Microbiology Research Journal International

22(2): 1-14, 2017; Article no.MRJI.36935
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Food and Health Potentials of Exopolysaccharides Derived from *Lactobacilli*

Priyanka Singh[1] and Pinki Saini[1*]

[1]Centre of Food Technology, University of Allahabad, Allahabad, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author Priyanka Singh performed the analyses of the study, managed the literature searches and statistical analysis and wrote the first draft of the manuscript. Author Pinki Saini designed the study, wrote the protocols and prepared the manuscript. Both the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2017/36935
Editor(s):
(1) Lidija Kozacinski, Professor, Department of Hygiene, Technology and Food Safety, Faculty of Veterinary Medicine, University of Zagreb, Croatia.
Reviewers:
(1) Eliene Penha Rodrigues Pereira, Universidade Federal Fluminense, Brazil.
(2) S.Thenmozhi, Periyar university, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/22004

Review Article

Received 23rd September 2017
Accepted 6th November 2017
Published 21st November 2017

ABSTRACT

A biofilm is any group of microorganism in which cells stick to each other on a surface. While forming these biofilms microorganisms excrete out extracellular polymeric substances and form a film like structure around them. These extracellular polymeric substance are generally composed of extracellular DNA, proteins, and polysaccharides. The interest has been increased now in exploring valuable EPS due to its various industrial applications, and hence attention on EPS-producing biofilm-forming bacteria has also been greatly enhanced. The wide structural, physical and rheological diversity and other unique properties of EPS produced by biofilm-forming bacteria make it industrially and biotechnologically important. EPS has already been widely used as bioflocculants, bioabsorbents, encapsulating materials, heavy metal removing agents, drug delivery agents, ion exchange resins, and a natural immunomodulator. In addition, the distinct biophysicochemical properties of bacterial EPS proves its importance in the food industry as viscosifying, stabilizing, emulsifying, antioxidant and antibiofilm agents.

Keywords: EPS; biofilms; rheology; emulsifying; stabilizing.

**Corresponding author: E-mail: pspinki55@gmail.com;*

1. INTRODUCTION

Microorganisms are often studied as simple creatures which are characterized and identified as planktonic, single cells [1,2]. However, the study of development of microbes, in various environments has lead to the conclusion that in nature planktonic microbial growth rarely exists. Antonie van Leeuwenhoek identified the biofilms formed by microbial community on the tooth surface [1,3,4]. Heukelekian and Heller found that microbial activity and growth were increased, in marine by the presence of a surface onto which they could adhere [5]. It has been observed that the formation of surface-attached microbial communities, known as biofilms, serves as an excellent model system for the study of microbial development.

Bacterial biofilms are the microbial community that reside in a self-produced matrix composed of extracellular polymeric substances (EPS) [4,6]. EPS is mainly made up of polysaccharides, proteins, extracellular DNA and lipids [6]. Microbial polysaccharides or EPS play a major role in structural development of ‘biofilm’ while attaching to the solid-liquid interface. The EPS synthesized by microorganisms either remains attached to the cell surface or is excreted out in the medium. The polymers are responsible for virulence, as in case of plant and animal pathogen or even protects the cells against desiccation and bacteriophages [7]. The polysaccharides are biopolymers and are widely distributed among all organism like animals, plants, fungi and bacteria. They are responsible for various biological function such as storage of energy, cell wall structure and cellular communication etc. These macromolecules possess high molecular weights in the range of several million Daltons. The polysaccharides can be homopolymers or heteropolymers of neutral sugars (pentoses and hexoses) or anionic sugars (hexoses) [8].

Bacterial exopolysaccharides are now described for their structural variability containing a wide range of physicochemical functions. However, many of them are still unknown and only few of them are explored for the industrial application [9]. Until now three bacterial polymers (xanthan, gellan and curdlan) are employed in food industry as additives [10,11]. These commercially available polysaccharides are produced by *Xanthomonas campestris* and *Pseudomonas elodea*, respectively. Products containing these EPS are required to be labelled since these bacteria are plant pathogens. Moreover, lactobacilli are food-grade bacteria and are associated with many fermented foods; particularly milk based products such as curd, yoghurt, sour cream, cheese and buttermilk where they contribute to develop taste, flavour and shelf life of fermented foods [12]. They known as GRAS (generally recognized as safe) and their EPS could be easily utilized in foods in a juridical point of view.

2. LAB AS EPS PRODUCER

The EPS producing lactic acid bacteria belongs to the genera *Streptococcus, Lactobacillus, Lactococcus, Leuconostoc,* and *Pediococcus* and the best known species among them are *L. casei, L. acidophillus, L. brevis, L. curvatus, L. delbrueckii bulgaricus, L. helveticus, L. rhamnosus, L. plantarum, L. johnsonii,* etc. Some non starter LAB like *Bifidobacteria* also produces a considerable amount of EPS [8]. Based on the location in the cell, these bacteria produce intracellular and extracellular polysaccharides [13]. Exopolysaccharides produced by Lactic acid bacteria (LAB) posses an important property of improving the rheology, mouthfeel and texture of different dairy and non dairy products such as curd, yoghurt, breads etc. In case of fermented milk products a smooth and creamy texture of the products is required which is usually achieved by adding fat, sugars, protein or stabilizers (eg. Starch, Alginate, pectin, or gelatin). EPS can be healthy and cheap alternative for the same purpose. It also increases the time of retention of the milk products in the mouth and hence improves the perception of the taste [14]. EPS also remains in the gastrointestinal tract for longer time and increases the number of colonies of probiotic bacteria [15]. Now a days cereal-base fermented products are also considered beneficial because of their high nutritional value and the availability of both soluble and insoluble dietary fiber [16,17]. LAB producing 2-substituted (1,3)- β-glucan are used chiefly for non-dairy fermented food such as oats based fermented foods. EPS from LAB also have been claimed to posses therapeutic properties like, antitumor [18], anticancer, cholestrol lowering properties [19] and immunostimulatory activity [20,21].

3. CLASSIFICATION OF EPS PRODUCED BY LAB

On the basis of chemical composition and biosynthesis mechanisms, EPS synthesized by

LAB are classified into two distinct groups: homopolysaccharides (HoPS) and heteropolysaccharides (HePS) [22]. HoPS consist of repeated units of only one type of monosaccharide and have molecular weight range from $4x10^4$ to $6.0x\ 10^6$ Da (Figs. 1,2). HoPs produced by lactobacillus sp. only contain glucose and fructose as their monosaccharides and which are classified as D- glucopyranose (glucans) or D-fructopyranose (fructans) [8]. They have various degrees and types of chain length, branching and linking sites. They posses molecular masses like for reuteran (2.8×10^7 Da), levan (2×10^6 Da) or inulin-type fructan (10^7 Da) [23,24,25]. Glucans consisting of glucose polymer produced by different strains belonging to the genera *Lactobacillus, Leuconostoc* and *Streptococcus* and can be classified as α- and β-D-glucans. According to the linkages in the main chain, the α-glucans are subdivided into dextrans (α-1,6), mutans (α-1,3), glucans (α-1,2), reuterans (α-1,4) and alternans (α-1,3 and α-1,6). Dextran synthesized by *Leuconostoc mesenteroides* is the only HoPs which is widely used in industrial market. It has specific applications as gel filtration compound (Sephadex) and as a blood plasma substitutes (Dextran 70) [26,27] . The second most important HoPS is "mutan". This water insoluble polymer is produced by *Lactobacillus reuteri*, and responsible for the adhesion of the producing bacteria, on teeth surface. Reuteren is a soluble compound and comprise of 70% α-1,4 linkages. It is used in baking industry in association with levan produced by *Lactobacillus reuteri*, and *L. sanfranciscensis*. These polysaccharides improves bread flavor, texture and shelf life [28].

Based on the structure they posses, fructans can be catagorised into two groups: (i) inulins (linked β-2,1) and (ii) levans (linked β-2,6), both are synthesized by different species of the genera *Leuconostoc*, *Lactobacillus*, *Streptococcus* and *Weissella.* Inulin-type fructans generally contains β-(2,6) or β-(2,1) linkages when they are excreted by *L. reuteri* 121. They are linear when *L. johnsonii NCC 533* is the producer [29]. Levan consists of fructose as sole monosaccharide linked by β-(2,6) glycosidic bonds.

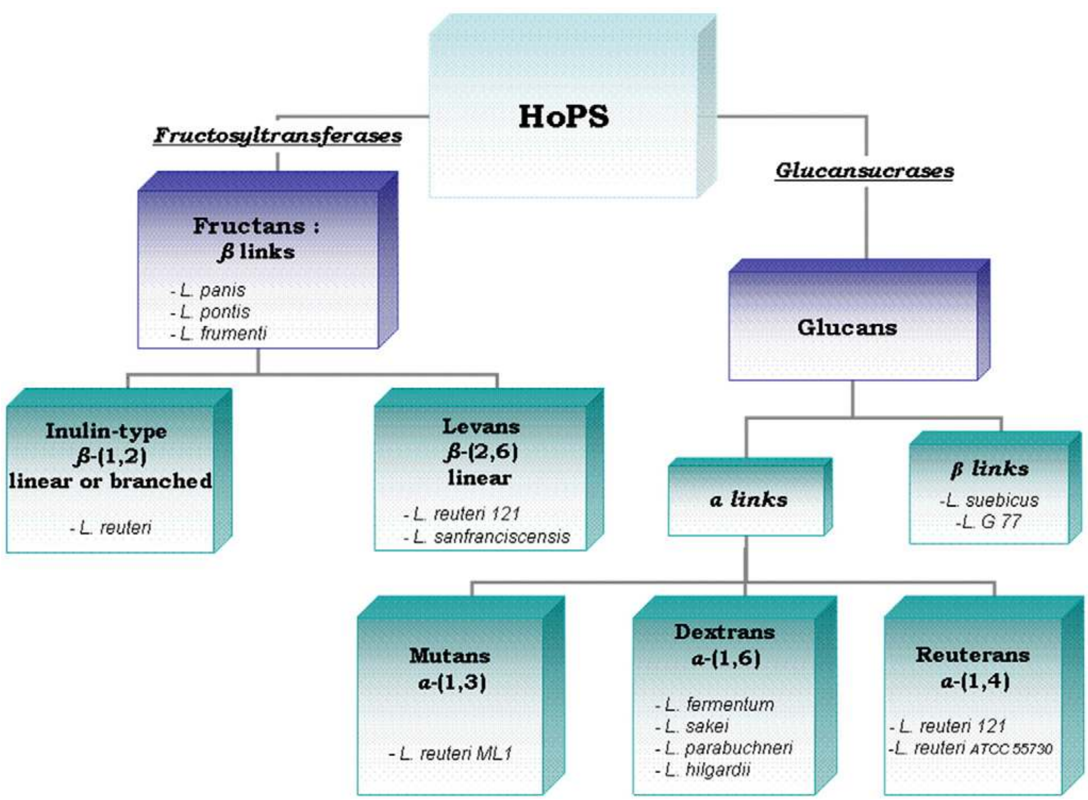

Fig. 1. Classification of homopolysaccharides produced by *Lactobacillus sp.* (Source: Badel et al. 2011)

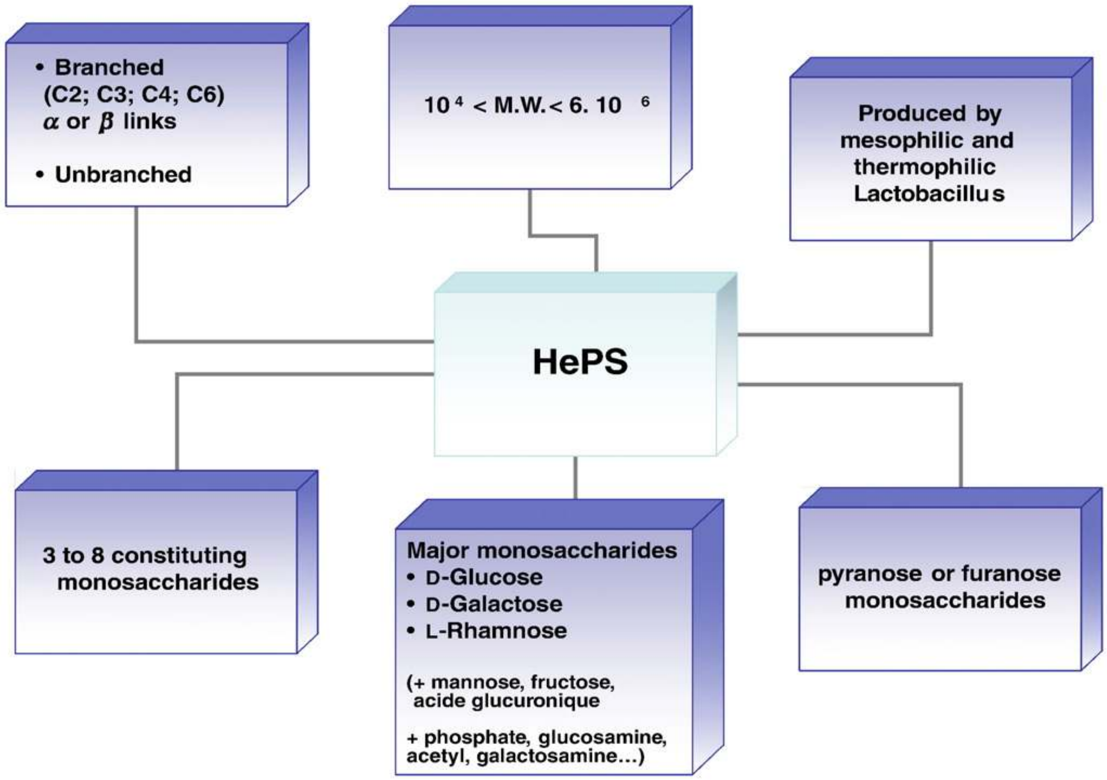

Fig. 2. Characteristics of heteropolysaccharides produced by *Lactobacillus sp.* (Source: Badel et al. 2011)

HePS are constructed by repeated subunits that of variable molecular masses 10^4 Da *(L. plantarum)* to 6×10^6 Da *(L. sakei 0-1*) [30,31] and can be linear or branched. These subunits can contain upto three to eight different monosaccharides linked with a range of different patterns. The monosaccharides are found as the α- or β-anomer in the pyranose or furanose form and D-glucose, D-galactose and L-rhamnose occur most frequently. In some cases, N-acetylglucosamine, glucuronic acid, manose, fucose, and noncarbohydrate substituents (phosphate, acetyl and glycerol) are also present [32,33]. Different species of LAB associated with dairy products, cereals and alcoholic beverages excretes HePS. These belong to the genera *Lactococcus* (*L. lactis* subsp. *cremoris-lactis*, *Lactobacillus* (*Lb. delbrueckii* subsp. *bulgaricus, Lb. acidophilus, Lb. casei, Lb. sakei,Lb. rhamnosus, Lb. helveticus*), *Streptococcus* (*S. thermophilus, S. macedonicus*) and *Leuconostoc* (*Lc. mesenteroides*) [34-36]. EPS from *Lactobacillus spp. (L. rhamnosus, L. delbrueckii bulgaricus and L. helveticus)* contains repeating units composed of seven monosaccharides, where glucose, galactose and rhamnose are the main sugars. HePSs are found to be partially anionic due to the presence of glucuronic acid and phosphate in their structure [8].

4. APPLICATIONS OF EPS

In the recent years the interest in exploring EPS produced by microorganism has increased focusing the attention on various industrial applications of biofilms. There is a vast scope of implication of EPS of microorganisms in food, pharmaceutical and biotechnology field due to their structural diversity, physical and rheological properties.

4.1 Improves Product Rheology

EPS may, improve the rheology of a final product by acting as a texturizer as well as physical stabilizers by binding hydration water and interacting with other milk constituents (ions and proteins) to avoid syneresis. Rheology constitute two major characteristics viz. viscosity and elasticity. Viscosity is the property of material to resist deformation and elasticity is the property to recover after the deformation occurred. Both the properties are important for organoleptic quality, its appealing appearance and pleasant mouthfeel. The physical and rheological properties are based on the chemical composition, molecular size, charge, presence of side chains, rigidity of the molecules and 3D-structures of the EPS polymers. The interactions between EPS and various components in food products are also responsible for development of the final product. In many studies it has been found that rheological properties of fermented milk products does not depend on the amount of EPS content [14,37,38]. The interaction among charged polysaccharides and proteins were studied by [39]. Neutral EPS increases the viscosity of a product with time to about 10 times higher than the viscosity of a control product produced with a non-EPS producing strain. But the viscosity of the product with the charged EPS was found comparable to the control product. The negatively charged polysaccharide by contrast, tends to increase the storage modulus G or elasticity of the product. Hence it is concluded that linear neutral EPS contributes to the viscosity of the product but not to the elasticity as it weakly interacts with the positively charged proteins molecules but dissolves in the serum phase uniformly. On the other hand, negatively charged polysaccharides contributes to the elasticity, but not to the viscosity of the product, due to their interaction with the positively charged casein molecules via electrostatic interactions, increasing the strength of the network and consequently increasing modulus of elasticity. They do not contribute to the viscosity as they are poorly dispersed in the serum phase. Since LAB produces polysaccharides of different charges and composition they apparently contribute to the viscosity and elasticity of the fermented product [14].

4.2 Texturing Agent

The most important textural property of dairy and non dairy fermented products in their firmness and water holding capacity These characters are related to the gel structure and are influenced by the type of culture. The EPS producing strains reduce the firmness and cohesiveness of yoghurt as the amount of EPS increases [40,41]. The EPS could interfere with the association between casein micelles resulting in a less firm coagulum. Studies of yoghurt microstructure [42,43] show void spaces around EPS producing bacteria in confocal scanning laser micrographies that can affect the integrity of the protein matrix. Yoghurts made with ropy cultures exhibited the highest water holding capacity [40] which decrease the susceptibility to syneresis. Similarly in case of bakery product when polymers produced from *lactobacilli* like levan, dextran or reuteran are externally added or formed in situ in the dough is responsible for increased water absorption of the dough, improved bread quality [28].

5. PRODUCT EXAMPLES

5.1 Curd

Curd is a popular fermented milk product of India having 3.5-8% fat content [44]. Now a days health awareness among consumer has made them to demand for low fat low calorie and fat free dairy products. However milk fat is responsible for flavour, texture and body development of the product and removal of it leads to the lack of flavour, body, loss of texture and other flavour defects in the product. In this context EPS producing strains of *Lactobacilli* act as “biothickners” and help to reduce total solids in the product without effecting the sensory attributes. Low calorie dahi prepared with different EPS producing strains of *L. lactis subsp.PM 23, L. lactis NCDC191*was found to be improved in terms of body, texture and flavour compared to dahi made with EPS negative culture [45]. Dahi made with EPS producing strain was found to have more porous and open structure with discontinuous casein matrix than the controlled dahi on the basis of microstructural studies, and hence showed increased water holding capacity [46].

5.2 Cheese

In the production and maturation of semi soft cheeses (e.g. Gouda), pressed cheeses (e.g. low fat Cheddar cheeses) and blue-veined cheeses (e.g. Roquefort), the development of *Lactobacillus sp*. was studied. EPS excreted by *lactobacilli strains* such as, *L. helveticus, L. delbrueckii bulgaricus* and *L.casei* increases water retention and improved the overall texture of cheese [47]. To overcome the functional defects manufacturers have used texture promoting or ropy cultures for low fat cheese production for many years in case of prohibition of stabilizers. The carbohydrate produced by these ropy strains imparts higher flavour intensity in the fermented milk, mouth feel and other attributes are also affected by them. In a study, viscosity of skim milk gel prepared by two ropy strains of *Lc. Lactis* subsp. *Lactis* was enhanced in comparison to that made by non-ropy cultures [45,48]. Perry et al. [49] studied that moisture increases on adding EPS-producing starter cultures in the preparation of low fat mozzarella. This water retention capacity is responsible for the texture improvement of cheese and permits reduction of calories in the final product.

5.3 Yoghurt

Yogurt is a fermented milk by starter cultures of *L. delbrueckii bulgaricus* and *S. thermophilus* in ratio 1:1. Both the bacteria are EPS producers. *Streptococcus thermophillus* produces EPS in the range of 30 to 890 mg/L and *Lactobacillus bulgaricus* in the range of 60 to 150 mg/L [50]. Polysaccharides obtained from EPS producing strains are claimed to reduce the amount of added milk solids, to improve the viscosity, to enhance texture and mouth feel and to avoid syneresis during fermentation or upon storage of yoghurt. However, EPS does not have any taste of their own but it improves texture perception by consumers in the final product [51]. It also avoids the need of stabilizers, which are forbidden especially in many countries. Yogurt fermented with EPS producing cultures has higher water holding capacity, which reduces the product's susceptibility to syneresis [52,53]. A study has shown that partial or total replacement of *L. delbrueckii* subsp. *bulgaricus* with EPS-producing *L. rhamnosus JAAS8* resulted in significant increases of about 16% and 21%, respectively, in apparent viscosity and an increase of about 2% in the WHC of the fermented products [54]. Studies done to elucidate the role of EPS in the texture of yoghurt has revealed that, the viscosity values were found higher in presence of EPS-producing strains rather than non EPS microorganism [50,53].

Other results have also shown the contribution of polysaccharide with high molecular mass and a stiff conformation in order to obtain an increase in viscosity [37]. However, no simple correlation has been found between viscosity and quantity of EPS produced. [55] have found a weak relation between texture and EPS concentration produced by *S. thermophilus* at different temperatures. Hence literature has shown different conclusion regarding EPS concentration and product rheology but authors are agreed with the fact, that interactions with caseins according to pH value and conformation of EPS are the key point of texture improvement. Application of pure EPS in the yoghurt has not been yet studied and at present, conclusions concerning their role as texturing agent are not very definite.

5.4 Kefir

Kefir is a self carbonated, slightly alcoholic, yoghurt like fermented milk product from Eastern European countries. It is prepared by Kefir grains which is an aggregation of microorganisms such as Lactobacillus sp. (*L. acidophilus, L. kefirgranum, L. kefir, and L. parakefir*) *Candida kefir, Saccharomyces sp., Acetobacter sp.*, etc. embedded in a polysaccharide matrix called Kefiran. Kefiran, a slimy polysaccharide is produced by *L. kefiranofaciens* in the centre of grain under anaerobic conditions is found to affect texture of kefir and act as natural viscosifying agent. Proportion of kefiran in grains is about 45% and it is composed of D-Glc and D-Gal in ratio 1:1 [56]. This biopolymer has a branched hexa- or heptasaccharidic unit. One or two residues are branched at the main chain composed of 5 monosaccharides. Rimada and Abraham, performed comparisons of rheological behaviour of skim-milk gels with and without kefiran. The mixture with kefiran offers viscosity and viscoelasticity improved up to 300 mg/L of polysaccharide. It was concluded that, this natural polysaccharide might be employed as an alternative thickening agent in dairy products [57].

5.5 Dough and Bread

Many patents have been found to claim that addition of plant polysaccharides like fructan or fructo oligosaccharides improves the rheological and textural properties of wheat dough and increases the shelf life of bread [58,59]. The study on addition of reutaran, dextran and levan from *lactobacilli* in baking application has proved that EPS effectively improves the rheological properties of dough and bread quality [60,28]. Dextran was found to be more effective in improving the viscoelastic property of dough on addition of the same quantities of reutaran and levan in the flour [28]. Formation of in situ EPS was found to be more effective than addition of Levan in the flour as it produces more metabolites (mannitol and glucose etc.) which contributes to the improved bread quality [61]. Similarly, the EPS produced by strains of *Weissella* in sourdoughs fermentation have improved the textural properties and quality of bread [62,63]. Polysaccharides produced from *lactobacilli* have now proved to beneficially affect one or more of the following technological properties of dough and bread such as water absorption of the dough, dough rheology and machinability, dough stability during frozen storage, loaf volume and bread staling.

5.6 Idli

Addition of Xanthan at a concentration of 0.1% to the idli batter has improved the textural properties resulting in sensory analysis scores that are higher compared to idli without additives [64]. The defect where idli batters start collapsing and whey is separated that leaves idli with a hard, unwanted texture is caused by prolonged fermentation and storage. Addition of different hydrocolloids to the batter may help to increase the viscosity of batter and hence improve the texture of the final product [65]. The addition of agar and guar gum was found to acceptable from a sensory point of view with the right mouth feel and texture of idli. The EPS produced by starter cultures in situ may be used to provide natural stabilizing agents. EPS formed by these microorganism in the batter can work as natural thickening agents, increasing the viscosity of the product and reducing syneresis) thus giving an improved product without using any additives.

6. EPS AS FOOD ADDITIVES

EPS produced by microorganisms mainly lactobacillus species posses GRAS status and are allowed to be incorporated in food without labeling. EPS impart highly desirable rheological properties to the food matrix like increased viscosity, improved texture and reduced syneresis that proves them as food additives. Dextran, a polysaccharide produced by *Leuconostoc,* Streptococcus and *Lactobacillus* species is the first industrial polysaccharide which is used in confectionary to improve viscosity, moisture retention and inhibit sugar crystallization. It also act as gelling agent in gum and candies and can be used as crystallization inhibitor in ice-creams. It provides viscosity and mouthfeel to the pudding mixes. It is a gel and hence can be used as molecular sieve for purification and separation of macromolecules such as proteins, nucleic acids and polysaccharides. It can be safely consumed hence used in clinical research and medical application as blood plasma extenders and also be used as a stationary phase in many chromatographic techniques. Xanthan is the second most important commercially available polysaccharides produced by *Xanthomonas compestris* and it was approved as food additive in 1969. It is important in both food and non- food applications as in dairy products, drinks, confectionary, dressing, bakery products, syrups and pet foods as well as the cosmetic, pharmaceutical, paper, paint and textile industry . Due to its highly pseudoplastic and suspending properties it possess large industrial importance as suspending and emulsifying agent. Gellan is a multifunctional gelling agent found from a non pathogenic bacteria *Sphingomonas paucinibilis*. Due to its native nature of forming elastic gel in the solution, it is used as a gelling agent for solidifying culture media mainly for studying marine organism. It is commercially available as Gelrite (a substitute of agar). In food applications it is used as stabilizer and suspending agent. Kelcogel ® F and kelcogel® LT100 are food grade gellan.

Curdlan obtained from *Rhizobium meliloti* and *Agrobacterium radiobacter* has a unique ability to form an elastic gel, when its aqueous solution is heated above 55^0C making it used as gelling agent and immobilization matrix. Curdlan along with Zidovudine (AZT) shows a high antiretroviral activity (anti AIDS-drug) and hence having promising role in pharmaceuticals.

7. HEALTH BENEFITS

EPS has found to posses many health benefits and exert beneficial effect on gut, blood ,stomach and immunsystem. It could be used as natural immunomodulator, drug delivery agents, ion exchange resins, antioxidant and antibiofilm agents, antitumor and anticarcinogenic agents (Fig. 3).

7.1 EPS as Prebiotics

The prebiotics are defined as “non-digestible 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” [66]. Non-digestible oligosaccharides (NDO) are intermediate in nature between simple sugars and polysaccharides and are claimed to behave as dietary fibres and prebiotics. They are having a configuration that makes their osidic bonds non-digestible by the hydrolytic activity of the human digestive enzymes [67]. They have functional effects similar to soluble dietary fiber such as enhancement of a healthy gastrointestinal tract, improvement of glucose control, and modulation of the metabolism of triglycerides. These compounds are easily incorporated into processed foods and hold much promise as functional ingredients in nutraceutical products. As polysaccharides are the main source of bioactive oligosaccharides and hence there is always a requirement of continuously investigating their new sources. In this regard LAB have become the most promising because of its GRAS status. A fructan-type EPS was found to be produced by strain of *L. sanfranciscensis* which has proven the possibility of LAB having a prebiotic property [61]. The strain of *L. reuteri 121* was also studied for abundant production of β-(2,1) fructans (inulin like polysaccharide) [25]. The linear type of Fructan was found to be produced by *L. johnsonii* NCC 533 [29]. There are also evidence of production of levan-type EPS produced by another strain of of *L. sanfranciscensis and L. reuteri* [68]. The EPS synthesized by intestinal *Bifidobacteria also* act as fermentable substrates for microorganisms in the human gut environment and improving the intestinal populations [69].

7.2 Inhibit Pathogenic Biofilm Formation

Lactic acid bacteria (LAB) like *Lactobacillus acidophilus* exhibit functions which are of importance to the health and immune system of the host cells [70]. It is well known that these bacteria prove to be effective tools for controlling the growth of pathogens and hence controlling or preventing infections [70,71]. Extra polysaccharides (EPS), is the primary metabolic products of LAB, and have received a large amount of attention in past few years as it have been attributed to positive health effects [70]. However, the functional role that EPS plays in bacterial ecology still remains uncertain. A recent study reported that different EPS isolated from commercial fermented milk ''villi" were capable of inhibiting the adhesion of several enteric pathogens [72]. Fracchia et al. [73] isolated *lactobacillus spp* from fresh fruits and vegetables and tested for inhibition of pathogenic biofilm produced by *Candida albicans* and found to reduce the biofilm formation by 82%. Similarly, EPS of several other microbes have shown antibiofilm activity against different pathogens. Bendaoud et al. [74] have observed potent antibiofilm activity by the cell free extract prepared from gram negative oral bacteria of *Kingella kingae*. Its biofilm were found to inhibit biofilm formation by *Aggregatibacter actinomycetemcomitans, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Candida albicans.* The anti biofilm activity of EPS isolated from *O. iheyensis* was checked on multidrug resistant clinical isolate of *S. aureus*. Biofilm formation in *S. aureus* was decreased by 62.3% [75].

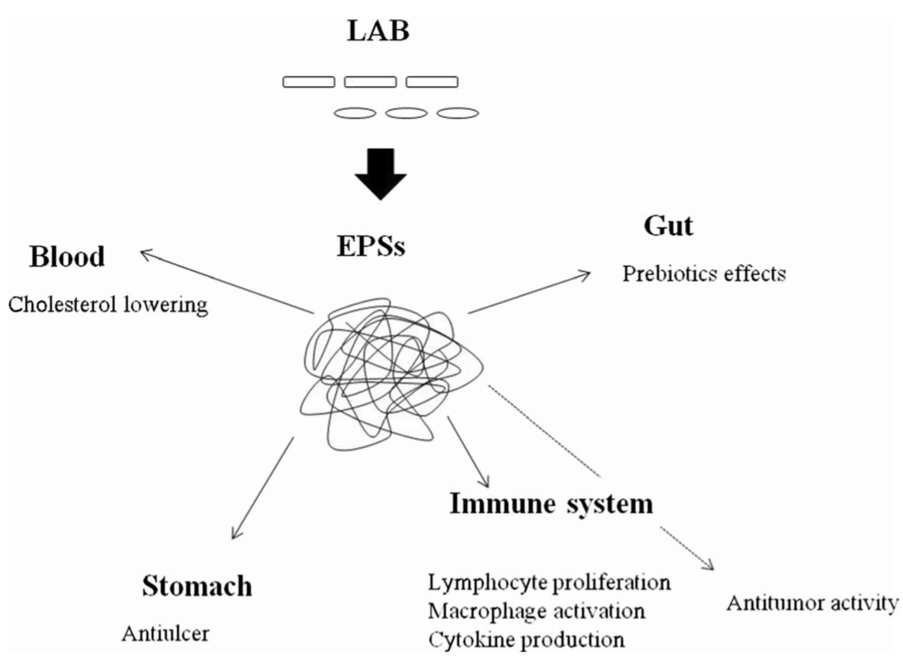

Fig. 3. Schematic representation of the possible health-promoting properties of LAB EPS

7.3 Anticarcenogenic and Antitumor Activity

Haroun et al. have observed the various degree of activity of EPS isolated from prebiotic *L. plantarum NRRLB-4496* against human tumor cell lines [76]. They checked the inhibitory effect of different concentration of EPS against the seven cell lines namely CACO (intestinal carcinoma cell line), HELA (cervical carcinoma cell line), HEPG2 (liver carcinoma cell line), HEP2 (larynx carcinoma cell line), MCF7 (breast carcinoma cell lines) and HFB4 (normal melanocytes) and found that EPS inhibited the proliferation of six cell lines. Ogawa et al. have observed EPS of L casei significantly increased the natural killer cell's (NK cells) cytotoxicity in spleen cells of test mice [77]. Similarly, Kitazawa et al. showed the EPS forming *Lc. Lactis ssp. cremori*s had strong antitumor activity [78]. Forsen et al. [79] showed that the lipoteichoic acids, found on the cell surface of *Lc. Lactis* ssp. *cremoris* T5 produced T-cell mitosis in human lymphocytes. Some other studies also showed immunomodulating, and antitumor activities of EPS [80]. The immunomodifying effects were shown on mouse splennocytes by the slime produced by *B. adolescentis* [81].

7.4 Antiulcer /Antigastritis Properties

EPS isolated from *Streptococcus thermophillus* strains were investigated for immunostimulatory /antiulcer effects in the host cells of mice and found to treat the stomach ulcer caused by antiinflammatery/analgesic drugs [80]. Similarly Nagaoka et al. [82] have observed the antiulcer effect of the polysaccharide fraction of *Bifidobacterium, Lactobacilli* and *Streptococci* against the acetic acid induced gastric ulcer and ethanol induced erosion models in rats. The result indicated that the polysaccharides induce host repair and protective system in the gastric ulcer model.

7.5 Cholestrol Lowering Property

Nakajima et al. [83] has reported that the milk fermented by EPS producing strain of *Lc. Lactis ssp.cremoris* has a property of lowering blood cholesterol. Similarly Soh et al. [84] has shown cholesterol adsorption property of microbial polysaccharides, through in vitro studies by enzymatic reaction and polysaccharide precipitation procedure. They observed the adsorption capacities of different polysaccharides at different concentration and found that zooglan was able to absorb the whole cholesterol (3mg/dL) at a concentration of 0.2% (wt/vol) in distilled water. Tok et al. [85] has observed that out of 5 strains of *L. delbrueckii* the highest EPS producing three strains have higher ability of removing cholesterol from the medium.

7.6 Immunomodulating Effect

Substances effecting the immune system are called as immunomodulator. This system induces or inhibit the immune response by the use of different ways like immunosuppressors or immunostimulants. These substances are used as food additive to suppress infections, to prevent digestive tract cancers or to treat sicknesses due to immunodeficiency, such as inflammatory bowel diseases (Crohn's disease and ulcerative colitis). The immunomodulators alter the activity of immune function by the dynamic regulation of informational molecules such as cytokines [86]. An important way of modulating the immune system is to modulate the cytokine expression through the use of herbal medicines. Some of the biopolymers synthesized by LAB have the potential to be used as immunomodulating food additives. β-glucans are able to activate the immune system hence known as "biological response modifiers" [87]. β-glucans are integral cell wall components of a variety of fungi, plants and bacteria. It has been found that LAB belonging to the *Lactobacillus* and *Pediococcus* genera producing 2-substituted (1,3) β-glucan are able for immunomodulating the macrophages *in vitro* [88,89]. Purified biopolymer treatment of macrophages has also increased the secretion of antiinflamatory IL-10 cytokine [88]. β-glucan producing strains have also found to be resistant against gastrointestinal stress. EPS producing species have also showed increased capability to adhere to Caco-2 human epithelial intestinal cells. Hence 2-substituted (1,3) β-glucan can be used as food additives to act as immunomodulator and alleviating inflammatory bowel diseases. Dextrans have also been proved as a good immunomodulator. Many studies on dextran-70 have shown its contribution in the prevention of acute respiratory distress syndrome after trauma and sepsis as well as pancreatitis [90]. The α- glucan was able to reduce the systemic inflammatory response and the release of the cardiac troponin-I after cardiac operation [91]. An α-glucan found from the edible mushroom *Tricholoma matsutake* has been reported to have excellent biological activities; exerting modulating effects on the immune competence of mice and rats. Therefore, the high production of α-glucans by LAB and the immunomodulatory properties of these bio-molecules as described above, predict that in the near future studies will be performed to evaluate the beneficial properties of these EPS, with the aim to use them as food additives.

8. CONCLUSION

However, it is well known that the lactic acid bacteria are very beneficial for human health and also exist in clusters or multicellular community attached to a number of different surfaces in nature but still there is a need of exploring interacting behavior among them in the complex systems in order to understand their actions and nature of resulting metabolites excreated by them. The extra polysaccharides produced by them have been found to have divergent properties which may be incorporated in different food, medical, cosmetics and pharmaceutical products. But production cost is found to be the biggest constraints for application at industrial scale. Hence the search for a high EPS producing variety and the optimization of the fermenting condition is very necessary. Overall, there should be a rapid expansion in the development of novel LAB probiotic organisms and their prebiotic EPS products, though their ultimate success in the market place will require rigorous scientific evaluations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Donlan RM. Biofilms: Microbial life on surfaces. Emerging Infect. Dis. 2002;8: 881-890.
2. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. Annu. Rev. Microbiol. 1995;49:711-745.
3. Socransky SS, Haffajee AD. Dental biofilms: Difficult therapeutic targets. Periodontol. 2000;2008(28):12–55.
4. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistant infection. Microbes, Immunity and Disease. 1999;284:1318-1322.
5. Heukelekian H, Heller A. Relation between food concentration and surface for bacterial growth. J Bacteriol. 1940;40:547–58.
6. Flemming HC, Wingender J. Relevance of microbial extracellular polymeric sub-stances (EPSs)– Part II: Technical aspects. Water Sci. Technol. 2001;43:9-16.
7. Sutherland IW. Novel and established applications of microbial polysaccharides. Trends Biotechnol. 1998;16:41-46.
8. Badel S, Bernardi T, Michaud P. New perspectives for *Lactobacilli* exopolysaccharides. Biotechnol Adv. 2010;29:54-66.
9. Suresh Kumar A, Mody K, Jha B. Bacterial exopolysaccharides—a perception. J Basic Microbiol. 2007;47:103–17.
10. Sutherland IW. Microbial polysaccharides from Gram negative bacteria. Int Dairy J. 2001;11:663–74.
11. Sutherland IW. Bacterial exopolysaccharides. In: Kamerling JP, Editor. Comprehensive Glycoscience. Oxford: Elsevier. 2007;2:521–57.
12. Shah N, Prajapati JB. Effect of carbon dioxide on sensory attributes, physico-chemical parameters and viability of probiotic *L. helveticus* MTCC 5463 in fermented milk. J Food Sci Technol; 2013.
13. Degeest B, Janssens B, De Vuyst L. Exopolysaccharide (EPS) biosynthesis by *Lactobacillus sakei* 0-1: Production kinetics, enzyme activities and EPS yields. J Appl Microbiol. 2001;91:470–417.
14. Duboc P, Mollet B. Applications of exopolysaccharides in the dairy industry. Int. Dairy J. 2001;11:759–768.
15. German B, Schiffrin E, Reniero R, Mollet B, Pfeifer A, Neeser JR. The development of functional foods: Lessons from the gut. Trends in Biotechnology. 1999;17:492–499.
16. Angelov A, Gotcheva V, Hristozova T, Gargova S. Application of pure mixed probiotic lactic acid bacteria and yeast cultures for oat fermentation. Journal of the Science of Food and Agriculture. 2005;85(12):2134-2141.
17. Martensson O, Biörklund M, Lambo AM, Dueñas-Chasco M, Irastorza A, Holst O, Norin E, Welling G, Öste R, Önning G. Fermented ropy, oat-based products reduce cholesterol levels and stimulate the bifidobacteria flora in humans. Nutrition Research. 2005;25:429-442.
18. Kitazawa H, Harata T, Uemura J, Saito T, Kaneko T, Itoh T. Phosphate group requirement for mitogenic activation of lymphocytes by an extracellular

phosphopolysaccharide from *Lactobacillus delbrueckii spp bulgaricus*. Int J Food Microbiol. 1998;40:169-175.
19. Nakajima H, et al. Cholesterol lowering activity of ropy fermented milk. J. Food Sci. 1992;57:1327–1329.
20. Chabot S, Yu HL, De Leseleuc L, Cloutier D, van Calsteren MR. Exopolysaccharides from *Lactobacillus rhamnosus* RW-9595M stimulate TNF, IL-6 and IL-12 in human and mouse cultured immunocompetent cells, and IFN- γ in mouse splenocytes. Lait. 2001;81:683-697.
21. Hosono A, Lee J, Ametani A, Natsume M, Hirayama M, Adachi T, Kaminogawa S. Characterization of a watersoluble polysaccharide fraction with immuno-potentiating activity from Bifidobacterium adolescentis M101-4. Bioscience. Biotechnology and Biochemistry. 1997;61: 312–316.
22. Sutherland IW. Bacterial exopolysaccharides. Adv Microb Physiol. 1972;8:143–213.
23. Kralj S, Stripling E, Sanders P, van Geel-Schutten GH, Dijkhuizen L. Highly hydrolytic reuteransucrase from probiotic Lactobacillus reuteri Strain ATCC 55730. Appl Environ Microbiol.2005;71:3942–50.
24. Van Hijum AFT, van Geel-Schutten GH, Rahaoui H, van der Maarel MJEC, Dijkhuizen L. Characterization of a novel fructosyltransferase from Lactobacillus reuteri that synthesizes high molecular weight inulin and inulin oligosacchrides. Appl Environ Microbiol 2002;68:4390–8.
25. Van Hijum AFT, Szalowska E, van der Maarel MJEC, Dijkhuizen L. Biochemical and molecular characterization of a levansucrase from Lactobacillus reuteri. Microbiology. 2004;150:621–30.
26. Leathers TD. In: Steinbüchel A, editor. Dextran. Biopolymers Weinheim: Wiley VCH. 2001;299–321.
27. Monsan P, Bozonnet S, Albenne C, Joucla G, Willemot RM, Remaud Simeon M. Homopolysaccharides from lactic acid bacteria. Int Dairy J. 2001;11:675–85.
28. Tieking M, Ehrmann MA, Vogel RF, Ga¨nzle MG. Molecular and functional characterization of a levansucrase from the sourdough isolate *Lactobacillus sanfranciscensis* TMW 1.392. Applied Microbiology and Technology; 2005.
29. Anwar MA, Kralj S, van der Maarel MJEC, Dijkuizen L. The probiotic *Lactobacillus johnsonii* NCC 533 produces high molecular mass inulin from sucrose by using an inulosucrase enzyme. Appl Environ Microbiol. 2008;74:3426–33.
30. Robijn GW, van den Berg DJC, Haas H, Kamerling JP, Vliegenthart JFG. Determination of the structure of exopolysaccharides produced by *Lactobacillus sakei* 0-1. Carbohydr Res. 1995;276:117–36.
31. Talon R, Bressollier P, Urdaci MC. Isolation and characterization of two exopolysaccharides produced by *Lactobacillus plantarum* EP56. Res Microbiol. 2003;154:705–12.
32. De Vuyst L, Degeest B. Heteropolysaccharides from lactic acid bacteria. FEMS Microbiology Reviews. 1999;23(2):153-17.
33. De Vuyst L, De Vin F, Vaningelmem F, Degeest B. Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. International Dairy Journal. 2001;11(9):687-707.
34. Montersino S, Prieto A, Muñoz R, de las Rivas B. Evaluation of exopolysaccharide production by *Leuconostoc mesenteroides* strains isolated from wine. Journal of Food Science. 2008;73(4):196-199.
35. Mozzi F, Vaningelgem F, Hebert EM, Van der Meulen R, Foulquie Moreno MR, Font de Valdez G, De Vuyst L. Diversity of heteropolysaccharide-producing lactic acid bacterium strains and their biopolymers. Applied and Environmental Microbiology. 2006;72:4431-4435.
36. Van der Meulen R, Grosu-Tudor S, Mozzi F, Vaningelgem F, Zamfir M, Font de Valdez G, De Vuyst L. Screening of lactic acid bacteria isolates from dairyand cereal products for exopolysaccharide production and genes involved. International Journal of Food Microbiology. 2007;118(3):250-258.
37. De Vuyst L, De Vin F, Kamerling JP. Exopolysaccharides from lactic acid bacteria. In: Kamerling JP, Editor. Comprehensive Glycoscience, Oxford: Elsevier. 2007;2:477–518.
38. Folkenberg DM, Dejmek P, Skriver A, Guldager HS, Ipsen R. Sensory and rheological screening of exopolysaccharide producing strains of bacterial yoghurt cultures. International Dairy Journal. 2006;16:111-118.
39. Pleijsier MT, de Bont PW, Vreeker R, Ledeboer AM. Functional properties of

exocellular polysaccharides in dairy based foods. 2nd International Symposium on Food Rheology and Structure, Z. Urich, Switzerland. 2000;12–16.
40. Hassan AN, Frank JF, Schmidt KA, Shalabi SI. Rheological properties of yoghurt made with encapsulated nonropy lactic cultures. Journal of Dairy Science. 1996;79:2091–2097.
41. Marshall VM, Rawson HL. Effects of exopolysaccharide-producing strains on thermophilic lactic acid bacteria on the texture of stirredyoghurt. International Journal of Food Science and Technology.1999;34:137–143.
42. Hassan AN, Frank JF, Farmer MA, Schmidt KA, Shalabi SI. Observation of encapsulated lactic acid bacteria using confocal scanning laser microscopy. J Dairy Sci .1995;78:2624-2628.
43. Van Marle, M. E., Zoon P. Permeability andrheological properties of microbially andchemically acidifiedskim-milk gels. Netherlands Milk and Dairy Journal. 1995; 49:47–65.
44. Prajapati JB, Nair BM. The history of fermented foods In: Fermented Functional Food. (Eds Edwards and R Farnworth) CRC Press. 2003;1–25.
45. Behare P, Singh R, Singh RP. Exopolysaccharide-producing mesophilic lactic cultures for preparation of fat-free Dahi - An Indian fermented milk. J Dairy Res. 2009;76:90-97.
46. Praveen K. Physico-chemical and microstructural properties of dahi using EPS producing strains. M Sc Thesis, National Dairy Research Institute, Karnal, Haryana, India; 2000.
47. Zisu B, Shah NP. Texture characteristics and pizza bake properties of low-fat mozzarella cheese as influenced by pre-acidification with citric acid and use of encapsulated and ropy exopolysaccharide producing cultures. Int Dairy J. 2007;17: 985–97.
48. Dabour N, Kheadr E, Fliss I, LaPointe G. Impact of ropy and capsular exopolysaccharide-producing strains of *Lactococcus lactis* subsp. *cremoris* on reduced-fat Cheddar cheese production and whey composition. Int Dairy J. 2005; 15:459-471.
49. Perry DB, Mc Mahon DJ, Oberg CJ. Effect of exopolysaccharide-producing cultures on moisture retention in low fat mozzarella cheese. J Dairy Sci. 1997;80:799–805.
50. Bouzar F, Cerning J, Desmazeaud M. Exopolysaccharide production and texturepromoting abilities of mixed-strain starter cultures in yogurt production. J Dairy Sci. 1997;80:2310–2317.
51. Jolly L, Vincent SJF, Duboc P, Neeser JR. Exploiting exopolysaccharides from lactic acid bacteria. Antonie Leeuwenhoek. 2002;82:367–74.
52. Hassan AN, Ipsen R, Janzen T, Qvist KB. Microstructure and rheology of yogurt made with cultures differing only in their ability to produce exopolysaccharides. J Dairy Sci. 2003;86:1632-1638.
53. Amatayakul T, Halmos AL, Sherkat F, Shah NP. Physical characteristics of yoghurts made using exopolysaccharide-producing starter cultures and varying casein to whey protein ratios. Int Dairy J. 2005;16:40-51.
54. Yang Z, Li S, Zhang X, Zeng X, Li D.Capsular and slime-polysaccharide production by *Lactobacillus rhamnosus* JAAS8 isolated from Chinese sauerkraut: Potential application in fermented milk products. J Biosci Bioeng. 2010;110:53-57.
55. Purwandari U, Shah NP, Vasiljevic T. Effects of exopolysaccharide-producing strains of Streptococcus thermophilus on technological and rheological properties of set-type yoghurt. Int Dairy J. 2007;17: 1344–52.
56. Micheli L, Uccelletti D, Palleschi C, Crescenzi V. Isolation and characterisation of a ropy *Lactobacillus* strain producing the exopolysaccharide kefiran. Appl Microbiol Biotechnol .1999;53:69–74.
57. Rimada PS, Abraham AG. Kefiran improves rheological properties of glucono-δ-lactone induced skim-milk gels. Int Dairy J. 2006;16:33–9.
58. Takehiro U, Teruo N, Akemi W, Terumi U. Modifier for frozen bread dough and production of frozen bread dough. Japanese Patent 08009892 A; 1994.
59. Yasushi M, Akifumi Y. Bread dough improver and production of bread dough. Japanese Patent 07046956A; 1993.
60. Brandt MJ, Roth K, Hammes WP. Effect of an exopolysaccharide produced by *Lactobacillus sanfranciscensis* LTH1729 on dough and bread quality. In L. de Vuyst (Ed.), Sourdough from fundamentals to applications Brussels: Vrije Universiteit Brussel (VUB), IMDO. 2003;80.
61. Korakli M, Pavlovic M, Ga¨nzle MG, Vogel RF. Exopolysaccharide and kestose

production by Lactobacillus sanfranciscensis LTH2590. Applied and Environmental Microbiology. 2003;69: 2073–2079.
62. Galle S, Schwab C, Arendt E, Ganzle M. Exopolysaccharide-forming *Weissella* strains as starter cultures for sorghum and wheat sourdoughs. J Agric Food Chem. 2010;58:5834-5841.
63. Di Cagno R, De Angelis M, Limitone A, Minervini P, Carnevali, A. Corsetti, M. Gänzle, R. Ciati, Gobbetti M . Glucan and fructan production by sourdough *Weissella cibaria* and *LactoBacillus plantarum*. J Agric Food Chem. 2006;54:9873-9881.
64. Thakur S, Prasad MS, Rastogi NK. Effect of Xanthan on textural properties of idli (traditional south Indian food). Food Hydrocolloids.1995;9:141-145.
65. Nisha P, Ananthanarayan L, Singhal RS. Effect of stabilizers on stabilization of idli (traditional south Indian food) batter during storage. Food Hydrocolloids. 2005;19: 179-186.
66. Gibson GR, Roberfroid M. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. The Journal of Nutrition. 1995; 125:1401-1412.
67. Roberfroid M, Slavin J. Nondigestible oligosaccharides. Critical Reviews in Food Science and Nutrition. 2000;40(6):461-480.
68. Bello FD, Walter J, Hertel C, Hammes WP. In vitro study of prebiotic properties of levan-type exopolysaccharides from *Lactobacilli* and non-digestible carbo-hydrates using denaturing gradient gel electrophoresis. Syst Appl Microbiol. 2001; 24:232-237.
69. Salazar N, Gueimonde M, Hernandez-Barranco AM, Ruas-Madiedo P, de los Reyes-Gavilan CG. Exopolysaccharides produced by intestinal *Bifidobacterium* strains act as fermentable substrates for human intestinal bacteria. Applied and Environmental Microbiology. 2008;74: 4737-4745.
70. Kim Y, Han KS, Imm JY, Oh You S, Park S, Kim SH. Inhibitory effects of Lactobacillus acidophilus lysates on the cytotoxic activity of shiga-like toxin 2 produced from *Escherichia coli* O157:H7, Lett. Appl. Microbiol. 2006;43:502–507.
71. Oh S, Kim SH, Worobo RW. Characterization and purification of a bacteriocin produced by a potential probiotic culture, *Lactobacillus acidophilus* 30SC, J. Dairy Sci. 2000;83:2747–2752.
72. Ruas-Madiedo P, Gueimonde M, de los Reyes-Gavilán CG, Salminen S. Short communication: Effect of exopolysaccharide isolated from ‘‘viili” on the adhesion of probiotics and pathogens to intestinal mucus, J. Dairy Sci.2006; 89 : 2355–2358.
73. Fracchia L, Cavallo M, Allegrone G, Martinotti MG. A *Lactobacillus*-derived biosurfactant inhibits biofilm formation of human pathogenic *Candida albicans* biofilm producers. Communicating current research and educational topics and trends in applied microbiology. A. Mendez-Vilas (Ed.) FORMATEX, Badajoz Spain. 2010; 827-837.
74. Bendaoud M, Vinogradov E, Balashova NV, Kadouri DE, Kachlany SC, Kaplan JB. Broad-spectrum biofilm inhibition by *Kingella kingae* exopolysaccharide. Journal of Bacteriology. 2011;3879–3886.
75. Kavitaa K, Singha VK, Mishraa A, Jha B. Characterisation and anti-biofilm activity of extracellular polymericsubstances from *Oceanobacillus iheyensis*. Carbohydrate Polymers. 2014;101:29–35.
76. Haroun M. Structure analysis and antitumor activity of the exopolysaccharide from probiotic *Lactobacillus plantarum* NRRL B- 4496 *in vitro* and *in vivo*. Journal of Applied Sciences Research. 2013;9(1): 425-434.
77. Ogawa T, Asai Y, Tamai R, Makimura Y, Sakamoto H, Hashikawa S, Yasuda K. Natural killer cell activities of symbiotic *Lactobacillus casei* ssp. *Casei* in conjunction with dextran. Clin Exp Immunol. 2006;143(1):103–109.
78. Kitazawa H, Yamaguchi T, Itoh T. B-cell mitogenic activity of slime products produced from slime-forming, encapsulated *Lactococcus lactis* ssp. *cremoris*. J Dairy Sci. 1992;75:2946-2951.
79. Forsén R, Heiska E, Herva E, Arvilommi H. Immunobiological effects of *Streptococcus cremoris* from cultured milk ‘viili’; application of human lymphocyte culture techniques. Int J Food Microbiol. 1987;5: 41-47.
80. Rodrigues L, van der Mei H, Banat IM, Teixeira J, Oliveira R. Inhibition of microbial adhesion to silicone rubber treated with biosurfactant from *Streptococcus thermophilus* A. FEMS Immunol Med Microbiol. 2006;46:107–112.

81. Gómez E, Melgar MM, Pérez Silva G, Portolés A, Gil I. (1988). Exocellular products from *Bifidobacterium adolescentis* as immunomodifiers in the lympho-proliferative responses of mouse splenocytes. FEMS Microbiol Let. 1998; 56:47-52.
82. Nagaoka M, Hashimoto S, Watanabe T, Yokokura T, Mori Y. Antiulcer effects of lactic acid bacteria and their cell wall polysaccharides. Biol. Pharm Bull. 1994; 17:1012-17.
83. Nakajima H, Hirota T, Toba T, Itoh T, Adachi S. Structure of the extracellular polysaccharide from slime-forming *Lactococcus lactis* subsp *cremoris* SBT 0495. Carbohydr Res. 1992;224:245–253.
84. Soh HS, Kim CS, Lee SP. A new *in vitro* assay of cholesterol adsorption by food and microbial polysaccharides. J Med Food. 2003;6:225-23.
85. Tok E, Aslim B. Cholesterol removal by some lactic acid bacteria that can be used as probiotic. Microbiol Immunol. 2010;54: 257-264.
86. Spelman K, Burns J, Nichols D, Winters N, Ottersberg S, Tenborg M. Modulation of cytokine expression by traditional medicines: A review of herbal immunomodulators. Alternative Medicine Review. 2006;11(2):128-150.
87. Wasser SP. Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Applied Microbiology and Biotechnology. 2011; 89(5):1323-1332.
88. Fernández de Palencia P, Werning ML, Sierra-Filardi E, Dueñas MT, Irastorza A, Corbí A, López P . Probiotic properties of the 2-substituted (1,3)-β-Dglucan producing bacterium *Pediococcus parvulus* 2.6. Applied and Environmental Microbioly. 2009;75:4887-4891.
89. Garai-Ibabe G, Werning ML, López P, Corbí AL, Fernández de Palencia P. Naturally occurring 2-substituted (1,3)-β-D-glucan producing *Lactobacillus suebicus* and *Pediococcus parvulus* strains with potential utility in the food industry. Bioresource Technology. 2010;101:9254-9263.
90. Modig J. Comparison of effects of dextran-70 and ringer's acetate on pulmonary function, hemodynamics, and survival in experimental septic shock. Critical Care Medicine. 1998;16:266-271.
91. Gombocz K, Beledi A, Alotti N, Kecskes, Gabor V, Bogar L, Koszegi T, Garai J. Influence of dextran-70 on systemic inflammatory response and myocardial ischaemia-reperfusion following cardiac operations. Critical Care. 2007;11:87.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/22004