Health Benefits of Exopolysaccharides in Fermented Foods

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4 authors, including:

K. Madhavan Nampoothiri
National Institute for Interdisciplinary Science and Technology

Bindhumol Ismail
University of Kerala

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Jayakumar Beena Divya*
Kontham Kulangara Varsha*
Kesavan Madhavan Nampoothiri
Bindhumol Ismail
Ashok Pandey

Biotechnology Division, National Institute for Interdisciplinary Science and Technology (NIIST), CSIR, Trivandrum, Kerala, India

Review

Probiotic fermented foods for health benefits

The history of fermented foods used by humans can be traced back to centuries. The medicinal as well as flavor enhancing properties of fermented foods are mainly due to the presence of bacteria known as probiotics. Probiotics aid in digestion and nutrient assimilation. These bacteria are also known for their beneficial effects for the immune system and health. Many of them produce antimicrobial bioactive molecules that make them effective biopreservatives and produce nutraceuticals to create functional foods with increased bioavailability of nutrients. Thus, these lactic acid bacteria have undeniable favorable effects. This review will summarize the health benefits of probiotic fermented foods.

Keywords: Functional foods / Lactic acid bacteria / Nutraceuticals / Prebiotics / Probiotics

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1 Introduction

Lactic acid bacteria (LAB) are a group of gram-positive, catalase negative, microaerophilic, nonsporing cocci, coccobacilli, or rods with lactic acid as the main product of carbohydrate fermentation. Man has consumed foods fermented with LAB for thousands of years. Because of their long history of safe use in foods, LAB are considered as nontoxic, food-grade microorganisms, and most of them have a GRAS status (generally recognized as safe). LAB have an important role in food production due to their positive contribution to flavor and preservation of the final product. LAB comprise of the genera *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Globicatella*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*. Lactobacilli, carnobacteria, and some *Weissella* are rods while the rest of the genera are cocci [1,2]. In addition to traditional fermentation, LAB have been used extensively as a preservative, acidulant, and flavorant in food processing, as an intermediate in pharmaceutical and cosmetic manufacture (e.g. surgical dressing), in the manufacture of biodegradable polylactic acid polymers [3] and for the development of probiotics and nutraceuticals.

Probiotics are living microorganisms that, following ingestion, form part of the colonic microbiota, at least temporarily, and are used with a view to improve the health and well being of the host. A number of uses of probiotics in gastrointestinal (GI) diseases have been proposed, including modulation of gut mucosal immunity and the prevention and treatment of intestinal infections. The products with proven biological activity that originate from biological compounds are often described as bioactives, whereas the subgroup of products that bridge the gap between food products and drugs are also termed nutraceuticals or functional foods. Probiotic bacteria are incorporated mainly in fermented foods and dairy products are the major carriers of probiotics. While adding probiotics to fermented foods, several factors must be considered that may influence the viability of probiotics in those foods as well as their survival upon entering the GI tract[4]. Since the *Lactobacillus* spp. found in each probiotic food are not the same, the fact that every bacterium plays a different role in the body, it is a good idea to include a variety of naturally fermented foods in our diet.

2 LAB in food fermentation

Food fermentation is regarded as one of the oldest ways of food processing and preservation. Throughout the ancient history, health-promoting fermented foods have played a role in sustaining thriving civilizations. Fermentation enhances the flavor and nutritional quality of food and increases its shelf life. The natural fermentation process takes place in presence of a mixed colony of microorganisms such as moulds, bacteria, and yeast

Correspondence: Dr. K. M. Nampoothiri (madhavan85@hotmail.com), Biotechnology Division, National Institute for Interdisciplinary Science and Technology (NIIST), CSIR, Trivandrum-695 019, Kerala, India

Abbreviations: CAGR, compound annual growth rate; **CVD**, cardiovascular disease; **DMAB**, 3,2-dimethyl-4-amino-biphenyl; **EPS**, exopolysaccharides; **FOS**, fructooligosaccharides; **GI**, gastrointestinal; **GOS**, galactooligosaccharides; **HMECs**, human mammary epithelial cells; **IBD**, inflammatory bowel disease; **IBS**, irritable bowel syndrome; **IDF**, International Dairy Federation; **LAB**, lactic acid bacteria; **LDL**, low-density lipoprotein; **MBV**, minimum of bio value; **NEC**, necrotizing enterocolitis; **NICE**, nisin-induced controlled expression system; **4-NQO**, 4-nitroquinoline-*N*-oxide; **NSLAB**, non starter lactic acid bacteria; **ODC**, ornithine decarboxylase; **SSAT**, spermidine/spermine N1-acetyltransferase

*These authors contributed equally to this work.

[5, 6]. The products of fermentation depend on the microorganisms involved, substrates used, and also on the fermentation conditions. Some of the products of fermentation include organic acids (e.g. palmitic, pyruvic, lactic, acetic, propionic, and butyric acids), alcohols (mainly ethanol), aldehydes, and ketones (acetaldehyde, acetoin, 2-methyl butanol, diacetyl) [7]. The microorganisms used for food fermentation are nonpathogenic and the enzymes such as proteases, amylases, and lipases produced by them help in the breakdown of complex food materials into simple nontoxic products with desirable flavor and texture [8]. LAB are the most common and dominant microorganisms present in fermented foods and therefore, lactic acid fermentation is considered as the major contributor to the beneficial characteristics observed in those foods [5]. Their importance is associated mainly with their safe metabolic activity thereby giving various functional attributes to the food.

Based on their fermentation pattern, LAB are classified as homofermentative (e.g. *Lactococcus, Streptococcus*) and heterofermentative (e.g. *Weissella, Leuconostoc*). Homofermenters generate two moles of lactate per mole of glucose via EMP pathway whereas heterofermentors utilize pentose phosphate pathway to produce equimolar amounts of lactate, CO_2, and ethanol from glucose [9].

2.1 LAB as starter cultures

A starter culture consists of a large number of live microorganisms of a single strain or a mixed culture that initiates and speeds up the food fermentation. LAB have a pivotal role in this and have long been used as starters in various food fermentation processes [9, 10]. A starter culture can provide particular characteristics in a more controlled and predictable fermentation. The primary function is the rapid acidification of the food by producing mainly lactic acid as well as small quantities of acetic acid. In addition, LAB produce substances such as bacteriocins, exopolysaccharides (EPS), aroma compounds, enzymes, etc. thereby enhancing the flavor, texture, and nutritive content of the food [11].

Traditionally, spontaneous fermentation was employed which was optimized by a process known as "backslopping." In backslopping, a small amount of previously fermented batch is used to inoculate a new batch. This results in the domination of the best-adapted strains. It is a cheap and reliable method and is still used especially for products for which the microbial ecology and the exact role of successions in microbial population are not well known [12]. There are also non starter lactic acid bacteria (NSLAB) that are not involved in the starter but develop as a secondary biota during maturation. Flavor of the fermented food is intensified by the action of these NSLAB [13]. As an example in sauerkraut process, the heterofermentative *Leuconostoc mesenteroides* predominates in the early stages of fermentation. It can grow over a wide range of temperatures and tolerate higher salt concentration than the subsequent species. As the total acidity increases during the later stages, lactobacilli take over the fermentation. Heterofermentative LAB strains such as *Lactobacillus brevis*, *Pediococcus,* and *Enterococcus* also contribute toward the kraut formation [14].

2.2 Traditional versus commercial fermentation

Most of the natural food fermentations employ a mixed culture indigenously present in the raw material. However, in an industrial scale where there is a large demand for maintaining the quality of the final product a defined starter culture is preferred. The daily propagation of these starter cultures may result in the disappearance of certain strains and this in turn may affect the properties and functionality of the final product. Also, there is a chance of losing some of the important metabolic traits that are plasmid encoded during propagation or due to adaptation to the food matrix. Thus, there is a limited biodiversity in these commercial starters [9, 10].

Interestingly, in traditional fermented foods more resistant wild-type strains of LAB are used. Since they have to compete with other microorganisms most of them will be able to produce antimicrobial substances such as bacteriocins. This paved the way for functional starter cultures [15]. Functional starter cultures possess at least one intrinsic functional property. They can add to food safety and/or offer one or more industrial, nutritional, or health advantages [11]. Promising examples are LAB that produce antimicrobial substances such as bacteriocins to assure food safety, sugar polymers to improve texture, desirable aroma compounds to enhance taste properties, or strains that display probiotic effects [10]. Also the recent advances in gene technology opened new possibilities for the microbiologists to develop starter cultures with desired qualities. Some of the fermented foods and associated LAB strains are listed in Table 1 and as an example; an overview on the manufacture of cheese is given in Fig. 1.

3 LAB as probiotics

The word probiotic means "for life" and the world health organization defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host." In the last two decades, research in the probiotic area has achieved major progresses in the selection and characterization of specific probiotic cultures and confirmed the health benefits associated with them. Traditionally, fermented foods are the main source of probiotics and hence one of the major dietary supplements of modern world.

LAB are the most important group of microorganisms commercially used as starter cultures for the manufacture of dairy-based probiotic foods [16] and have been established as a natural consumer. Strains of the genera *Lactobacillus*, *Bifidobacterium*, and *Propionibacterium* are the most widely used and commonly studied probiotic bacteria. LAB fulfill the criteria that have to be met by the organisms to be selected as probiotics like resistance to the enzymes in the oral cavity, survival through the GI tract, arrival at the site of action in a viable physiological state and adherence to the host cell surface [17]. Nowadays probiotics are consumed as fermented dairy products or as freeze-dried cultures. Probiotics, upon reaching the lower part of small intestine and colon, colonize and multiply to exert their beneficial effects. Health claims provided by LAB range from the regulation of intestinal microbial homeostasis to the modulation of immune responses. Alleviation of lactose intolerance, a reduction in the

Table 1. LAB strains in common fermented foods.

Fermented foods	Reported LAB strains
Yoghurt	*Lb. delbrueckii* subsp. *bulgaricus, S. thermophilus*
Cheddar cheese	*L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris, S. thermophilus*
Italian cheese such as Mozzarella	*Lb. delbrueckii* subsp. *bulgaricus, Lb. helveticus, Lb. lactis, S. thermophilus*
Swiss cheese types	*Lb. delbrueckii* subsp. *bulgaricus, Lb. lactis, Propionibacterium shermanii, L. lactis* subsp. *biovar diacetylactis, Leuc. mesenteroides* subsp. *cremoris, L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris, S. thermophilus*
Goat cheese and sheep cheese	*L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris, L. lactis* subsp. *biovar diacetylactis, Leuc. mesenteroides* subsp. *cremoris*
Butter and buttermilk	*L. lactis* subsp. *lactis, L. lactis* subsp. *lactis biovar diacetylactis, L. lactis* subsp. *cremoris, Leuc. menesteroides* subsp. *cremoris*
Kefir	*Lb. kefir, Lb. kefiranofacies, Lb. brevis*
Fermented, probiotic milk	*Lb. casei, Lb. acidophilus, Lb. rhamnosus, Lb. johnsonii, B. lactis, B. bifidum, B. breve*
Fermented sausages	*Lb. sakei, Lb. curvatus, P. acidilactici, P. pentosaceus*
Sauerkraut	*Lb. plantarum, Lb. brevis, P. acidilactici, P. cerevisiae, Leuc. mesenteroides*
Pickles	*Lb. plantarum, Lb. pentosus, Lb. plantarum, Leuc. mesenteroides*
Kimchi (Korea)	*Lb. plantarum, Leuc. mesenteroides, L. brevis*
Idli/dosa (India)	*Leuc. mesenteroides, E. faecalis*
Wine (malolactic fermentation)	*O. oeni*
Rice wine	*Lb. Sakei*

B. = Bifidobacterium; E. = Enterococcus; L. = Lactococcus; Lb. = Lactobacillus; Leuc. = Leuconostoc, O. = Oenococcus, P. = Pediococcus, S. = Streptococcus.

risk of diarrhea caused by bacteria and virus as well as lowering serum cholesterol are some other positive effects obtained with the consumption of probiotic [18]. Table 2 shows some of the probiotics present in the market, the LAB used in each preparation, and the health benefit that it provides.

The possible mechanisms of action of probiotics include production of antimicrobial compounds, competitive exclusion of pathogen binding, competition for nutrients and immunomodulation [19]. Figure 2 summarizes the important mechanisms.

The mechanisms by which LAB differentially modulate the host cell functions depend upon the cell wall constituents of bacteria and corresponding host cell receptors that modulate downstream processes. High tolerance to acid and bile helps them survive the harsh physical–chemical conditions of GI tract. The ability of the bacteria to colonize the mucosal surfaces by adhering to the host GI epithelial cells and extracellular matrix proteins depends on cell surface hydrophobicity [20]. This prevents pathogen access by steric interactions or specific blockage on cell receptors. Probiotic interaction with the mucin layer of the host epithelial cells increases their retention time in the host [17].

The antimicrobial activity of LAB has been attributed to the production of organic acids [21,22], reuterin [23], proteinaceous compounds [24], and cyclic dipeptides [25]. Bacteriocins of less than 20 kDa cause depolarization of the target cell membrane and/or inhibit cell wall synthesis and those with more than 20 kDa degrade the murein layer [26]. Reuterin, a broad spectrum antimicrobial agent is hypothesized to competitively inhibit the enzyme ribonucleotide reductase [27] and also inactivate proteins and other small molecules [28] thereby preventing microbial growth. Organic acids in their undissociated, hydrophobic form diffuse across the cell membrane of the pathogens, neutralize the electrochemical potential, and increase its permeability resulting in bacteriostasis and death [29, 30].

4 Development of probiotic foods

Probiotics are incorporated in foods such as yoghurt, cheese, ice cream, infant formulas, breakfast cereals, sausages, luncheon meats, chocolates, puddings, and also sold as capsules containing freeze-dried cell powders and tablets. While adding probiotics to a food product, several factors must be considered that may influence the viability of the culture as well as its activation in the intestine. These factors include (i) the physiological state of the probiotic organisms added (growth phase), (ii) storage conditions (e.g. temperature, humidity), (iii) chemical composition of the food matrix (e.g. titrable acidity, available carbohydrate content, nitrogen sources, vitamins, minerals, prebiotics, food additives, water activity, and oxygen content), and (iv) possible interaction between the probiotics and the starter cultures (e.g. antagonism, mostly caused by the production of bacteriocins, and synergism) [4].

The major challenge in probiotic food preparation is the retention of viability of the cultures. Typical methods for preserving sensitive biological materials include freeze drying, cryopreservation, and spray drying. These techniques involve the use of extreme temperatures that may initiate structural damages to the cell membranes, protein denaturation, and/or DNA damage, and can lead to a decrease in cell viability [31]. The minimum of bio value (MBV) index represents the minimum number of probiotic cells (CFU/g) in the product at the moment of consumption that is necessary for the beneficial effects [32]. According to the recommendation by the International Dairy Federation (IDF), MBV should be $\geq 10^7$ CFU/g up to the date of minimum durability [33]. In order to increase the resistance of probiotic bacteria against the detrimental food processing conditions, several approaches such as selection of acid- and bile-tolerant strains, microencapsulation, packaging in oxygen protected materials, double-step fermentations, preadaptation to stress conditions, and addition of micronutrients are

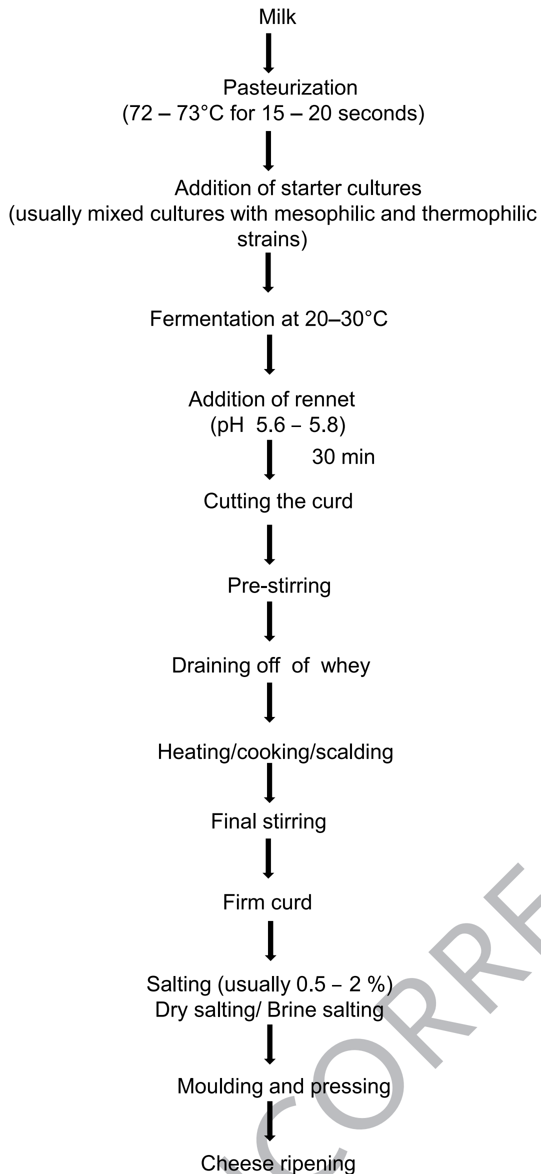

Figure 1. General steps in cheese making.

being employed [34]. Recently, researchers from Technische Universität München (TUM) developed an environmental friendly low-temperature vacuum drying process to increase the rate of cell viability (http://www.sciencedaily.com).

Microencapsulation of the probiotic bacteria using various biopolymers can noticeably increase the viability of the bacteria thereby increasing the shelf life of the probiotic food. Microencapsulated cells are easier to handle and the number of cells in each bead can be quantified thus allowing controlled dosages. Some of the important techniques of microencapsulation are given in Table 3. It was found that microencapsulation of probiotic along with a prebiotic (coencapsulation) increases the viability of the probiotic microbiota. Once the matrix beads have been dried, a surface coating by polymers such as chitosan, alginate, or carrageenan can be applied (double encapsulation) providing extra protection for the cells that may also enhance the sensory properties of the product. According to some studies, functionality of a multistrain probiotic could be more effective and more consistent than that of monostrain probiotic provided the strains are compatible and preferably synergistic. In a clinical study conducted, it was found that a multistrain probiotic preparation significantly reduced the symptoms of irritable bowel syndrome (IBS) [35]. The selection of suitable probiotic strains, coating material, and prebiotics has a major role in the efficiency of the process.

5 Health benefits of probiotics

Infectious diarrhea is a major health concern both in developing and developed countries. Studies show probiotic intake can be an important means to reduce this problem. Research groups working under various conditions reported that consumption of probiotic fermented dairy products have shortened the episodes or reduced the risk of rotavirus-induced diarrhea in humans. Many strains of LAB are reported to reduce the risk of acute diarrhea. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* reduced the incidence of acute diarrhea and rotavirus shedding in infants admitted to hospital [36]. Children with acute diarrhea have been administered with an oral rehydration solution containing *Lactobacillus* GG that resulted in shorter duration of diarrhea and less chance of a protracted course [37]. In a more wide study, 112 newborn infants in rural India were randomized to receive a daily oral dose of 10^8 CFU/g *Lb. sporogenes* or a placebo for 1 year. Results have shown that feeding *Lb. sporogenes* on a prophylactic basis in the first year of life has a significant preventive impact on the incidence and duration of diarrhea as well as the total number of days of illness [38]. A combination of nitazoxanide and *Lactobacillus* was found to be efficient in the treatment of acute rotavirus diarrhea in children [39].

Traveler's diarrhea is generally caused by enterotoxigenic *Escherichia coli*. *Shigella*, *Campylobacter*, *Salmonella*, and *Aeromonas* species as well as *Plesiomonas shigelloides* and noncholera vibrios have also been isolated from travelers [40]. LAB preparations have been used in the treatment since they can interfere with the gut colonization by pathogenic microorganisms. The efficacy of *Lactobacillus* GG strain isolated from healthy humans in decreasing the incidence of traveler's diarrhea in Finnish travelers [41] has been studied. In a recent study, the inefficiency of nonviable *Lb. acidophilus* in prevention of traveler's diarrhea has been found by a randomized, double-blind, controlled study [42]. More clinical trials are required to confirm the usefulness of probiotic preparations to prevent traveler's diarrhea.

Excessive use of antibiotics may lead to colitis caused by *Clostridium difficile*. This is a common inhabitant of intestine but an imbalance in the indigenous microbiota leads to an elevation in their number and production of toxin. In such cases, probiotics can be administered to restore the lost intestinal microbiota. Intake of *Lactobacillus* GG is reported to be very effective in alleviating the signs and symptoms of *C. difficile* infection [43]. A daily supplementation with two strains of *Lb. acidophilus* (CUL60 and CUL21) and two strains of *Bifidobacterium* spp. during and post antibiotic therapy reduced the extent of disruption to the intestinal microbiota as well as the incidence

Table 2. Probiotics in the market.

Trade name	Organism	Health benefit
Culturelle probiotic (Amerifit Brands)	*Lactobacillus* GG	Restores natural balance of intestinal microbiota, boost immune system
Kyo-Dophilus (Kyolic)	*Lb. acidophilus*, *B. bifidum*, *B. longum*	Promotes healthy intestinal function
Dannon DanActive	*Lb. casei* Immunitas	Antibiotic-associated diarrhea and immune system function
RepHresh Pro-B	*Lb. rhamnosus*, *Lb. reuteri*	Maintain the microbiota important to overall vaginal health in females
Ideal bowel support 299V	*Lb. plantarum*	Reduces bloating, gas, and intestinal discomfort
Probiotic health food	*Lactobacillus Bifodobacterium*	Helps to maintain healthy intestinal biota, nutritionally supports immune function

Lb. = Lactobacillus; B. = Bifidobacterium.

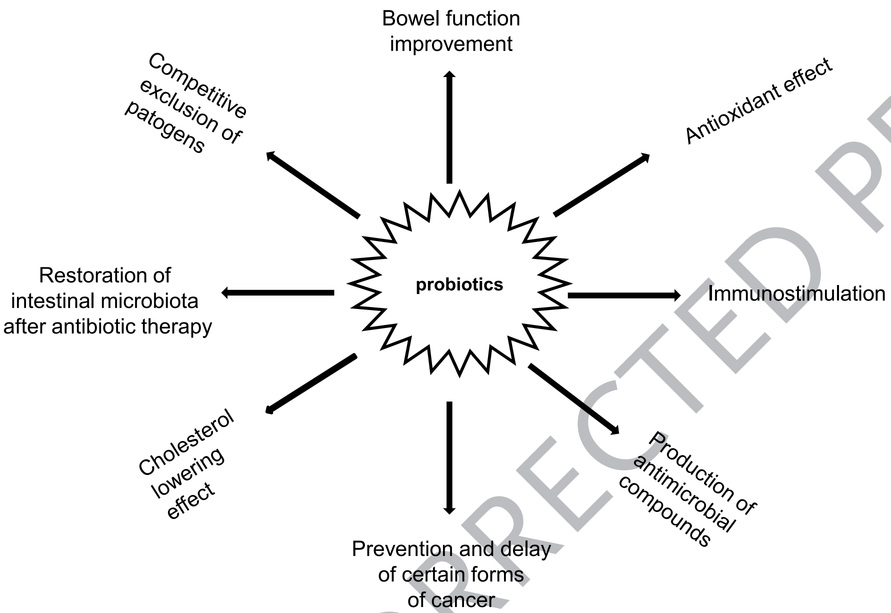

Figure 2. Mode of action of probiotics.

and total numbers of antibiotic-resistant strains in the regrowth population [44, 45].

Helicobacter pylori is a gram-negative pathogen that causes peptic ulcers and type B gastritis. *Lactobacillus johnsonii* La1, when administered as a whey-based culture supernatant, is reported to have attenuated the colonization of *H. pylori* in the gut [46]. Administration of *Lb. rhamnosus, Propionibacterium freudenreichii,* and *Bifidobacterium breve* after an anti *H. pylori* treatment resulted in minor changes in intestinal microbiota and slightly diminished the microbial disturbances [47]. In a mouse study, the strain *Lb. casei* Shirota (LcS) administered for 9 months reduced colonization of *H. pylori* in the antrum and corpus regions of the stomach, associated with a reduced intensity of mucosal inflammation [48].

Alterations in the gut biota including infection may lead to inflammatory bowel disease (IBD). IBD is chronic inflammation of the terminal ileum and colon (e.g. Crohn's disease and ulcerative colitis) [49]. The intestinal microbiota plays a critical role in inflammatory conditions in the gut and hence the therapeutic restoration of the altered microbiota with appropriate probiotics can be an ideal treatment for IBD. The ileal microbial biota of IBD patients have increased amount of mucosal bacteria including *E. coli, Bacteroides* spp., and Enterococci, and suggest that the changes in mucosal biota are the result of specific host response [50]. Several studies show interesting effects of probiotics on IBD. Children with mild-to-moderate active Crohn's disease were given enterocoated *Lb. rhamnosus* GG twice a day for 6 months and had a notably reduced inflammation after 4 weeks [51]. Another proof that demonstrates the potential role of probiotics in prevention or treatment of IBD came from a clinical study in which an IL-10-deficient mouse was pretreated with *Lb. reuteri* and *Lb. paracasei* and then infected with IBD causing *H. hepaticus.* Intestinal inflammation was reduced and the levels of proinflammatory colonic cytokines were lowered after the pretreatment [52].

IBS is a functional GI disorder that is portrayed by chronic abdominal discomfort or pain associated with bowel habit change such as diarrhea and constipation, without obvious organic abnormalities. The low-grade inflammation related with IBS may be restrained or the normal local immune function was restored by probiotics. Deconjugation and absorbtion of bile acids can be performed by lactobacilli and bifidobacteria, possibly reducing

Table 3. Methods for microencapsulation of probiotics.

Microencapsulation techniques	Coating materials	Process	Advantages	Disadvantages
Extrusion	Water soluble and water insoluble polymers	Preparation of coating solution materials Dispersion of core material Cooling or passing of the mixture through dehydrating fluid	Simple and cheap method No use of deleterious solvents Can be used in aerobic and anaerobic conditions No damage to probiotic cells	Difficult to use in large-scale productions due to slow formation of the micro beads
Emulsion	Alginate, k-carrageenan, gelatin etc.	Dispersion of an aqueous phase containing the bacterial cells and polymer suspension into an organic phase, such as oil, resulting in an emulsion The dispersed aqueous droplets are hardened by cooling or by addition of a gelling agent or a cross-linking agent The beads are partitioned into water and washed to remove oil	Results in smaller diameter beads Suitable for scale up applications	Not suitable for the development of low-fat products
Drying				
Spray drying	Water soluble polymers (e.g. gum arabic, starch)	Dispersion of probiotics into the polymer solution Homogenization of the liquid Atomization of the mixture into the drying chamber Evaporation of the solvent Separation of microcapsules	Rapidity Relatively low cost Reproducibility Can be used in industrial scale	Use of high temperature affects the viability of the probiotic bacteria
Freeze drying	Trehalose, lactose, maltose etc.	Initial freezing Primary drying (sublimation phase) Secondary drying	Heat injuries to cells are minimal	Relatively expensive Difficult to be performed on industrial scale
Fluidized bed drying	Skim milk, potato starch, alginate, casein etc.	The encapsulated cells are dried by air that is blown through a hole resulting in a suspension	Residence time can be extended allowing longer drying at low temperatures, thus reducing the risk of heat inactivation	Oxidative stress affects the viability of the cells
Spray freeze drying	Water soluble polymers (maltodextrose, trehalose)	Combines spray and freeze drying Homogenized solution of probiotic cells is atomized into a cold vapor phase of a cryogenic liquid generating frozen droplets Drying in freeze dryer	Controlled size and larger specific area of the microcapsules	Long processing time Use of high energy Expensive

the colonic mucosal secretion of mucin and fluids that may add to functional diarrhea or IBS with diarrhea [53].

Among the many health-promoting functions of probiotics, much interest has given to their immune modulatory activity. LAB induce immune responses and intestinal barrier integrity. The immunomodulation roles may involve the activation of both specific and nonspecific immune responses. The cell wall components of probiotics include peptidoglycans, polysaccharides, and teichoic acid that are previously reported to have immunostimulatory effects. Knowledge of the normal intestinal microbiota, contribution of LAB and their role in numerous functions in the digestive tract, as well as the functioning of the mucosal immune system form the basis for the study and selection of a probiotic strain with immunostimulatory properties [54].

Probiotics boost the mucosal barrier function by innate immune molecules, including goblet cell-derived mucins and trefoil factors and defensins produced by intestinal paneth cells or by promoting adaptive immune responses [55]. In a mouse study, 7 days of *Lb. casei* CRL 431 administration decreased the severity of infection with *Salmonella enterica* serovar Typhimurium. This continuous administration diminished the counts of the pathogens in the intestine as well as their spreads outside this organ [56]. Experiments by means of LcS detected enhancement of innate immune responses and promotion of Th1-mediated immune activity [57]. In a human study, the effect of yogurt containing *Lb. casei* as the main probiotic component on immune system function was checked. Administration of LAB strains resulted in an increase of NK activity and proliferative response to *Candida albicans* in adults and secretory-IgA in children saliva [58]. In another study, administration of *B. bifidum* increased expression of TLR-2, COX-2, and PGE2, and significantly reduced apoptosis in the intestinal epithelial cell line (IEC-6) in the intestinal epithelium of rat necrotizing enterocolitis (NEC) model [59].

Experiments show that probiotic microorganisms can preclude or delay the start of certain cancers. The 6-day-old extracts of fermented milk product kefir depressed the growth of human mammary cancer cells (MCF-7) in a dose-dependent manner, showing 29% inhibition of proliferation at a concentration as low as 0.63% without any antiproliferative effect against normal human mammary epithelial cells (HMECs) [60]. Ornithine decarboxylase (ODC) and spermidine/spermine N1-acetyltransferase (SSAT), the key enzymes involved in polyamine biosynthesis and catabolism, respectively, are associated with cancer risk and are the specific markers for neoplastic proliferation. Administration of *Lb. rhamnosus* GG homogenate significantly reduced ODC mRNA and activity as well as polyamine content and neoplastic proliferation. In addition, an increase in both SSAT mRNA and activity was observed after *Lb. rhamnosus* GG administration in HGC-27 human gastric cell lines [61].

Lactobacillus acidophilus VM 20 caused a decrease of the mycotoxins ochratoxin A and *B. animalis* VM 12 reduced patulin level from a liquid medium [62]. In another study, antimutagenic effect of soymilk fermented with *S. thermophilus*, *Lb. acidophilus*, *B. infantis,* and *B. longum* was checked against mutagenesis induced by 4-nitroquinoline-*N*-oxide (4-NQO), a direct-acting mutagen, and 3,2-dimethyl-4-amino-biphenyl (DMAB), an indirect-acting mutagen, on *S. typhimurium* TA 100 and concluded with lack of mutagenic activity of the fermented soymilk [63].

Preliminary evidences show that lactobacilli, bifidobacteria, and their metabolic products lower serum cholesterol level thus reduce the risk of cardiovascular diseases due to hypercholestremia. The LAB strains *B. longum*, *Lb. plantarum*, *Lb. paracasei*, *S. thermophilus,* and *Lb. delbreuckii* brought about significant lowering of the serum concentrations of total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides in rats and hamsters. In humans, a decrease in serum total cholesterol was observed as a result of the activity of probiotics [64, 65]. The hypocholesterolemic effects exerted may be because of removal of cholesterol through incorporation into the cellular membranes [66] of live or dead lactobacilli or by deconjugation of bile via bile salt hydrolase and coprecipitation of cholesterol with the deconjugated bile [67, 68]. Bile salts are prepared from cholesterol and stored as conjugated bile salts in gall bladder. The deconjugation of bile forms free bile salts that are less hydrophilic resulting in lower absorption in intestine and thus a reduced amount is excreted in feces. To maintain the physiological balance, lost bile has to be replaced by production of new bile and cause a reduction in the amount of cholesterol. Not all investigations support this outcome. More in vivo trials and experimental data have to be generated to confirm the cholesterol-reducing effect of probiotics.

6 Prebiotics and its significance

A prebiotic was originally defined as a “nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” [69]. A recent definition states that “prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the GI microbiota that confers benefits upon host well being and health” [70]. Prebiotics beneficially affect the host by stimulating the growth of intestinal bacteria especially *Bifidobacterium* and *Lactobacillus* and can enhance their viability after fermentation and during cold storage [71]. They escape digestion in the small intestine and reach the large intestine where they provide fermentable substrate for gut microbiota.

To be considered as prebiotic, some criteria have to be fulfilled by the food ingredient are (i) resistance to gastric acidity, to hydrolysis by mammalian enzymes, and to GI absorption; (ii) fermentation by intestinal microbiota; and (iii) selective stimulation of the growth and/or activity of those intestinal bacteria that contribute to health and well being [69, 70]. Many dietary fibers especially soluble fibers exhibit some prebiotic activity. They are of plant origin and found in natural food. Main prebiotic dietary sources include soybeans and other cereals, chicory root, unrefined grains, and oats. Common prebiotics in use include inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), soy oligosaccharides, xylooligosaccharides, pyrodextrins, isomaltooligosaccharides, and lactulose [72]. A range of new prebiotic compounds has been emerged that include pecticoligosaccharides, lactosucrose, the sugar alcohols, glucooligosaccharides, levans, resistant starch, xylosaccharides, and soy oligosaccharides [70, 73]. Of this, inulin-type prebiotics and galactooligosaccharides are common in use. Inulin-type fructans resist digestion in the upper part of GI tract because of the ß-configuration of the anomeric C-2 in their fructose monomers and can be called colonic food since they are not absorbed to any significant extent [18]. Oligodextran, oligoalternan, and FOS were checked for their ability to support the growth of *Bifidobacterium* and observed with their potential prebiotic properties [74, 75]. GOS have been successfully used to preserve *Lb. delbreuckii* in a freeze-dried form [76].

Some nondigestible carbohydrates facilitate mineral absorption in colon and transfers water into the large bowel increasing the fluid volume. FOS supplementation resulted in increased hepatic zinc and femoral magnesium levels and also boosted calcium absorption in mice [77]. Inulin-type fructans increase HDL cholesterol, iron absorption, and reduce enterobacteria in rats [78]. Mineral absorption role of prebiotics was noticed in humans also. Inulin, oligofructose, and transgalactooligosaccharides significantly increased calcium absorption and enhanced bone mineralization during pubertal growth in adolescents [79, 80] and postmenopausal women [81].

By combining the rationale of pro- and prebiotics, the concept of synbiotics has been proposed to characterize nutritional food with some health-enhancing activity called functional food. Synbiotics could beneficially affect the host by improving survival and implantation of live microbial dietary supplements in the GI biota. A mixture of 0.5% inulin and *Weissella cibaria* in the diet of *Pseudoplatystoma* hybrid surubins trimmed down the number of pathogenic bacteria and stimulated the beneficial intestinal microbiota [82]. Recent studies show the significance of prebiotics in reducing the risk of diseases. A specific mixture of neutral oligosaccharides and pectin-derived acidic oligosaccharides reduced the occurrence of early atopic dermatitis among low-atopy-risk infants [83]. Modulation of cholesterol metabolism is another important function of prebiotics. A synbiotic product containing *Lb. Acidophilus* CHO-220 and inulin reduced plasma total cholesterol and LDL cholesterol by 7.84% and 9.27%, respectively, thus lessen the risk of artherosclerosis [84]. The effect of a mixture of four probiotic bacterial strains along with GOS in preventing allergic disease was carried out in infants till 2 years of age from 2–4 weeks before delivery. Even though no effect was observed in all allergic diseases, the treatment significantly prevented eczema and especially atopic eczema [85].

7 Nutraceuticals from LAB and functional foods

The term “nutraceutical” was coined from “nutrition” and “pharmaceutical” in 1989 by Stephen DeFelice, and defined as, “a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease” [86, 87]. These products may range from dietary supplements, herbal products, functional foods (yoghurt, cereals, enriched foods) to genetically engineered “designer” foods [88]. The word “nutraceutical” in the food industry has no regulatory definition. The terms nutraceuticals, functional or medical foods, or dietary supplements are often used interchangeably. However, according to different perspectives these concepts can be distinguished, e.g. functional food is a term to emphasize foods that may have a beneficial effect on the health [89]. But when a functional food is associated with the prevention and/or treatment of disease(s) other than anemia, it is called a nutraceutical. In other terms, a functional food for one consumer can be a nutraceutical for the other [90].

Since nutraceuticals provide nutrition and health benefits, they can be considered as food. At the same time they can be used for the prevention, treatment, or cure of a disease and hence can be considered as drugs. But actually nutraceuticals occupy a gray area between food and drugs. Food is GRAS whereas nutraceuticals, even though they contain “natural” substances may not be GRAS. In order to be approved, a drug must demonstrate its safety and effectiveness. Nutraceuticals are not drugs simply because they have not gone through an approval process [91].

Table 4. Nutraceuticals produced by LAB and their health benefits.

Nutraceuticals	Health benefits
B vitamins	
Folate	Involved in nucleotide biosynthesis
	Prevents neural tube defects in newborns
Riboflavin	Prevents liver and skin disorders, disturbed metabolism of the red blood cells
Cobalamin	Prevents pernicious anemia
Low-calorie sugars	
Sorbitol	Acts as low-calorie sweetener and have anticancer properties
Mannitol	Acts as antioxidant and low-calorie sweetener
Tagatose	Acts as low-calorie sugar, prebiotic, and anti-plaque agent
Exopolysaccharides (EPS)	Increases flavor and texture of food and used as food additives

Nutraceuticals can be grouped into the following three broad categories: (i) Nutrients—substances with nutritional value such as vitamins, minerals, amino acids, and fatty acids; (ii) Herbal—plant extracts and concentrates; (iii) Dietary supplements—nutritional supplements derived from other sources (e.g. pyruvate, chondroitin sulphate, steroid hormone precursors) that can have specific functions, such as sports nutrition, weight-loss supplements, and meal replacements [92]. The health benefits of nutraceuticals are primarily in several areas including cancer, atherosclerosis and other cardiovascular disease (CVD), the aging process and immune response enhancing effect, diabetes, and mental health [93]. They also prevent or treat hypertension, hypercholesterolemia, obesity, arthritis, osteoporosis, macular degeneration (leading to irreversible blindness), cataracts, menopausal symptoms, insomnia, diminished memory and lack of concentration, digestive upsets and constipation, and headaches [94].

Besides lactic acid, LAB can also produce other compounds that contribute to the unique product characteristics such as flavor, texture, and nutrition. LAB produce various nutraceuticals such as B vitamins (mainly folate, riboflavin, and cobalamin), low-calorie sugars (mannitol, sorbitol, tagatose), L-alanine, EPS, etc. [95]. Dairy industry is rapidly evolving in the area of nutraceuticals. Bio yoghurts containing *Lb. acidophilus* and bifidobacteria and other specialist fermented products such as yakult (providing LcS), nestles LC1 (providing *Lb. johnsonii*), and the culturelle (providing *Lactobacillus* GG) are leaders in this sector [96]. Table 4 shows the important nutaceuticals reported from LAB and their health benefits.

The advancement in the field of metabolic engineering has contributed much in the nutraceutical production in LAB. The biosynthetic capacity, metabolic versatility, and relatively simple physiology of LAB make them suitable organisms for metabolic engineering. Modern metabolic engineering approaches mainly focus on more complex, biosynthetic pathways leading to nutraceuticals [97]. Various cloning systems, chromosome modification systems, and expression systems have been developed to generate GM-LAB [98]. The most popular transformation

Table 5. Successful metabolic engineering strategies employed in LAB.

Nutraceuticals	Host LAB strain	Modified genes	Expression system/vector	Reference
B vitamins				
Folate (Vitamin B9 or B11)	*L. lactis*	Folate gene cluster and pABA synthesis genes	NICE	[101, 104]
Riboflavin (Vitamin B2)	*L. lactis*	GTP cyclohydrolase II (*ribA*)	NICE	[95]
Cobalamin (Vitamin B12)	*P. freudenreichii*	ALA dehydratase (*hemB*) and uroporphyrinogen III methyltransferase (*cobA*)	pPK705 vector and *Propionibacterium* promoter	[105]
Low-calorie sugars				
Mannitol	*L. lactis*	Mannitol-1- phosphatase gene	NICE	[106]
Sorbitol	*Lb. plantarum*	sorbitol 6-phosphate dehydrogenase genes (*srlD1*, *srlD2*)	pGIZ90 vector	[107]
Exopolysaccharides	*L. lactis* NIZO9000	EPS gene cluster	NICE	[108]

L. = Lactococcus; Lb. = Lactobacillus; P. = Propionibacterium.

system is electroporation with self-replicating vectors. In addition to efficient cloning systems, suitable expression systems have been developed for the controlled expression of homologous and heterologous genes. Controlled constitutive expression is achieved by using a system of synthetic promoters [99], while a nisin-induced controlled expression system (NICE) allows the gradual over expression of genes [100]. Nisin is the only bacteriocin with GRAS status for use in specific foods and has a history of 25 years of safe use in many European countries. This was supported by the results of various studies proving its nontoxic and nonallergenic nature [11]. The NICE system has been successfully used in *L. lactis* for the overexpression of folate biosynthetic genes [101] and also for the overexpression of genes involved in riboflavin synthesis [95]. Metabolic engineering strategies resulting in the simultaneous overproduction of folate and riboflavin in *L. lactis* could eventually lead to "multivitamin LAB" [97]. Other systems are controlled by promoters based on sugar utilization, e.g. the lactose operon promoter [102].

The key challenge in finding an effective nutraceutical is its bioavailabilty or absorption rate. The bioavailability of these nutrients will be higher in foods in its natural state. Even unprocessed foods are not broken down and digested as effectively. Hence, nutraceuticals with poor absorption rates results in nutrients being eliminated from the body without providing any health benefit [103]. Studies have shown that by adapting suitable metabolic engineering strategies, it is possible to increase the bioavailability of certain nutraceuticals such as folate [101]. Future analysis of other complex pathways will provide us with valuable knowledge concerning the potential of LAB as a better producer of nutraceuticals. Some of the metabolic engineering landmarks resulted in better yield of selected nutraceuticals were listed in Table 5.

Nutraceutical industry is a rapidly evolving (7–12% per year) billion-dollar industry. The increased awareness toward health, nutrition, and lifestyle diseases among people resulted in an increased interest toward nutraceuticals. According to the report published by GBI research in 2010, the global nutraceuticals market was estimated to be worth $128.6 billion, after increasing at a compound annual growth rate (CAGR) of 4.4% during 2002–2010. Moreover, the market is predicted to reach $180.1 billion by 2017, after growing at a CAGR of 4.9% throughout 2010–2017 (http://www.marketresearch.com). Even if the total market size of nutraceuticals in India is very small compared to the global market, it is expected to be more than double in the next 4 years at over 95 billion INR.

A collective effort by health professionals, nutritionists, and regulatory toxicologists is necessary to provide the ultimate health and medicinal benefits of nutraceuticals to the mankind. Similar to drugs, there should be strict regulatory controls for nutraceuticals. Also the effect of different processing methods on the biological availability and effectiveness of nutraceuticals are to be evaluated [109].

8 LAB and biopreservation

Biopreservation is the use of natural or added microbiota and/or their antimicrobial products for extending the shelf life and enhanced food safety. LAB as biopreservation organisms are of particular interest since they greatly influence the nutritional, sensory, and shelf-life characteristics of fermented food products.

Fungal growth is one of the causes of spoilage in vegetables and baked foods causing significant reduction in their quality and quantity. In addition, allergenic fungal spores and mycotoxins can cause serious health problems [110, 111]. The frequently encountered mycotoxins in food systems come under six classes: aflatoxins, fumonisins, ochratoxins, patulin, trichothecenes, and zearalenone. These mycotoxins are reported to be carcinogenic, immunotoxic, teratogenic, neurotoxic, nephrotoxic, and hepatotoxic and hence the occurrence of these moulds in food is a major health concern and economic problem [12]. *Penicillium* and *Aspergillus* species have been reported as spoilage organisms during storage of wide range of food and feed and *Fusarium* species often contaminate cereal grains. Bacterial pathogens that account for food borne illness include *Salmonella*, *Campylobacter jejuni*, *E. coli* 0157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, and *C. botulinum* [112]. Physical and chemical methods have been developed to control the occurrence of these microorganisms and their toxins but some moulds have acquired resistance against some chemical treatments and preservatives. For example, some *Penicillium*, *Saccharomyces*, and *Zygosaccharomyces* can grow in the presence of potassium sorbate [113]. In addition, *P. roqueforti* isolates have been found to be resistant to benzoate and some other moulds possess the ability to degrade Q2 sorbate [114]. Some strains of the mould *P. discolor*, a species that causes spoilage of hard cheese were found to be natamycin resistant [24,115]. Yeasts, such as *Debaryomyces hansenii*, *C. versatilis,* and *Torulaspora delbrueckii*, have also shown strong resistance to chemical sanitizers and cleaning compounds in dairy environments [116]. Frequent use of antibiotics and preservatives may increase the resistance phenomena in the future. Instead of chemical preservatives or additives in food or feed, consumers want high-quality, preservative-free, safe, and mildly processed food with extended shelf life.

LAB produce some antagonistic compounds able to control the growth of pathogenic bacteria as well as unwanted spoilage fungi and offer effective means for food preservation and safety. Antimicrobial activities of LAB have been ascribed to the production of organic acids, bacteriocins, and also competition for nutrients. Organic acids with antifungal activity include 3-hydroxy fatty acids [117], phenyllacttic acid, and 4-hydroxy phenyllactic acid [118, 119], and a mixture of acetic, caproic, formic, propionic, butyric, and n-valeric acids, acting in a synergistic way [120]. Bread produced through sourdough fermentation with *Lb. plantarum* extended the shelf life under common storage conditions [121, 122]. Golden delicious apples and iceberg lettuce was bioprotected with direct inoculation of viable LAB strains by reducing the count of *S. typhimurium*, *E. coli*, and inhibiting *L. monocytogens* [123]. So far, nisin is the only bacteriocin licensed as a food preservative (E234). Many preliminary studies on the activity of bacteriocins in vitro or in food systems are carried out with partially purified preparations obtained from cultured broths [124]. Bacteriocin producing strains of *Lactobacillus*, *Enterococcus,* and *Pediococcus* have been effectively used for the preservation of refrigerated and vacuum-packed *Dicentrarchus labrax* [125], vacuum-packaged cold-smoked salmon [126], and chicken meat [127]. Enterocin AS-48 was found to reduce the number of *L. monocytogens* in artificially contaminated alfalfa and soybean sprouts as well as active against *Bacillus cereus* contamination in rice and vegetables [128, 129]. *Enterococcus raffinosus* PS99 and *Lb. reuteri* TA43 could lower counts of coliforms, *Pseudomonas* spp., proteolytic, and hemolytic bacteria in blood while preservation [130]. The long tradition of using LAB in food and feed along with recent scientific knowledge on encouraging health effects caused by probiotic LAB ingestion suggest them as potential alternatives to chemical preservatives.

9 Conclusions

LAB, one of the powerhouses of the food industry, is not only of major economic significance, but are also of value in maintaining and promoting human health. The increased health awareness among people and recent sophisticated studies support the role of LAB as probiotics. Basic knowledge on probiotic bacteria, their interactions with each other and the host are a prerequisite for future developments. For example, microencapsulation technology will lead to increased viability as well as controlled release of probiotic bacteria. Researchers are also now focusing on nanoencapsulation of probiotics that may offer further advantages. In addition, insight into the genetic blueprint of LAB offers more efficient tools to use genetic engineering strategies, which can result in improved strains with industrial relevance.

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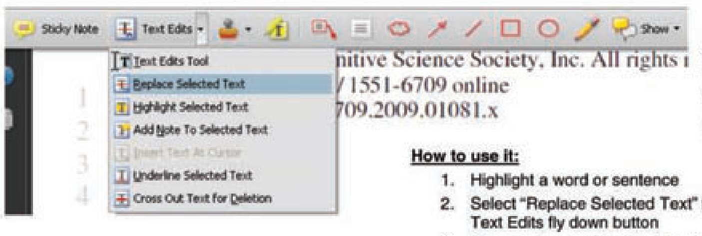

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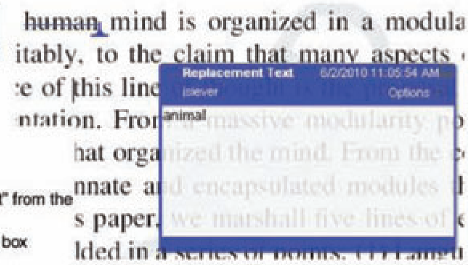

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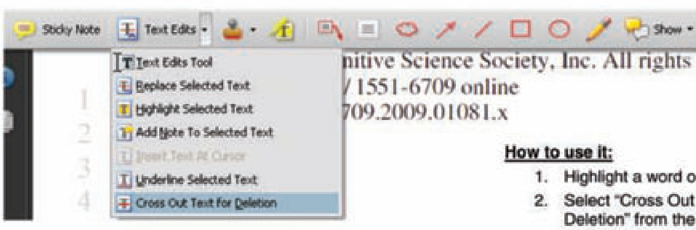

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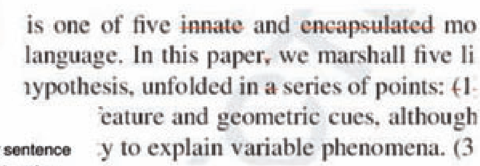

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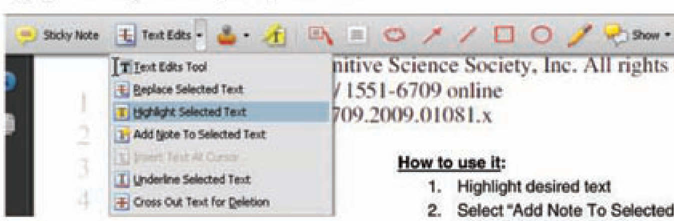

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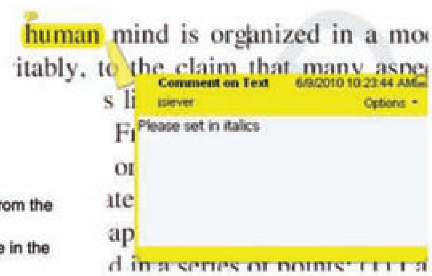

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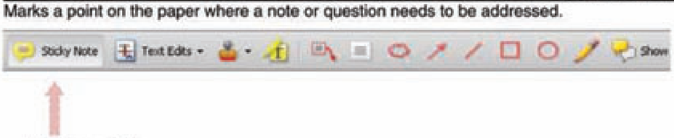

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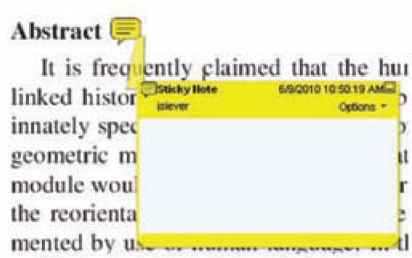

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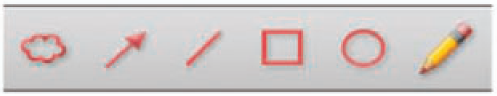

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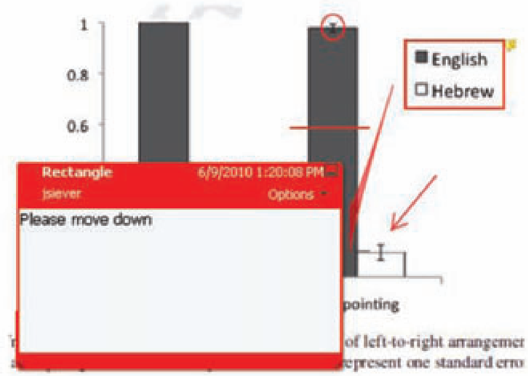

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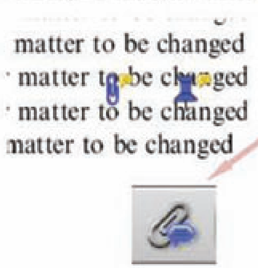

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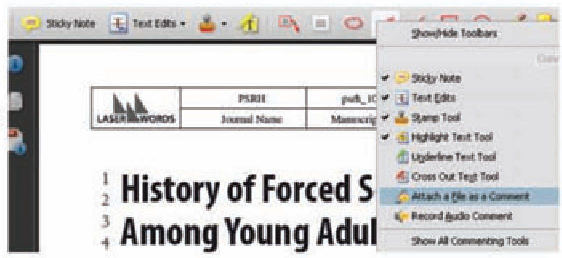

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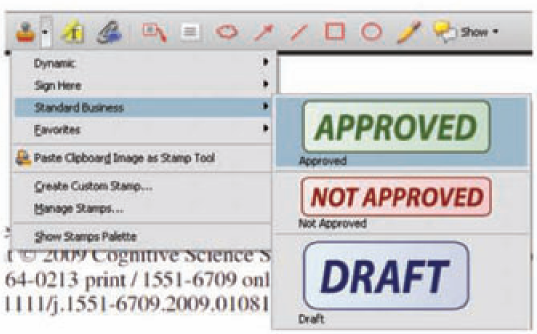

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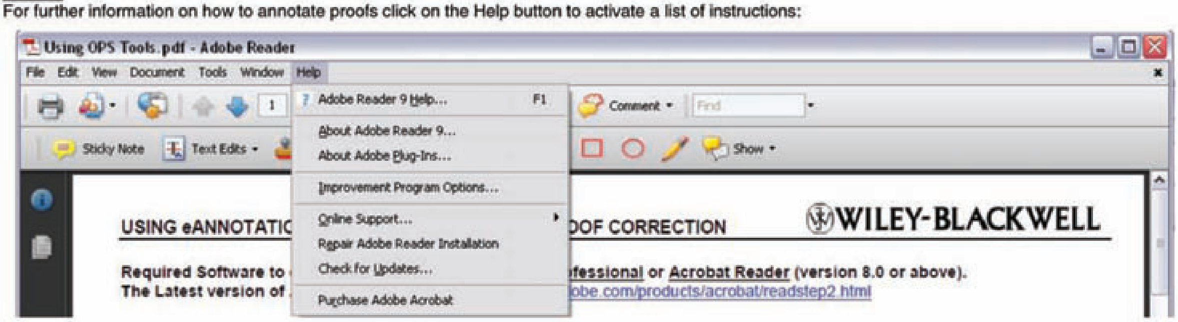

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