Role of Lactic Acid Bacteria as Probiotics in Health and Disease

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Abstract

In natural environment a delicate symbiosis evolves between endogenous bacteria and their host is very crucial for maintaining the internal flora of organisms. It stimulates immune system to respond rapidly to infection with pathogens and through bacterial antagonism it inhibits the colonization of the gut by harmful or pathogenic bacteria [2]. It consists of species belonging to families: - Bacteroides, Fusobacterium, Butyryrobid, Clostridium, Bifidobacterium, Eubacterium and Lactobacillius, Enterococcus and E.coli constitute less than 1% of all intestine micro-organism. Anaerobes dominate upon facultative anaerobes and microaerophilic at the ratio of 1000:1 [3]. A dominant flora represents 90% of the population, essentially composed of Bifidobacterium and Lactobacillii. In group they are called as Lactic Acid Bacteria (LAB) and are collectively called probiotics. These antibacterial effects of LAB are possible due to different antimicrobial compounds like organic acids, hydrogen peroxide (H2O2), carbon dioxide (CO2), fatty acids, reuterin, bacteriocins and other low molecular mass compounds produced by them. Probiotics is playing an important role in prevention of many bacterial diseases also. Probiotic bacteria may add a low-cost, low risk layer of protection from infection and disease.

Key words

LAB; Probiotics; GI tract; Bacteriocins; Diarrhea; Biopreservative agent

Introduction

Microorganisms are ubiquitous in biosphere. We generally have more than three pounds of bacteria inside our bodies. The human intestine is habitat for more than a trillion live bacteria from about 400 species. The average adult human body contains about 20 times more bacteria than it does cells [1]. In the natural environment a delicate symbiosis evolves between these endogenous bacteria and their host. Microflora of Gastrointestinal (GI) tract plays a crucial role in the anatomical, physiological and immunological development of the host. It stimulates the immune system to respond rapidly to infection with pathogen and through bacterial antagonism it inhibits the colonization of the gut by harmful or pathogenic bacteria [2]. It consists of species belonging to families: - Bacteroides, Fusobacterium, Butyryrobid, Clostridium, Bifidobacterium, Eubacterium and Lactobacillius, Enterococcus and E.coli constitute less than 1% of all intestine micro-organism. Anaerobes dominate upon facultative anaerobes and microaerophilic at the ratio of 1000:1 [3]. A dominant flora represents 90% of the population, essentially composed of Bifidobacterium and Lactobacillii. In group they are called as Lactic Acid Bacteria (LAB). LAB was first isolated from milk and have since been used as a biopreservative in food for centuries and are now added as starters in food products, vegetables, beverages, bakery products, soil, water, manures and sewage [4-8]. In addition, LAB produce small organic compound that give the aroma and flavour to the fermented product and have been used as a flavouring and texturizing agents as well as a preservative in food for centuries and are now added as starters in food [9]. Antimicrobial effect of LAB is mainly due to their lactic acid and organic acid production, resulting in reduced pH of the growth environment; apart from this it also produce acetaldehydes hydrogen peroxide, diacetyl, CO2, polysaccharides and bacteriocins [9,10].

<table>
<thead>
<tr>
<th>S. no</th>
<th>Health-effect</th>
<th>Mechanism</th>
<th>Strain example</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Relive lactose intolerance symptoms</td>
<td>Hydrolysing lactose into glucose and galactose and forming the physical appearance of milk into a thick substance, such as yogurt, that passes through the GI tract slowly reducing the lactose pulse in the colon</td>
<td>Lactobacillus rhamnosus GG</td>
<td>[80].</td>
</tr>
<tr>
<td>2</td>
<td>Control viral, bacterial and antibiotic associated diarrhoea in humans and animals</td>
<td>Reinforcing the local immune defence through specific IgA response to rotavirus and to other pathogens</td>
<td>L. rhamnosus GG L. reuteri Enterococcus faecium</td>
<td>[15, 53, 81, 82].</td>
</tr>
<tr>
<td>3</td>
<td>Prevention of allergy and atopic eczema</td>
<td>Prevention is partially due to serum antibodies IgG and IgE and IgM immune response enhanced by probiotics</td>
<td>L. rhamnosus GG Bifidobacterium lactis Bl-12</td>
<td>[82, 84].</td>
</tr>
<tr>
<td>4</td>
<td>Prevention of intestinal bacterial enzymes involved in the synthesis of colonic carcinogens</td>
<td>Enhancing host's immune response, binding and degrading carcinogens, producing antimutagenic compounds, alteration of metabolic activities of intestinal bacteria and alteration of physio-chemical conditions in colon might work to prevent cancer</td>
<td>B. bifidum B. infantis B. longum L. acidophilus L. paracasei</td>
<td>[81, 85, 86].</td>
</tr>
</tbody>
</table>

Table 1: Selected health-promoting Lactic-acid bacteria, their impacts and mechanisms.
Members of LAB share the property of being Gram-positive that ferment carbohydrate into energy and lactic acid [11]. Depending on the organism, metabolic pathway differ when glucose is the main carbon source: Homo-fermentative bacteria such as Lactococcus and Streptococcus yield two lactate molecule from one glucose molecule, whereas the Hetero-fermentative (i.e, Leuconostoc and Weissella) transform a glucose molecule into lactate, ethanol and carbon-dioxide [10,11]. Selected health promoting Lactic acid bacteria, their impacts and mechanisms is summarized in (Table 1).

Apart from antimicrobial activity LAB is also involved in maintaining healthy Gastrointestinal (GI) micro ecology and host metabolism [12]. It also controls intestinal disorder by producing Serum antibodies IgG and Secretary IgA and IgM enhancing immune responses [13,14,15] (Figure 1). LAB is one of the major class of Probiotics which is defined as “live micro-organism which, when administered in adequate amounts confer a health benefit on the host” [16]. While probiotics is defined as non-absorable or non-digestible food components that beneficially stimulate one or more of the gut beneficial microbe groups and thus have a positive effect on human health and Symbiotic is combined administration of specific probiotics with probiotics to provide definite health benefit by synergisitic action.

 оргонический кислоты и сопутствующее снижение pH. Уровни и виды оргонических кислот, образующихся в продуктах, зависят от вида и условий роста. Аммонная кислота влияет на pH, а также на неионизированную форму молекул.

Hydrogen peroxide (H$_2$O$_2$)

Hydrogen peroxide is produced by LAB in the presence of oxygen as a result of the action of flavoprotein oxidases or nicotinamide adenine dinucleotide (NADH) peroxidases. The antimicrobial effect of H$_2$O$_2$ may result from the oxidation of sulfhydryl groups causing denaturations of a number of enzymes and from the peroxidation of membrane lipids thus the increased membrane permeability [29]. H$_2$O$_2$ may also be as a precursor for the production of bactericidal acid free radicals such as superoxide, carbon-dioxide and hydroxyl (OH) radicals which can damage DNA [30]. The production of H$_2$O$_2$ by Lactobacillus and Lactococcus strains inhibited Staphylococcus aureus, Pseudomonas spp. and various psycho-trophic microorganisms in foods [31,32].

Carbon dioxide (CO$_2$)

CO$_2$ is mainly produced by heterofermentative LAB. CO$_2$ plays a role in creating anaerobic environment which inhibits enzymatic decarboxylations and the accumulation of CO$_2$ in the membrane lipid bilayer may cause a dysfunction in permeability [33]. CO$_2$ can effectively inhibit the growth of many food spoilage microorganisms, specially Gram-positive psychrotrophic bacteria [34,35]. The degree of inhibition by CO$_2$ varies considerably between the organisms.

Fatty acids

Under certain conditions some Lactobacilli and Lactococci possessing lipolytic activities may produce significant amounts of fatty acids i.e. in dry fermented sausage and fermented milk [36,37]. The unsaturated fatty acids are active against Gram-positive bacteria and the antifungal activity of fatty acids is dependent on chain length, concentration and pH of the medium [19]. The antimicrobial action of fatty acids is due to the undissociated molecule, not the anion since pH had profound effects on their activity [38].
Reuterin and other low molecular mass compounds

Reuterin is produced by Lactobacillus reuteri, a heterofermentative species inhibiting the GI tract of humans and animals [39]. It is formed during the anaerobic growth of L. reuteri by the action of glycerol dehydratase which catalyzes the conversion of glycerol into reuterin [40]. Reuterin has been chemically identified to be 3-hydroxy propanol (β-hydroxyl propionaldehyde) a highly soluble, pH-neutral compound which is in equilibrium with its hydrated monomeric and cyclic dimeric forms [41]. Reuterin exhibits a broad spectrum of antimicrobial activity against certain Gram-positive and Gram-negative bacteria, yeast, fungi and protozoa. Spoilage organisms sensitive to reuterin include species of Salmonella, Shigella, Clostridium, Staphylococcus, Listeria, Candida and Trypanosoma [42]. Besides reuterin which has been studied, there are several reports on the production of Low molecular mass (LMM) antimicrobial compounds by different species of LAB (Table 2).

Bacteriocins

These are ribosomally synthesized, extra cellularly released bioactive peptides or peptide complexes, having a bactericidal and bacteriostatic activity. Bacteriocins are proteins peptide or proteinaceous complexes secreted by many strains of Gram positive and Gram negative bacteria that show their inhibitory action against strains of the same species or species of that are closely related to producer strains [43]. Four general classes of antimicrobial peptides or proteins (Bacteriocins) from LAB (Table 3). The detailed source and spectrum of different bacteriocins is summarized in (Table 4).

<table>
<thead>
<tr>
<th>Producing Strain</th>
<th>Antimicrobial Compound</th>
<th>Anti microbial activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus sp.</td>
<td>Mixture of small antibiotics, peptides and organic acids</td>
<td>Acidolin</td>
<td>[87].</td>
</tr>
<tr>
<td>Casei ssp. Casei strains</td>
<td>Acidophilus 2181</td>
<td>Bulgarian</td>
<td>[88].</td>
</tr>
<tr>
<td>Delbrueckii ssp</td>
<td>Delbrueckii ssp DD 14</td>
<td>Non lactic compound possibly with an aromatic group</td>
<td>[88].</td>
</tr>
<tr>
<td>bulgaricus</td>
<td>bulgaricus –7994</td>
<td>Microcin – like compound</td>
<td>[90].</td>
</tr>
<tr>
<td>Rhamnosus GG</td>
<td>Rhamnosus GG</td>
<td>3-Hydroxy propanal and its hydrated and dries forms (reuterin)</td>
<td>[90].</td>
</tr>
<tr>
<td>Reiteuri 1063</td>
<td>Streptococcus spp.</td>
<td>Cationic Compounds</td>
<td>[90].</td>
</tr>
<tr>
<td>Diacetylactis DRC -1</td>
<td>Thermophilus Strain</td>
<td>Containing carbohydrates and Amines</td>
<td>[90].</td>
</tr>
</tbody>
</table>

Table 2: Low molecules mass (LMM) antimicrobial compounds produced by lactic acid bacteria.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Remarks</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLASS-I</td>
<td>Lanthionine containing bacteriocins/ lantibiotics</td>
<td>Include both single and two peptide lantibiotics; upto 11 subclasses have been proposed.</td>
</tr>
<tr>
<td>CLASS-II</td>
<td>Non-lanthionine containing bacteriocins</td>
<td>Heterogenous class of small heat stable peptides.</td>
</tr>
<tr>
<td>CLASS-III</td>
<td>Non-bacteriocinolytic proteins</td>
<td>Large heat proteins often marin hydrolysis</td>
</tr>
<tr>
<td>CLASS-IV</td>
<td>Bacteriocins (non-proteanacious moties)</td>
<td>No longer considered bacteriocin.</td>
</tr>
</tbody>
</table>

Table 3: Suggested classification scheme for Bacteriocins [95].

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Bacteriocin</th>
<th>Source</th>
<th>Spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nisin</td>
<td>Lactococcus lactis</td>
<td>Inhibits Gram positive bacteria like bacilli, Lactococci, Micrococcus, Clostridium, botulism.</td>
</tr>
<tr>
<td>2</td>
<td>Diplococcic</td>
<td>Lactococcus lactis subsp. cremoris in milk.</td>
<td>Inhibitory against closely related Lactococcus lactis and other strains of Lactococcus cremoris</td>
</tr>
</tbody>
</table>
These factors can include: A diet high in sugar and refined carbohydrates which are low in probiotic containing foods. Sugars encourage the growth of yeast and bad bacteria, enemies of probiotics. Over exposure to antibiotics, long term use of antibiotics, chlorinated water and over use of antacids can devastate our intestinal flora. The effects can be harmful to the probiotic bacteria resulting GI tract less acidic (less friendly for good bacteria) and stress by affecting our hormone levels which stress lowers probiotic level.

**Table 4: Bacteriocins of Lactococci, Lactobacilli, Pediococci and Leuconostacs.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Bacteriocin</th>
<th>Source</th>
<th>Action against strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Lectostrepsins</td>
<td>L. lactis Sub 3, P. diacetylactis L. lactis</td>
<td>Active against other lactococci, group A, C and G streptococci, Bacillus cereus, Lactobacillus helveticus, leuconostoc hexamorphs, L. lactis subsp cremoris and Leuconostoc paracitrocinum.</td>
</tr>
<tr>
<td>4</td>
<td>Lactocoecins</td>
<td>L. lactis subsp cremorid, L. lactis subsp. diacetylactis</td>
<td>Inhibit other Lactococci and some Clostridia.</td>
</tr>
<tr>
<td>5</td>
<td>Lactocin 27</td>
<td>L. helveticus LP 27</td>
<td>Inhibitory action against other Lactobacilli</td>
</tr>
<tr>
<td>6</td>
<td>Helvetins Helveticins J</td>
<td>Lactobacillus Helveticus 481 Lactobacillus Helveticus 1829</td>
<td>Active against limited related Lactobacilli Bactericidal against other Lactobacilli</td>
</tr>
<tr>
<td>7</td>
<td>Lantacins</td>
<td>Lactobacillus acidophilus 110088 Lactobacillus acidophilus N2</td>
<td>Inhibitory against L. delbereekii subsp. Bulgaricus, L. delbereekii Subsp. Lactis, L. helveticus, L. acidophilus, L. ferenturn and strain of L. feacalis.</td>
</tr>
<tr>
<td>8</td>
<td>Plantaricins D</td>
<td>Lactobacillus plantarium C-111Lactobacillus plantarium NCDO 1191</td>
<td>Inhibitory to other lactic acid bacteria Inhibitory against other strains of L. plantarum, L. mesenteroides. Pseudomonas</td>
</tr>
<tr>
<td>9</td>
<td>Fermentin</td>
<td>Lactobacillus Fermenti</td>
<td>Inhibitory to Lactobacilli</td>
</tr>
<tr>
<td>10</td>
<td>Lacticins</td>
<td>Lactobacillus Sake 245</td>
<td>Inhibitory against Pediococcus, Leuconostac and Lactobacillus</td>
</tr>
<tr>
<td>11</td>
<td>Brevecin 37</td>
<td>Lactobacillus Curvatus LTH 1174</td>
<td>Inhibitory against other Lactobacillus, Leuconostoc, Carnobacterium monocytogens weak action against Micrococcus and Staphylococci</td>
</tr>
<tr>
<td>12</td>
<td>Sakacins Sakacin A Sakacin M Sakacin P</td>
<td>Lactobacillus Sake 70B Lactobacillus Sake 148 Lactobacillus Sake LTH 673</td>
<td>Inhibits strains of LAB and L. mesenteroides Inhibits Lactobacilli, leuconostoc. Carnobacterium Monocytogens and Staphylococcus Cynobacteria and S. aureus Inhibits Lactobacilli, leuconostoc, Gasrobacter, Enterococci, Brochothrix thermosphacta and Listeria sp.</td>
</tr>
<tr>
<td>13</td>
<td>Carecin 80</td>
<td>Lactobacillus Casei B-80</td>
<td>Active against other strains of L. casei</td>
</tr>
<tr>
<td>14</td>
<td>Curvacin A</td>
<td>Lactobacillus Curvatus LTH 1174</td>
<td>Inhibitory against otter lactobacilli, Leuconostoc. Carnobacteria, L. monocytogens as well as weak action against Micrococcus and Staphylococci</td>
</tr>
<tr>
<td>15</td>
<td>Pediocin AcH</td>
<td>Pediococcus acidolactis strain H</td>
<td>Active against many Lactobacilli, Leuconostoc, S. aureus Clostridium perfringes, L. monocytogens and Pseudomonas spp.</td>
</tr>
<tr>
<td>16</td>
<td>Pediocin PA-1</td>
<td>Pediococcus acidolactis strain PAC-10</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>Mesenteriocin 5</td>
<td>Leuconostac mesenteroid 5</td>
<td>Inhibitory to L. monocytogens, S. faecalis, Brevibacterium</td>
</tr>
<tr>
<td>18</td>
<td>Leuconin A</td>
<td>Leuconostac geldum</td>
<td>Inhibitory to Leuconostac, Lactobacilli, Pediococci, E. faecalis and L. monocytogens</td>
</tr>
<tr>
<td>19</td>
<td>Leuconostac-S</td>
<td>Leuconostac parameentricoides OX</td>
<td>Inhibits L. monocytogens, S. aureus, Aeromonas spp., Clostridium.</td>
</tr>
<tr>
<td>20</td>
<td>Carnocin</td>
<td>Leuconostac carnosum LA-44A</td>
<td>Inhibits Pediococci, Enterococci, Leuconostac and Listeria spp.</td>
</tr>
</tbody>
</table>

### Why probiotic supplementation is needed?

In our modern world it is challenge to maintain a healthy quantity of good bacteria in the colon. Fermented foods including yogurt, miso, tempeh and sauerkraut can supply some healthy bacteria but, other factors in our life may be actively depleting our probiotic population. These factors can include: A diet high in sugar and refined carbohydrates which are low in probiotic containing foods. Sugars encourage the growth of yeast and bad bacteria, enemies of probiotics but vegetables and fruits contain prebiotics the building blocks of probiotics. Over exposure to antibiotics, long term use of antibiotics, chlorinated water and over use of antacids can devastate our intestinal flora. The effects can be harmful to the probiotic bacteria resulting GI tract less acidic (less friendly for good bacteria) and stress by affecting our hormone levels which stress lowers probiotic level.

### Potential benefits of Probiotics

#### Alleviation of lactose intolerance

About 70% of the world's population has lactose intolerance to have a low amount of intestinal β-galactosidases activity and it is a major problem and for whom lactose behaves like an osmotic nondigestible carbohydrates [44]. Lactic acid bacteria increase the lactose tolerance level in lactose intolerant individuals [45].

#### Cholesterol lowering

LAB removes cholesterol from culture media [46]. The cholesterol removal from culture media results precipitation of cholesterol with free bile acids, formed in the media because of the activity of the
bacterial enzyme bile salt hydrolase [47]. Enhanced bile salt hydrolase activity increases cholesterol excretion. However, consumption of a probiotic milk product increases cholesterol excretion [48].

Prevention of urinary & genital infections in women’s

Almost all infections of the vagina and bladder are caused by microorganisms that originate in the bowel. There is strong correlation between presence of commensals particularly Lactobacilli in the healthy vagina and an absence of these microorganisms in patients with urogenital infections. Disruption of the normal vaginal flora is caused by broad-spectrum antibiotics, hormones, dietary substances and factors not as yet fully understood. There is some evidence that probiotic microorganisms delivered as foods and tropical probiotics using Lactobacillus rhamnosus and Bifidobacterium lactis are thought to have several presumably beneficial effects on immune system by inactivation and reduction in activity increases cholesterol excretion. However, consumption of a probiotic milk product increases cholesterol excretion [48].

Prevention of disorders associated with the GI tract-Diarrhea

Infectious diarrhea is a major world health problem responsible for several million deaths each year. While the majority of deaths occur amongst children in developing countries, it is estimated that up to 30% of the population even in developed countries are affected by food-born diarrhea each year. Probiotics can potentially provide an important means to reduce these problems. The beneficial effect of probiotics using Lactobacillus rhamnosus and Bifidobacterium lactis for prevention and treatment of acute diarrhea mainly caused by rotaviruses in children [56-59].

In addition to rotavirus infection, many bacterial species cause death and morbidity in humans. Certain probiotics strains can inhibit the growth and adhesion of Salmonella and decreases urease activity necessary for the pathogens to remain in acidic environment of the stomach [66-68].

Helicobacter pylori infection and complication

A new development for probiotics application is activity against Helicobacter pylori, a Gram negative pathogen responsible for Type-B gastritis, peptic ulcers and gastric cancer. LAB inhibits the pathogen growth and adhesion of enteropathogens and animal studies have indicated beneficial effects against pathogens such as Salmonella and decreases urease activity necessary for the pathogens to remain in acidic environment of the stomach [66-68].

Acute diarrhea, mainly caused by Rotavirus

Rotavirus is the main virus of study with probiotics. Rotavirus infection causes gastroenteritis, characterized by acute diarrhea and vomiting. There are ample evidence that probiotics reduce the duration and severity of rotavirus diarrhea. Consumption of Lactobacillus decreased the diarrheal phase in children infected with rotavirus infection [56, 69-71].

Traveler’s diarrhea

Traveler’s diarrhea is defined as the passage of greater than 3 unformed stools in a 24 hour period in people who normally live in industrialized countries and travel to tropical and sub tropical countries [72]. Although it is not possible to medically recommend any probiotic at the present time to prevent traveller’s diarrhea, the likelihood for some agents to be effective is high and more through studies need to be performed.
Inflammatory disease and bowel syndromes

Inflammatory bowel diseases, such as Pouchitis and Crohn’s disease as well as Irritable bowel disease, may be caused by alterations in the gut flora including infection [73]. A large body of evidence suggests that the enteric microbiota may be an important factor in driving the abnormal inflammatory response in the susceptible host leading to chronic inflammatory bowel diseases due to incidence of inflammation is greatest in the part of the intestine which harbours the highest concentration of bacteria (colon, caecum and terminal ileum), fecal stream has been implicated in disease activity, its interruption has been associated with the disease improvement, enhanced mucosal permeability may play a vital role in maintaining a chronic inflammatory state due to genetic predisposition or direct contact with bacterial or their products, loss of immunological tolerance to commensal bacteria in genetically susceptible patients.

The discovery of the role of the enteric microbiota is a common denominator in the pathogenesis of inflammatory bowel disease has lead to the interest and increasing scientific evaluation of the use of probiotics as a means to reconstitute microbial and immunological hemostasis [74].

Role of LAB in food preservation as a biopreservative agent

Now a day’s consumers are concerned about the synthetic chemicals used as preservatives in food and there is resulting trend towards less processed foods. The untreated foods can harbour dangerous pathogen, which can multiply under refrigeration and without oxygen. A solution to this dilemma is the use of antimicrobial metabolites of fermentative microorganisms. In common fermented products such as yogurt, lactic acid is produced by the starter bacterial culture to prevent growth of undesirable microorganism [75]. LAB has contributed in the increased volume of fermented foods worldwide especially in foods containing probiotics or health promoting bacteria.

The development of novel approaches in food allows broadening the potential for using LAB in food [76,77]. The nature of genetic modification in LAB as Genetically modified organism (GMOs) can be divided into 3 groups including one-step genetic events like deletion, gene amplification, plasmid insertion and losses, multistep genetic rearrangements with DNA of same species and trans –species genetic modifications has emphasized the effective use of gene manipulated LAB in battle against food–spoilage and pathogenic bacteria [10,78]. An example genetically modified LAB have been utilized to improve cheese ripening [79].

Future prospects of probiotics

It includes for determining the physiological role, mechanisms of action and extent of influence of probiotics in human health using human feeding studies, used for the validating the extent of its importance in the areas of immune system, cancer and gut micro ecology and also assess the effects of probiotics on populations its activity on gut microbes and in selection of dose responses for different strains of bacteria.

Conclusion

The interest in establishing scientific credibility for probiotic effects is of high importance to companies and scientists. Research to support health claims will have to take into account the intestinal microbiota and its interaction with the host. The information gleaned from sequence data will provide opportunity to improve probiotic functionality and expand understanding of mechanisms. The health promoting benefits and efficacy of probiotics has been demonstrated in many models of gastrointestinal disease and indeed in diseases and conditions at other anatomically distinct locations. The use of probiotics in the treatment of many forms of diarrheal disease appears especially promising. The inherent physiological and technological fragility of what are often promising candidate probiotic strains can render them ineffective for clinical use. Formulation of food products with additional vitamins, non-digestible carbohydrates, soluble fiber, phytochemicals, or other bioactive ingredients could further enhance the dietary value of the product.

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