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Microbial Exopolysaccharides: Natural Modulators of Dairy Products

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ABSTRACT

Microbial exopolysaccharides (EPS) are considered as natural bio-thickeners abundantly used in dairy and fermented food industries for quality improvement. In fermentation based dairy industries, researchers are seeking their attention on substitution of artificial food stabilizers with naturals by exploring EPS, especially for improving the rheology of fermented food products. Lactic acid bacteria (LAB) being a starter player for fermented food products are believed to be as one of the best natural producers of EPS. These EPS are basically sugar residues, secreted by microbes in their surrounding environment but their *in-vitro* production is not economical due to the media and processing costs involved. The process of production is also complex due to the involvement of various enzymes and regulatory proteins. With modernization in dairy sector, our understanding towards EPS needs improvement. The review focuses on a brief explanation on the behavior and functionality of EPS from lactic acid bacteria.

INTRODUCTION

In many fermentation based dairy industries the property of any matter in terms of rheology is one of the main characteristic that defines the quality of fermented dairy products. By the definition the firmness, consistency, stability, steadiness, texture and viscosity are the important rheological parameters that govern the quality of fermented dairy product. In dairy industries yogurt, curd, cheese are the most popular fermented food product prepared by milk fermentation. In milk fermentation, the raw materials are converted by microorganisms (bacteria, yeast and molds) to products that have the acceptable qualities like food. One of the most important group of microbes in the food industry is the Lactic Acid Bacteria (LAB). Most commonly LAB is used in making starter culture for dairy products. A starter culture is a microbiological culture which actually performs fermentation and is supplemented with one or more species or strains of LAB. In milk fermentation lactic acid is produced by starter bacteria, which create acidity and prevent the growth of undesirable microorganisms. Presently in many fermented dairy products, LAB is used, for their contribution in the textural characteristic of the fermented food product by the production of exopolysaccharides (EPS). EPS are polymeric compounds which are considered as natural biothickeners which contribute to the structure of fermented milk product (Mostefaoui *et al.*, 2014). Because of they are produced in situ by the LAB starters which possessing General Recognized as Safe status (GRAS). LAB has contributed their role in the increased volume of fermented foods worldwide especially in foods containing probiotics or health promoting bacteria. Approximately 30 species of lactobacilli are described as EPS producers. Among them, the best known are *L. casei*, *L. acidophilus*, *L.brevis*, *L. curvatus*, *L. delbrueckii bulgaricus*, *L.helveticus*, *L. rhamnosus*, *L plantarum*, *L. johnsonii*, etc. They are principally cultivated between 30 and 37ºC on rich media as MRS (Man Rogosa Sharp), milk or milk derivatives; therefore, many strains of dairy LAB manufacture extracellular polysaccharides (Tabibloghmany and Ehsandoost, 2014). They can grow extremely rapid in milk at temperatures 37 to 45°C. A special characteristic of the metabolism of these bacteria is that they utilizes only the glucose moiety from sugars like lactose and sucrose, resulting in fermented product lactic acid containing residual galactose or fructose. These residual moieties further metabolize in the formation of other beneficial metabolic compounds. The specific flavor compound produced by these bacteria during milk fermentation is acetaldehyde, diacetyl and anti bacterial compound bacteriocins. Apart from this, these bacteria are well known for their ability to produce EPS resulting in a desired slimy & rheological structure of the fermented product.

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Several strains of LAB are commercially used, which are able to produce EPS, these compounds have attracted great interest since they can act as natural thickeners which improve the properties of texture, decrease syneresis and reduce the fat level in fermented dairy foods. In the food industry, many EPS are commonly used as food additives for their gelling, stabilizing or thickening properties. Today there is an increasing demand of microbes producing these polysaccharides (Patel *et al.,* 2010).

EXOPOLYSACCHARIDES

Bacterial polysaccharides synthesized and secreted into the external environment or are synthesized extracellularly by cell wall-anchored enzymes referred to as EPS. Microbial polysaccharides or bacterial polysaccharides are multifunctional and can be divided into intracellular polysaccharides, structural polysaccharides and extracellular polysaccharides or exopolysaccharides. These polysaccharides are produced by plants (cellulose, pectin and starch), seaweeds (alginate and carragenan) and microbes (alginate, dextran, gellan, pullulan, and xanthan) (De Vuyst *et al.,* 1998).

Sundman (Sundman, 1953) first studied the slimy nature (EPS) of LAB. These EPS are long-chain, high molecular weight polymers that dissolve or disperse in water to give thickening or gelling properties, and contributes their property as indispensable tools in food product formulation.

It is reported that LAB produces both homopolysaccharides and hetropolysaccharides (Cerning, 1990; Dunican and Seeley, 1965). Homopolysaccharides are present in the form of glucans which is mainly contain a-1,6- and a-1,3-linked glucose residues, known as dextrans (*L. mesenteroides* subsp. *mesenteroides* and *L. mesenteroides* subsp. *dextranicum*), fructans contain b-2,6-linked fructose molecules, such as levan (*S. salivarius*), (*L. mesenteroide and Streptococci*), and mutans (*S. mutans* and *S. Sobrinus*). Heteropolysaccharides produced by both mesophilic (*L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*) and thermophilic bacterial strains (*L. delbrueckii* subsp. *bulgaricus*, *L. helveticus* and *S. thermophilus*) (Cerning, 1990; Kranenburg 1997).

CHEMICAL AND STRUCTURAL COMPOSITION OF EXOPOLYSACCHRIDE

Exopolysaccharide (EPSs) is generally heteropolymeric (made of different monomeric units), non-sugar components like uronic acid, methyl esters, sulphates, pyruvates, proteins, nucleic acids and lipids (Shankar *et al.,* 2014). The repeating sugar units are mainly composed of glucose, galactose, mannose, N-acetylglucosamine, N-acetyl galactosamine and rhamnose, in variable ratios and structurally these polymeric carbohydrate molecules form long chains of monosaccharide units which are bound together by glycosidic linkage and on hydrolysis give the constituent sugars or monosaccharides. EPS are the construction material of bacterial settlements and either remains attached to the outer surface of the cell, or are secreted into its surrounding or in growth medium. EPS of some strains of LAB contribute a gelatinous texture to fermented milk products and these polysaccharides are also digestible.

TYPES OF EXOPOLYSACCHARIDES

Based on their composition EPS are divided in to Homo polysaccharides & hetro polysaccharides.

Homopolysaccharides

Homopolysaccharides consist of one monosaccharide (mostly fructose or glucose), and are usually produced in large amount from sugars by the action of glycansucrases e.g. dextran, levan, alternan, reuteran etc.

Heteropolysaccharides

Heteropolysaccharides are mostly composed of identical repeating units consisting of two or more monosaccharides e.g. galactose, glucose, rhamnose and fructose. Several linkages can occur at the same time in one polysaccharide. Sugar nucleotides play an essential role in the synthesis of heteropolysaccharides due to their function in sugar inter-conversions as well as sugar activation.

It is reported that hetropolysaccharide; Kafiran which is produced by many *Lactobacillus* species such as *L. kefiranofaciens*, *L. kefirgranum*, *L. parakefir*, *L. kefir* and *L. delbrueckii subsp. bulgaricus* is a water soluble heteropolysaccharide and is consists of equal amount of glucose and galactose (Micheli *et al.,* 1999).The composition and nature of any EPS is affected by the environmental conditions, biosynthetic pathways or rate of microbial growth in the medium. EPS of *Streptococcus thermophilus* was the first heteropolysaccharide studied and described in details (Doco *et al.,* 1990).

BIOSYNTHESIS

Biosynthesis of bacterial EPS is a complex process involving large number of enzymes and regulatory proteins. The gene which encodes the proteins or enzyme requires for the biosynthesis of EPS are located on chromosomes in those which are thermopiles and are plasmid origin in mesophiles (Broadbent *et al.,* 2003; Laws *et al.,* 2001).

EPS producing microorganisms utilize sugars as their carbon and energy source; ammonium salts and amino acids are their source of nitrogen (Gandhi, 1997; Czaczyk and Wojciechowska, 2003). The synthesis of EPS by microbial cells basically depends on the carbon and nitrogen availability in the culture medium (Cerning *et al.,* 1994).

Biosynthesis of homopolysacchraide and heteropolysaccharide are different with each other. EPS biosynthesis involves four major steps, sugar nucleotide synthesis, repeating unit synthesis, and polymerization of the repeating units (de Vuyst *et al.,* 2001). It is Glycosyl transferase (glucansucrase) which is involved in the synthesis of EPS in LAB (De Vuyst and Degeest, 1999; Kralj *et al.,* 2004; van Hijum *et al.,* 2004).

A glycosyltransferase (GTs) is a catalytic enzyme that catalyzes the transfer of a sugar moiety from an activated donor sugar onto acceptors molecules which are saccharides or nonsaccharides and establishes natural glycosidic linkages. Glucansucrase (GS) & fructosucrases (FS) are the enzymes of family glucosyltransferase used by LAB to split sugars and use resulting glucose molecules to build long sticky EPS molecule (van Hijum *et al.,* 2006).

GROWTH MEDIUM FOR EXOPOLYSACCHARIDE PRODUCING STARTER CULTURE

Composition of the medium used for the cultivation of LAB has significant value on both lactic acid and EPS production. The basic fermenting sugar for LAB in the growth medium is lactose which is naturally present in milk. LAB also ferments sucrose and dextrose to produce lactic acid. Yeast extract, tryptone, casein enzyme hydrolysate, peptones are the basic nitrogenous sources of the growth medium.

These are the basic ingredient of any growth medium providing high quality and very rich source of nitrogen, peptides, polypeptides and amino acids. MRS is the most significantly used growth media for laboratory cultivation of LAB contains compounds like beef extract, peptone, yeast extract, lactose dextrose and some salts.

Some studies explain another growth medium using Whey protein concentrate (WPC) and Skim milk powder (SMP) which had performed significant role in the EPS production. Higher EPS production during fermentation process occurs due to the availability of nitrogen sources in the growth media (Garicia-Garbay and Marshall, 1991).

FACTOR AFFECTING THE BIOSYNTHESIS OF EXOPOLYSACCHARIDES

It has been suggested by some research work, that a potential controlling factor in EPS biosynthesis is the availability of sugar nucleotides which is necessary for construction of the polymers (Boels *et al.,* 2001). It was studied that microbial EPS are not consumed as an energy source by the producing microorganism, but are released to protect the producer organism during starvation conditions and also at extreme pH and temperature conditions (Kim *et al.,* 2000).

The total yield of EPS produced by LAB mostly depends on the composition (carbon and nitrogen sources) of the growth medium and the physical conditions at which the strains grow, i.e. temperature, pH of medium and incubation time (duration of fermentation). Not only the nature of the carbon source, sometimes the combination of sugars and their concentration units also have a stimulating effect on EPS biosynthesis. Some experimental studies showed that EPS production and growth were initially obtained higher with the supplementation of medium by skim milk and whey protein concentrate (WPC) or whey protein hydrolysates (Vaningelgem *et al.,* 2004). Other medium components such as minerals, some amino acids, some bases and vitamins, can also affect the synthesis of EPS.

ROLE OF LACTIC ACID BACTERIA IN RHEOLOGY OF FERMENTED DAIRY PRODUCT

EPS producing lactic acid bacteria are most wildly used in the fermentation based industries for manufacturing of dairy products to improve texture and quality. They produce a wide range of EPS and lactic acid during milk fermentation. Because of their water binding and viscosity modulating properties, EPS have been intensely studied for their potential as natural stabilizers in fermented milk. Currently Cheese, curd and yogurt are the popular fermented dairy product prepared by fermenting the milk by LAB as a starter culture.

The role of LAB in dairy product manufacturing can be shortly summarized as milk fermentation, milk acidification, synthesis of aromatic compounds, development of texture and viscosity. Milk chemically acidified by lactic acid produced by the starter culture from sugars like lactose. It has been studied that lactic acid produced reduces the pH of the milk and leads to the solubilization of micelles of calcium phosphate present in milk (Zourari *et al.,* 1992). This causes the demineralization and destabilization of casein micelles, which generates the complete precipitation of casein in a pH range of 4.6-4.7 (Fox, 1989). Now the interaction between this precipitated casein and produced polysaccharide is important in relation with the milk gel quality and formation. According to Snoeren *et al.,* (1976) electrostatic attraction between charged polysaccharide and casein molecular is the major driving force for these interactions, some of mixed polysaccharide and milk showed that precipitated casein particle are absorbed on the polysaccharide chain. EPS have the potential to replace hydrocolloids currently used as bread improvers and meet so the consumer demands for a reduced use of food additives (Galle and Arendt, 2014)

PROCESS OF GELLING OF FERMENTED MILK PRODUCTS

The important factor which affects the rheological behavior of LAB is the heat treatment of milk and fermented product. Temperature affects the rheological parameters mainly due the denaturation of proteins present (Casein) in milk. This protein casein present in colloidal form and it is sustained by calcium bridges between the casein micelles. Apart of the casein, another protein substance is also present in milk is whey protein and is exist in soluble state and have no interaction with casein micelles (Mathur *et al.,* 1978; Jenness and Patton 1959). When milk is heated the whey protein gets denatured and interacts with casein micelles without affecting the calcium bridges. During fermentation process, starter bacteria produces lactic acid so that pH of the medium decreases. Due to which calcium of bridges goes from colloidal state to solution state. As the acidity rises and pH decreases, these calcium bridges between the protein particles get dissolved in solution state (Hassan, 2003). Now these precipitated casein-whey protein particles arrange themselves in a specific manner and forms a network. Interaction between this precipitated network and EPS is the important relationship in terms of rheological behavior of *S. thermophilus* and Lactobacillus.

The electrostatic attraction between charged polysaccharide and casein network provides three dimensional (gel like, firmness) structure to the fermented milk. Casein protein and EPS interaction is the basic structural quality of gel firmness and texture of the fermented milk. In dairy industries many processing problems during fermentation process such as high syneresis, low viscosity, are now solved by increasing amount of EPS. Apart from this the typical flavor of fermented milk is due to the various carbonyl compounds, such as acetaldehyde, acetone and diacetyl are also produced by LAB. Acetaldehyde and Diacetyl is considered as the major flavor component of fermented milk.

ISOLATION OF EXOPOLYSACCHARIDES

According to the studies of De Vuyst *et al.*, isolation of EPS could be carried out in flasks containing 500 ml of (milk) medium and performed in four steps. In first step milk proteins were removed from the fermentation liquor by precipitation with one volume of 20% trichloroacetic acid, followed by centrifugation of cells and proteins (25000 *g*, 20 min, 4°C). Then exopolysaccharides were precipitated overnight with an iso-volume of acetone, followed by centrifugation of the precipitate (25000 *g*, 30 min, 4 °C), after which the pellet was redissolved in ultra-pure water. Now the residual proteinaceous material was precipitated with trichloroacetic acid and removed by centrifugation as described above. Finally the exopolysaccharides were isolated by acetone precipitation (one volume) and harvested by spinning for further analysis or quantification.

APPLICATION OF EXOPOLYSACCHARIDES IN DAIRY INDUSTRIES

EPS from lactic acid bacteria have found their valuable application in the improvement of the rheological properties of fermented dairy products and have been studied extensively over the past decades. Microbial EPS function as biothikeners, stabilizers, gelling agents, viscosifying agents, or water binding agents.

Their production significantly contributes to texture, mouth feel, teats and stability of the final dairy product. Recently, it is observed that the consumer demand for products with low fat or sugar content and low levels of food additives, as well as cost factors, make microbial EPS a viable alternative of artificial food additives. To reduce the amount of added milk solids, to improve viscosity, to enhance texture and mouth feel and to avoid syneresis during fermentation or upon storage of the fermented dairy products, EPS producing functional starters have valuable applications in fermentation based dairy industries. It is studied that yogurt made with EPS producing starter cultures has better water binding capacity, which decreases the process of syneresis (whey separation) (Amatayakul *et al.*, 2005).

CONCLUSION

Exopolysaccharide improves rheology of fermented dairy product in terms of texture, stability, firmness and mouth feel. It reduces the problems like low viscosity, weak gel firmness or high syneresis (whey or water separation) during dairy product preparation. Also, these EPS stay in the gastrointestinal tract and facilitate colonization of the tract by probiotic bacteria like *Streptococcus*, *lactobacillus* and *lactococcus*. With the modernization of dairy and fermented food sector, these natural thickeners from lactic acid bacteria must be extensively explored targeting efficient and economical isolation and production.

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