peptides secreted by LAB have known to possess probiotic properties that preserve food as well as beneficial for human health. The Lactobacillus genus comprises of rod shaped, non spore forming, non pigmented, catalase negative and microaerophilic to strictly anaerobic bacteria which are widely used in the production of fermented foods. The LAB cultures grow optimal in temperature range of 30°C to 40°C, with an optimum pH range between 4.5-6.5.

**Classification:** Lactobacillus species can be divided into three groups on the basis of their metabolism.  
1. **Obligate homofermentative:** L.acidophilus, L.hulgaricus, L.salivarius, L. helveticus etc.
2. **Facultative heterofermentative:** L.casei, L. plantarum, L. curvatus, L. sakei
3. **Obligate heterofermentative:** L. brevis, L. buchneri, L. fermentum, L. reuteri etc.

The LAB can be identified on the basis of the morphological characteristics such as colony color, size, margin and its shape. The various biochemical characteristics for LAB culture identification are the fermentation type, carbohydrate metabolism and production of isomers of lactic acid. The phenotypic tests for the identification of Lactobacilli on the basis of the characteristics are respiratory type, motility, growth, growth in sodium chloride, and temperature. Identification of LAB cultures on the basis of morphological and biochemical tests could further be authenticated through certain specific tests such as milk coagulation ability and enzyme specific tests like arginine dihydrolase and sugar utilization pattern tests which when subjected to software named PIBWIN give tentative as well as confirmed identification of a Lactic Acid Bacteria by matching it with Bergey’s Manual of Determinative Bacteriology. The other important methods for the detection and characterization of LAB strains are protein fingerprinting using SDS gel electrophoresis, 16S rRNA, sequencing analysis, Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphism (RFLP) and Pulse-field gel electrophoresis (PFGE).

**LACTOBACILLI BACTERIA IN BENEFITING HUMAN HEALTH**

The health benefits of LAB in humans are well known. The gut bacteria interact directly with the host cells and exert their positive effects. LAB has a major role in the treatment of intestinal disorders, since, these cultures enhances immune response due to serum antibodies, IgG and secretory IgA and IgM.

There are several mechanisms which prevents the attachment of harmful bacteria on intestinal epithelium. Lactobacilli led to the fermentation of substrates such as lactose, biogenic amines and other compounds into short chain fatty acids, organic acids and gases. The production and secretion of substances by LAB cultures such as bacteriocins and organic acids that are antimicrobial agents adhere to intestinal epithelium after competing for binding sites and thereby eliminates harmful bacteria. A study for a period of two years was carried out which showed that, the micro flora of GI tract in case of infant is highly variable and changes rapidly in the first five months of infants life, while, in case of adults, the lactobacillus community is more stable.

The intestinal Lactobacilli constitutes an important effective mechanism for the metabolism and detoxification of foreign substances entering the biological system.

The functions of Lactobacilli are strain specific and results in different mechanisms to produce beneficial results for health. In available literature some additional information is present on the production of bacteriocin by probiotic bacteria that target pathogenic bacteria in vitro. Reutericyclin, an antibiotic produced by Lactobacillus reuteri LTH2584, is reported to inhibit spectrum of bacteria, its biological activity is
similar to that of Nisin. The colonized L. reuteri cells were recovered from the intestine of reconstituted lactobacillifree (RLF) mice in high cell counts. This strain has been investigated for its antibacterial role in the habitats of intestine\textsuperscript{32}. Below in Table-1 is the list of probiotic cultures of lactic acid bacteria and their administrative form, in line with clinical trial evidences.

Table 1. List of selected health-promoting lactic acid bacteria with clinical trial evidence

<table>
<thead>
<tr>
<th>Probiotic cultures used and its form</th>
<th>Participants, age (yr), Gender, Case/Control (n)</th>
<th>Observations</th>
<th>Ref.</th>
</tr>
</thead>
</table>
| Capsule: 
Lacticobacillus acidophilus, L. casei, L. rhamnosus, L. bulgaricus, Bifidobacterium breve, B. longum, Streptococcus thermophila, FOS |
T2DM, 35–70 yrs, Both, 27/27 |
Reduces FBS, HOMA-IR, HbA1C, hs-CRP
Increases FSI, improves oxidative stress biomarkers |
| | | | 33 |
| Probiotic capsule: 
L. acidophilus, Bifidobacterium bifidum, L. reuteri, L. fementum |
DN (T1DM & T2DM), 45–85, NA, 30/30 |
Reduces FBS, HOMA-IR, HbA1C, hs-CRP, BUN, Cr, urine protein, inflammatory markers, oxidative stress biomarkers |
| | | | 34 |
| Probiotic honey: 
Bacillus coagulans T4 |
DN (T1DM & T2DM), 45–85, NA, 30/30 |
Reduces FBS, HOMA-IR, lipid profile, hs-CRP, BUN, Cr, urine protein, inflammatory markers, oxidative stress biomarkers |
| | | | 35 |
| Probiotic capsule: 
L. acidophilus, Bifidobacterium bifidum, L. casei, L. fementum |
DF (T1DM & T2DM), 45–85, both, 30/30 |
Reduces FBS, HOMA-IR, lipid profile, hs-CRP, BUN, Cr, urinary protein, inflammatory markers, oxidative stress biomarkers |
| | | | 36 |
| Probiotic capsule: 
L. acidophilus, Bifidobacterium bifidum, L. casei, L. fementum |
T2DM, 40–85, Both, 30/30 |
Reduces FBS, HOMA-IR, lipid profile, hs-CRP, inflammatory markers, oxidative stress biomarkers |
| | | | 37 |
| Capsule: 
L. acidophilus, L. casei, L. rhamnosus, L. bulgaricus, B. breve, B. longum, S. Thermophila, FOS |
T2DM, 30–75 yrs, Both, 30/30 |
Reduces FBS, HbA1C, increase HDL-Cholesterol, no significant changes HOMA IR, TC & TG |
| | | | 38 |
| L. casei |
T2DM, 30–60 yrs, Both, 20/20 |
Reduces FBS, HOMA-IR, Fetuin-A, increases insulin/Sirtuin1 |
| | | | 39 |
| L. rhamnosusGG |
Bifidobacterium lactis Bb-12 |
Prevention is partially due to serum antibodies IgG and secretory IgA and IgM immune response enhanced by probiotics |
| | | | 40 |
| L. rhamnosus GG |
Prevention of allergies and atopic eczema |
| L. reuterii, Enterococcus faecium |
Reinforcing the local immune defence through specific IgA response to rotavirus and pathogens |
| | | | 41 |
| Lactobacillus rhamnosus GG |
Relieves lactose in tolerance symptoms |

CURRENT SCENARIO OF THE HEALTH EFFECTS OF LACTOBACILLUS BACTERIA:

It has been observed that the probiotics exert beneficial effects in the biological system\textsuperscript{43}. Recently, a large number of studies have been reported emphasizing probiotic properties of LAB.\textsuperscript{44} The naturally fermented drinks are a rich source of lactic acid bacteria, such as fermented milk available in Indonesia named, dangke and dadih, contains lactic acid bacteria belonging to the species Lactobacillus plantarum, Lactococcus lactis
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susceptibility and adherence to CaCo-2 colon cancer cells. The Brazilian Kefir is also a rich source of lactic acid bacteria (LAB), and thirty-four LAB isolates were identified and characterized. The isolated species belonged to Leuconostoc mesenteroides, Lactococcus lactis and Lactococcus paracasei. L. paracasei MRS59 showed adhesion to human Caco-2 epithelial cells thereby confirming their anti cancerous properties. Lactobacillus plantarum YS2 (LP - YS2) was isolated from yak yoghurt and was studied for its probiotic properties. It showed high acid resistant activity when compared to Lactobacillus bulgaricus. The traditionally fermented Xinjiang cheese serve as a great source of lactic acid bacteria such as Lactobacillus rhamnosus, Lactobacillus helveticus, and Enterococcus hirae. L. rhamnosus was found to be most effective probiotic as it degradescholesterol and triglyceride up to 50.97% and 28.92% respectively. L. rhamnosus GR-1 in combination with Streptococcus thermophilus and Weissella cibaria N20 when consumed on daily basis reduces the urinary concentration of aflatoxin. The binding of L. rhamnosus GG to aflatoxin B1, reduces intestinal absorption thereby reducing its pathogenicity, researchers also demonstrated its ability to prevent the growth of urogenital infectious bacteria and fungi. The Lactobacillus strains such as L. rhamnosus 4B15 and L. gasseri 4M13 are known to possess antioxidant properties alongwith the ability to inhibit nitric oxide production, lowering cholesterol and α-glucosidase activity inhibition. L. rhamnosus in combination with prebiotic such as almond exhibits probiotic properties. Amongst the different strains Lactobacillus rhamnosus NCDC17 was found to show antimicrobial activity, bile salt tolerance and cell surface hydrophobicity. The Lactobacillus rhamnosus L60 and Lactobacillus fermentum L23 are known to produce secondary metabolites such as bacteriocins, organic acids and hydrogen peroxide. The fungal growth of aflatoxin producing strains were inhibited completely by L. rhamnosus L60 and L. fermentum L23. The probiotic strains Lactobacillus, L. rhamnosus and L. casei exhibits acid resistance and tolerance of bile salts as compared to other lactic acid bacteria (LAB). The strains L. rhamnosus IMC501 and L. paracasei IMC 502 have shown protective effects against Candida albicans and multdrug-resistant gram-negative bacteria. The strains showed high adherence to HT-29 cells, these strains exhibit synergistic effects and also showed resistance to antibiotics such as vancomycin and kanamycin etc. during in vitro studies. Furthermore, probiotics have the ability to improve the protein as well as some minerals like calcium, iron, manganese, and copper absorption from the gut by making acidic pH of the intestine; regulate the production of mucus, regulate epithelial functions and increase intestinal motility.

MARKET SCOPE OF PROBIOTICS

Earlier in 2015, we have reported that, the probiotics marketed in India for Indians are mostly non-indigenous strains and their efficacy is debated, further we also reported that, the demand of probiotic food alone in global market was 27.9 billion USD in 2011 and is increases at a 6.8% CAGR by 2016 period. In the current global scenario, the probiotic market is estimated to grow with approx 7% CAGR during 2018-2026 period. The growth of the probiotic market is majorly driven by the following factors:

1. Awareness amongst the health conscious consumers/public
2. Rise in demand for functional food products
3. Investment in the R & D sector especially in nutraceuticals for functional foods
4. Rise in disposable income of developing nations
5. The awareness regarding health benefits of probiotics has been a major driver in the growth of the probiotics market. The probiotics are divided into different categories on the basis of the application

a) Food and Beverages
b) Dietary supplements
c) Animal feed products

Fig.1: Probiotics Market, (Source: www.marketsandmarkets.com)
According to “MarketsandMarkets”, the probiotics market to be estimated at USD 69.3 billion by 2023, recording a CAGR of 7.0% (Probiotics Market: Published Date: Jan 2019, Report Code: FB 2269). The consumption of probioticsupplemented food has been known to ameliorate digestive ailments, bloating and promotes strength of the immune system. The animal feed products containing probiotics market has shown a lot of potential in terms of growth, since a ban was imposed on synthetic antimicrobial growth promoters (AGPs) in Europe in 2006. So, now The antimicrobial drugs are not being promoted to increase the production of meat, dairy products and the growth of livestock. This paves the path for probiotics as a new entrant in the field of animal feed.

On the basis of the regional predominance of probiotics, it is the Asia Pacific region, which has shown promising market for probiotics due to public awareness amongst these regions a high demand for functional foods as well as for dairy products has also been noted in this region.

II. Conclusion

The Lactic acid bacteria especially bacteria of Lactobacillus genera shows a promising future in terms of their use as probiotics. With this in view, large number of health benefits have been explored for Lactobacillus bacteria, and their production as functional foods provides a great scope for the probiotics market in near future.

References


