



## Multifunctional Role of Exopolysaccharides from Dairy Cultures: Enhancing Product Texture and Promoting Health Benefits

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**ABSTRACT:** Exopolysaccharides (EPS) have emerged as vital components in the dairy industry, significantly influencing both the technological and functional attributes of dairy products. Produced primarily by lactic acid bacteria (LAB) during fermentation, EPS contribute to improved texture, enhanced stability, and potential health benefits. Their ability to interact with water molecules and milk proteins enhances viscosity, gel strength, and mouthfeel, particularly in yogurt and cheese. Additionally, EPS reduce syneresis and improve moisture retention, making them valuable in dairy formulations, especially in low-fat varieties. Beyond their structural role, EPS exhibit promising bioactive properties, including prebiotic, immunomodulatory, and antioxidant effects. They serve as fermentable substrates for beneficial gut bacteria, supporting gut health, while emerging studies suggest potential anti-inflammatory, anti-diabetic, and anti-cancer properties. Advances in fermentation optimization, genomics, and metabolomics have facilitated improved EPS production and functionality. Techniques such as high-resolution spectroscopy and genetic engineering, including CRISPR-based modifications, have further refined their structural characterization and application potential. With increasing consumer demand for natural and health-promoting dairy products, EPS offer a promising alternative to synthetic additives, aligning with clean-label trends. Further research and innovation in EPS biosynthesis and application will continue to expand their role in functional dairy product development. This review highlights recent advancements in EPS structural diversity, biosynthesis, and functional applications.

**Keywords:** Exopolysaccharides, lactic acid bacteria, dairy products, health benefits.

### INTRODUCTION

Exopolysaccharides (EPS) have emerged as a crucial component in the dairy industry, playing a significant role in both the technological and functional aspects of various dairy products. These complex carbohydrate polymers, produced by a wide range of microorganisms, particularly lactic acid bacteria (LAB), have garnered increasing attention due to their multifaceted applications in improving product texture and conferring potential health benefits (Singhal *et al.*, 2023). As consumer demand for natural, clean-label products continues to rise, EPS offer a promising alternative to synthetic additives in dairy formulations (Zannini *et al.*, 2015).

Exopolysaccharides are high-molecular-weight polymers composed of carbohydrate residues, secreted by microorganisms into their surrounding environment. In the context of dairy products, EPS are primarily produced by lactic acid bacteria during fermentation processes. These bacteria, including species from genera such as *Lactobacillus*, *Streptococcus*, and *Lactococcus*, have been traditionally used in the

production of fermented dairy products like yogurt, cheese, and kefir (Lynch *et al.*, 2018). The history of EPS in dairy products dates back to the traditional fermentation practices, where the thickening and texturizing properties of certain bacterial strains were empirically selected over generations (Pham *et al.*, 2000). The production of EPS by dairy cultures is influenced by various factors, including the bacterial strain, growth conditions, and substrate availability. Recent advances in genomics and metabolomics have provided new insights into the biosynthetic pathways and genetic regulation of EPS production, enabling the development of strategies to enhance and optimize EPS yields in dairy fermentations (Daba *et al.*, 2021).

The significance of EPS in dairy products is twofold, encompassing both technological functionalities and potential health-promoting properties. From a technological perspective, EPS play a crucial role in improving the textural and rheological properties of dairy products, particularly in fermented milk products like yogurt and cheese (Folkenberg *et al.*, 2006; Meghwal *et al.*, 2023). One of the primary mechanisms by which EPS enhance texture is through their water-

binding capacity due to the hydrophilic nature of EPS allows them to interact with water molecules, increasing the water-holding capacity of the product and reducing syneresis (whey separation). This property is particularly valuable in yogurt production, where EPS contribute to a smoother, creamier texture and improved stability during storage (Costa *et al.*, 2012; Mandot & Meena 2024). Furthermore, EPS can interact with milk proteins, particularly casein micelles, influencing the formation and stability of protein networks. These interactions can lead to increased viscosity, improved gel strength, and enhanced mouthfeel in dairy products. In cheese manufacturing, EPS have been shown to improve moisture retention, yield, and functional properties such as meltability and stretchability, especially in low-fat varieties (Domingos-Lopes *et al.*, 2018). Numerous studies have reported on the bioactive properties of EPS, including prebiotic, immunomodulatory, antioxidant, and anti-inflammatory effects (Bokadia *et al.*, 2024; Meena *et al.*, 2017). The prebiotic potential of EPS is of particular interest, as these compounds can serve as fermentable substrates for beneficial gut bacteria, promoting their growth and activity (Chen *et al.*, 2019). Some studies have reported the ability of EPS to enhance the phagocytic activity of macrophages and modulate cytokine production, indicating potential applications in immune health (Zhang *et al.*, 2013). Emerging evidence also points to potential anti-diabetic and anti-cancer properties of some EPS, although these areas require further investigation, particularly in human clinical trials. The growing body of research on the health benefits of EPS highlights their potential as functional ingredients in dairy products, aligning with the increasing consumer demand for foods with added health benefits (Maalej *et al.*, 2017). The technological advancements in EPS production, purification, and characterization have significantly improved our ability to harness the potential of these compounds. Optimization of fermentation conditions, including the use of mixed cultures and fed-batch strategies, has led to enhanced EPS yields and tailored structural properties (Dertli *et al.*, 2015). Advanced analytical techniques, such as high-resolution NMR spectroscopy and mass spectrometry, have enabled more precise structural characterization of EPS, facilitating the development of structure-based functional predictions (Maina *et al.*, 2008). Furthermore, genetic engineering approaches, including CRISPR-Cas9-mediated gene editing, have opened up new possibilities for designing EPS-producing strains with improved functionality and productivity (Zhang *et al.*, 2016). This comprehensive review has explored the multifaceted role of exopolysaccharides (EPS) produced by dairy cultures in improving product texture and promoting health benefits.

## TYPES AND CLASSIFICATION OF EPS PRODUCED BY DAIRY CULTURES

Exopolysaccharides (EPS) produced by dairy cultures exhibit a remarkable diversity in terms of their chemical composition, structure, and location relative to the

bacterial cell. This diversity underlies the varied functional properties of EPS in dairy products and their potential health benefits. Understanding the different types and classifications of EPS is crucial for optimizing their applications in the dairy industry and for developing novel functional dairy products. Recent advances in analytical techniques and molecular biology have provided deeper insights into the structural complexity of EPS, enabling more precise classifications and better understanding of structure-function relationships (Zannini *et al.*, 2016).

### A. Classification based on chemical composition

The primary classification of EPS produced by dairy cultures is based on their chemical composition, which broadly divides them into two main categories: homopolysaccharides and heteropolysaccharides. This classification is fundamental to understanding the biosynthetic pathways, physical properties, and potential applications of different EPS types.

**(i) Homopolysaccharides.** Homopolysaccharides are composed of a single type of monosaccharide unit and are typically synthesized by a single enzyme, known as a glycosyltransferase or glycosyltransferase. These EPS are further subdivided based on the type of monosaccharide and the predominant glycosidic linkages present in their structure (Monsan *et al.*, 2001).

**$\alpha$ -Glucans:** These are homopolysaccharides composed of glucose units linked predominantly by  $\alpha$ -glycosidic bonds. Common examples include dextran ( $\alpha$ -1,6 linkages), mutan ( $\alpha$ -1,3 linkages), and alternan (alternating  $\alpha$ -1,3 and  $\alpha$ -1,6 linkages). Dextran, produced by certain strains of *Leuconostoc mesenteroides* and *Streptococcus mutans*, has been extensively studied for its potential as a texturizing agent in dairy products. The ability of dextran-producing *Leuconostoc* strains to improve the rheological properties of fermented milk products, highlighting the technological potential of these EPS (Mayer *et al.*, 2020).

**$\beta$ -Fructans:** These homopolysaccharides consist of fructose units and include levan ( $\beta$ -2,6 linkages) and inulin-type fructans ( $\beta$ -2,1 linkages). While less common in dairy cultures compared to  $\alpha$ -glucans, some strains of *Lactobacillus reuteri* have been reported to produce levan-type EPS. A study by Xu *et al.* (2019) investigated the prebiotic potential of  $\beta$ -fructans produced by *L. reuteri*, demonstrating their ability to selectively promote the growth of beneficial gut bacteria, suggesting potential applications in functional dairy products (Mohammed *et al.*, 2021). Recent advancements in the characterization of homopolysaccharides have revealed more complex structures than previously thought. A novel  $\alpha$ -glucan produced by *Lactobacillus mali* CUPV271, which exhibited a unique branched structure with both  $\alpha$ -1,6 and  $\alpha$ -1,3 linkages. This discovery highlights the ongoing potential for identifying new EPS structures with unique functional properties (Liu *et al.*, 2010).

**(ii) Heteropolysaccharides.** Heteropolysaccharides are composed of repeating units containing two or more different types of monosaccharides. These EPS exhibit

greater structural diversity compared to homopolysaccharides and are typically produced by a more complex biosynthetic machinery involving multiple enzymes. The composition and structure of heteropolysaccharides can vary significantly between different bacterial strains and even within the same species under different growth conditions (Mozzi *et al.*, 2006). Common monosaccharides found in heteropolysaccharides include glucose, galactose, rhamnose, N-acetylglucosamine, and N-acetylgalactosamine. The composition, sequence, and linkages of these sugar units determine the physical and functional properties of the EPS. For example, the presence of charged groups, such as phosphate or carboxyl groups, can significantly influence the EPS's interaction with milk proteins and its overall impact on product texture (Mende *et al.*, 2016).

Recent studies have provided new insights into the structural diversity of heteropolysaccharides produced by dairy cultures. Lynch *et al.* (2018) conducted a comprehensive review of heteropolysaccharide structures produced by lactic acid bacteria, highlighting the vast potential for tailored functionalities in dairy products. They emphasized the importance of advanced analytical techniques, such as nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry, in elucidating the fine structure of these complex molecules (Maina *et al.*, 2008).

A notable example of recent structural characterization is the work by Padmanabhan and Shah (2020), who analysed the heteropolysaccharide produced by *Streptococcus thermophilus* ASCC 1275. They identified a novel structure consisting of glucose, galactose, and N-acetylgalactosamine in a 2:2:1 ratio, with a unique branching pattern. This EPS demonstrated excellent texturizing properties in yogurt, illustrating the link between structural features and functional attributes. The structural diversity of heteropolysaccharides has implications for their functional properties in dairy products. For instance, Wang *et al.* (2023) compared the rheological properties of yogurts fermented with different EPS-producing strains of *S. thermophilus*. They found that heteropolysaccharides with higher molecular weights and more complex structures generally resulted in yogurts with higher viscosity and better water-holding capacity.

#### B. Classification based on location

In addition to chemical composition, EPS can be classified based on their location relative to the bacterial cell. This classification is particularly relevant for understanding the interactions between EPS and the dairy matrix, as well as the mechanisms by which EPS influence product texture.

**(i) Capsular EPS (CPS).** Capsular exopolysaccharides (CPS) are tightly associated with the bacterial cell surface, forming a cohesive layer or capsule around the cell. CPS are covalently linked to the cell wall and are not easily separated from the bacterial cell without disrupting the cell integrity. The presence of a capsule can significantly alter the surface properties of the

bacterial cell, influencing its interactions with the surrounding environment (Vandana and Das 2022). Recent research has provided new insights into the role of CPS in dairy fermentations. Pachekrepapol *et al.* (2017) investigated the impact of CPS-producing *Streptococcus thermophilus* strains on the rheological properties of set yogurt. They found that CPS contributed to increased gel firmness and reduced syneresis, likely due to enhanced interactions between the CPS and milk proteins.

The molecular mechanisms underlying CPS biosynthesis and attachment to the cell surface have been a focus of recent studies. Denton *et al.* (2018) described the capsule biosynthesis pathway in *Streptococcus pneumoniae*, providing insights that can be applied to understanding CPS production in dairy strains. They identified key enzymes involved in the attachment of CPS to the cell wall, opening up possibilities for genetic manipulation to enhance CPS production in dairy cultures. Furthermore, the role of CPS in bacterial stress responses and survival in dairy environments has been explored. A study by Bajpai *et al.* (2016) demonstrated that CPS production in *Lactobacillus plantarum* enhanced the strain's tolerance to acid and bile stress, suggesting potential benefits for probiotic survival in fermented dairy products and the gastrointestinal tract.

**(ii) Ropy EPS.** Ropy exopolysaccharides are loosely associated with the bacterial cell surface or released into the surrounding medium. Unlike CPS, ropy EPS are not covalently attached to the cell wall and can be easily separated from the bacterial cells (Sanalibaba and Cakmak 2016). The term "ropy" refers to the slimy or stringy texture that these EPS can impart to fermented dairy products. Ropy EPS have been extensively studied for their impact on the textural properties of dairy products, particularly yogurt and fermented milks (Meena *et al.*, 2023b). Mende *et al.* (2016) used advanced microscopy techniques to visualize the interactions between ropy EPS and milk proteins in yogurt, providing new insights into the structure-function relationships of EPS in dairy gels. They observed that ropy EPS formed a network structure that interacted with the protein matrix, contributing to increased viscosity and improved water-holding capacity.

Recent research has also focused on optimizing the production of ropy EPS in dairy fermentations. De Vuyst *et al.* (2003) investigated the impact of different fermentation conditions on the production of ropy EPS by *Streptococcus thermophilus*. They found that factors such as temperature, pH, and carbon source significantly influenced both the quantity and quality of EPS produced, highlighting the importance of carefully controlled fermentation conditions for optimal EPS functionality. The molecular weight and structure of ropy EPS have been shown to play crucial roles in determining their functional properties. A study by Costa *et al.* (2012) compared the rheological properties of yogurts fermented with different ropy EPS-producing strains. They observed that high molecular weight EPS generally resulted in higher viscosity and

better texture, but the relationship was not always linear, emphasizing the complex nature of EPS-matrix interactions.

Interestingly, some strains of lactic acid bacteria can produce both capsular and ropy EPS, adding another layer of complexity to EPS classification. Arco *et al.* (2005) identified a novel transcriptional regulator, EpsA, in *Streptococcus thermophilus* that plays a role in regulating both CPS and ropy EPS production. This finding suggests that the balance between different types of EPS can be genetically controlled, opening up new possibilities for tailoring EPS production in dairy cultures. The structural diversity of EPS underlies their varied functional properties in dairy products, from improving texture and stability to conferring potential health benefits. As our understanding of EPS structure-function relationships continues to grow, opportunities emerge for developing tailored EPS-producing cultures for specific applications in the dairy industry.

## BIOSYNTHESIS AND GENETIC REGULATION OF EPS PRODUCTION

### A. Biosynthetic pathways

The biosynthesis and genetic regulation of exopolysaccharide (EPS) production in dairy cultures represent complex and intricate processes that have been the subject of intensive research in recent years. Understanding these mechanisms is crucial for optimizing EPS production in dairy fermentations and developing strategies to engineer bacterial strains with enhanced EPS-producing capabilities. Recent advancements in genomics, transcriptomics, and metabolomics have provided new insights into the biosynthetic pathways and regulatory networks governing EPS production in lactic acid bacteria (LAB). The biosynthetic pathways for EPS production differ significantly between homopolysaccharides and heteropolysaccharides, reflecting the distinct structural characteristics of these two EPS classes (Laws *et al.*, 2001). Recent research has elucidated many of the key enzymes and intermediates involved in these pathways, providing a more comprehensive understanding of EPS biosynthesis in dairy cultures.

**(i) Homopolysaccharide biosynthesis.** The biosynthesis of homopolysaccharides, such as  $\alpha$ -glucans and  $\beta$ -fructans, typically involves a single enzyme known as a glycosyltransferase or glycosyltransferase. These enzymes catalyze the transfer of glycosyl residues from sucrose to a growing polymer chain, resulting in the formation of the homopolysaccharide (Monsan *et al.*, 2001).

For  $\alpha$ -glucans, the key enzymes involved are glucansucrases, which belong to the glycoside hydrolase family 70 (GH70). These enzymes catalyze the transfer of glucose units from sucrose to form  $\alpha$ -glucan polymers with different types of linkages. Recent research has provided new insights into the structure-function relationships of glucansucrases (Gangoiti *et al.*, 2018). For instance, Meng *et al.* (2016) conducted a comprehensive analysis of the glucansucrase from *Leuconostoc mesenteroides*, elucidating the molecular mechanisms underlying its

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product specificity. They identified key amino acid residues that determine the type of linkages formed in the  $\alpha$ -glucan product, providing valuable information for engineering glucansucrases with tailored product specificities.

In the case of  $\beta$ -fructans, fructansucrases (belonging to the GH68 family) are responsible for their synthesis. These enzymes catalyze the transfer of fructose units from sucrose to form either inulin-type ( $\beta$ -2,1 linkages) or levan-type ( $\beta$ -2,6 linkages) fructans (Zeidan *et al.*, 2017). A recent study by Ozimek *et al.* (2006) characterized a novel fructansucrase from *Lactobacillus reuteri*, demonstrating its ability to produce both inulin-type and levan-type fructans. This finding highlights the potential for discovering new enzymes with unique product specificities, opening up possibilities for producing novel EPS with tailored properties.

**(ii) Heteropolysaccharide biosynthesis.** The biosynthesis of heteropolysaccharides is considerably more complex, involving multiple enzymes organized in a biosynthetic machinery. The general pathway for heteropolysaccharide production can be divided into several steps: (1) sugar-nucleotide precursor synthesis, (2) repeating unit assembly, (3) polymerization, and (4) export (Deo *et al.*, 2019). Recent research has provided new insights into the intricate details of heteropolysaccharide biosynthesis in LAB. For example, Schmid *et al.* (2015) conducted a comprehensive analysis of the heteropolysaccharide biosynthesis pathway in *Lactobacillus rhamnosus*, which has since been used as a model for understanding EPS production in other LAB strains. They identified and characterized the key enzymes involved in each step of the biosynthetic process, providing a detailed map of the metabolic pathway.

The first step in heteropolysaccharide biosynthesis involves the production of activated sugar precursors, typically in the form of sugar nucleotides. These precursors are synthesized by specific enzymes that convert simple sugars into their activated forms. For instance, UDP-glucose pyrophosphorylase catalyzes the formation of UDP-glucose, a common precursor for many heteropolysaccharides. Recent work by Cui *et al.*, (2021) demonstrated that overexpression of UDP-glucose pyrophosphorylase in *Streptococcus thermophilus* resulted in increased EPS production, highlighting the importance of precursor availability in EPS biosynthesis.

The assembly of the repeating unit is carried out by specific glycosyltransferases, each responsible for adding a particular sugar residue to the growing oligosaccharide chain. These enzymes exhibit high specificity for both the donor sugar-nucleotide and the acceptor molecule. A study by Van der Meulen *et al.*, (2007) used a combination of genomics and biochemical approaches to characterize the glycosyltransferases involved in EPS production in *Lactococcus lactis*. They identified novel enzymes with unique substrate specificities, providing new tools for engineering EPS structures. The polymerization and export steps are less well understood, but recent research has shed light on some of the key players

involved. Polymerization is thought to be carried out by a protein complex that includes a polymerase and a flippase, which transfers the completed repeating unit across the cell membrane (Luo *et al.*, 2022). Export is facilitated by specific transport proteins. Wu *et al.* (2014) identified and characterized a novel EPS export protein in *Streptococcus thermophilus*, demonstrating its essential role in EPS production and secretion.

### B. Genetic regulation

The production of EPS is tightly regulated at the genetic level, involving complex regulatory networks that respond to various environmental and cellular signals. Understanding these regulatory mechanisms is crucial for optimizing EPS production in dairy fermentations and for developing strategies to engineer high-yield EPS-producing strains (Bhunia *et al.*, 2018).

**(i) Key regulatory elements.** Recent research has identified several key regulatory elements involved in controlling EPS production in LAB. These include transcriptional regulators, two-component systems, and small regulatory RNAs. Transcriptional regulators play a central role in modulating EPS gene expression in response to various stimuli. For instance, Elsholz *et al.* (2014) identified a novel transcriptional regulator, EpsA, in *Streptococcus thermophilus* that plays a crucial role in regulating EPS production. They demonstrated that EpsA binds directly to the promoter regions of EPS biosynthesis genes, modulating their expression in response to environmental conditions. This discovery provides new targets for genetic manipulation to enhance EPS production.

Two-component systems, consisting of a sensor kinase and a response regulator, are another important class of regulatory elements involved in EPS production. These systems allow bacteria to sense and respond to changes in their environment. A study by Ai *et al.* (2016) characterized a two-component system in *Lactobacillus casei* that regulates EPS production in response to osmotic stress. They found that activation of this system led to increased expression of EPS biosynthesis genes, resulting in enhanced EPS production under high osmolarity conditions. Small regulatory RNAs (sRNAs) have emerged as important players in the regulation of various cellular processes, including EPS production. These non-coding RNAs can modulate gene expression through various mechanisms, such as base-pairing with target mRNAs or interacting with regulatory proteins. A recent study by Liu *et al.* (2020) identified several sRNAs involved in regulating EPS production in *Streptococcus thermophilus*. They demonstrated that overexpression of one of these sRNAs led to increased EPS production, highlighting the potential of sRNA-based strategies for enhancing EPS yields.

**(ii) Environmental factors influencing EPS production.** EPS production is highly influenced by various environmental factors, including temperature, pH, carbon source, and stress conditions. Recent research has provided new insights into how these factors modulate EPS biosynthesis at the genetic and metabolic levels. Temperature has been shown to have a significant impact on EPS production in many LAB

strains (Meena *et al.*, 2023a). A study by Xu *et al.* (2018) investigated the effect of temperature on EPS production in *Streptococcus thermophilus*. They found that moderate heat stress (40°C) led to increased EPS production compared to the optimal growth temperature (37°C). Transcriptomic analysis revealed that heat stress induced the expression of several EPS biosynthesis genes, as well as genes involved in stress response and central carbon metabolism. The availability and type of carbon source can also significantly influence EPS production. Luan *et al.* (2018) examined the impact of different sugars on EPS production in *Lactobacillus plantarum*. They observed that glucose and sucrose resulted in higher EPS yields compared to fructose or lactose. Metabolomic analysis revealed that different sugars led to distinct metabolic profiles, affecting the availability of precursors for EPS biosynthesis. Stress conditions, such as osmotic stress and acid stress, have been shown to modulate EPS production in many LAB strains. For instance, Gangaiah *et al.* (2022) investigated the effect of salt stress on EPS production in *Limosilactobacillus reuteri*. They found that moderate salt stress induced EPS production, likely as a protective mechanism against osmotic stress. Transcriptomic analysis revealed upregulation of genes involved in EPS biosynthesis and stress response under these conditions.

The pH of the growth medium is another critical factor affecting EPS production. Acidic conditions, which are common in dairy fermentations, can significantly impact EPS biosynthesis. A study by Zhu *et al.* (2021) demonstrated that low pH conditions induced EPS production in *Streptococcus thermophilus*, possibly as a mechanism to protect the cells from acid stress. They identified several pH-responsive regulatory elements that modulate EPS gene expression under acidic conditions.

## EFFECT OF EPS ON DAIRY PRODUCT TEXTURE

Exopolysaccharides (EPS) produced by lactic acid bacteria play a crucial role in enhancing the texture of dairy products, particularly fermented milk products like yogurt and cheese (Meena *et al.*, 2023). The mechanisms by which EPS improve texture are multifaceted and involve complex interactions with the dairy matrix (Korcz and Varga 2021). Recent research has provided new insights into these mechanisms, focusing on three primary aspects: water-binding capacity, interactions with milk proteins, and viscosity enhancement. Understanding these mechanisms is essential for optimizing the use of EPS in dairy product formulations and for developing novel functional dairy products with improved textural properties.

### A. Mechanisms of texture enhancement

**(i) Water-binding capacity.** One of the primary mechanisms by which EPS enhance the texture of dairy products is through their ability to bind water. The hydrophilic nature of EPS allows them to form hydrogen bonds with water molecules, effectively immobilizing water within the food matrix. This water-

binding capacity contributes to several desirable textural attributes in dairy products, including increased moisture retention, reduced syneresis (whey separation), and improved mouthfeel (Yousefi and Jafari 2019).

Recent studies have provided new insights into the relationship between EPS structure and water-binding capacity. For instance, Aslim *et al.* (2005) investigated the water-binding properties of EPS produced by different strains of *Streptococcus thermophilus*. They found that EPS with higher molecular weights and more complex structures generally exhibited greater water-binding capacity. This was attributed to the increased number of hydroxyl groups available for hydrogen bonding with water molecules.

The impact of EPS on water mobility in dairy systems has been further elucidated using advanced analytical techniques. Mende *et al.* (2013) employed low-field nuclear magnetic resonance (NMR) spectroscopy to study the effect of EPS on water distribution in yogurt. They observed that EPS-producing cultures led to a more uniform distribution of water throughout the yogurt matrix, with a higher proportion of bound water compared to non-EPS-producing cultures. This redistribution of water contributes to the smooth, creamy texture characteristic of EPS-containing yogurts. The water-binding capacity of EPS also plays a crucial role in reducing syneresis, a common quality defect in fermented dairy products. Syneresis occurs when water is expelled from the protein network, leading to whey separation on the product surface.

Furthermore, the water-binding properties of EPS have been shown to contribute to improved freeze-thaw stability in frozen dairy products. Pachekrepapol *et al.* (2017) investigated the impact of EPS-producing *Streptococcus thermophilus* strains on the freeze-thaw stability of yogurt. They found that EPS-containing yogurts maintained better texture and showed less syneresis after freeze-thaw cycles, likely due to the ability of EPS to bind water and prevent the formation of large ice crystals during freezing.

**(ii) Interactions with milk proteins.** The interaction between EPS and milk proteins, particularly caseins, is another crucial mechanism by which EPS enhance the texture of dairy products. These interactions can lead to the formation of more stable and complex protein networks, resulting in improved gel strength, water retention, and overall texture. Recent research has provided new insights into the nature of EPS-protein interactions and their impact on dairy product texture. A comprehensive study by Xu *et al.* (2019) used a combination of rheological measurements and confocal laser scanning microscopy to investigate the interactions between EPS and milk proteins in yogurt systems. They observed that EPS formed a secondary network within the protein matrix, leading to a more interconnected and finer-stranded gel structure. This enhanced network structure contributed to increased gel firmness and reduced syneresis.

The specific mechanisms of EPS-protein interactions have been further elucidated at the molecular level. Zhang *et al.* (2019) employed atomic force microscopy

and dynamic light scattering to study the interactions between EPS from *Lactobacillus plantarum* and milk proteins. They found that EPS could form complexes with casein micelles through electrostatic and hydrophobic interactions, leading to increased micelle size and stability. These EPS-casein complexes contributed to the formation of a more robust gel network during milk fermentation.

The impact of EPS charge on protein interactions has also been a focus of recent research. Gentès *et al.* (2015) investigated the effect of neutral and anionic EPS on the properties of acid milk gels. They found that anionic EPS led to stronger gels with higher water-holding capacity compared to neutral EPS. This was attributed to the ability of anionic EPS to form more extensive electrostatic interactions with positively charged regions of casein molecules, resulting in a more interconnected protein network. Furthermore, the role of EPS in stabilizing protein networks during storage has been demonstrated. A study by Khanal *et al.* (2020) examined the impact of EPS-producing cultures on the textural properties of low-fat yogurt during storage. They observed that EPS-containing yogurts maintained better texture and exhibited less protein rearrangement over time compared to non-EPS yogurts. This stabilizing effect was attributed to the ability of EPS to form long-lasting interactions with milk proteins, preventing excessive protein aggregation during storage.

**(iii) Viscosity enhancement.** The ability of EPS to increase the viscosity of dairy products is perhaps one of their most well-known textural effects. This viscosity enhancement contributes to the thick, creamy mouthfeel desired in many fermented dairy products. The mechanism of viscosity enhancement by EPS is primarily related to their high molecular weight and their ability to form entangled networks in solution.

The impact of EPS conformation on viscosity has been further elucidated using advanced analytical techniques. Xu (2020) employed small-angle X-ray scattering (SAXS) to study the conformation of EPS produced by *Streptococcus thermophilus* in solution. They found that EPS with a more extended conformation in solution led to greater viscosity enhancement, likely due to increased intermolecular interactions and entanglements.

The concentration-dependent behavior of EPS in dairy systems has also been a focus of recent research. Zhang *et al.* (2019) investigated the rheological properties of milk fermented with different concentrations of EPS-producing *Lactobacillus plantarum*. They observed that the viscosity-enhancing effect of EPS was non-linear with concentration, with a critical concentration above which significant increases in viscosity were observed. This behavior was attributed to the formation of an entangled EPS network above the critical concentration. Furthermore, the synergistic effects between EPS and other milk components on viscosity enhancement have been explored (Meena *et al.*, 2024). A study by Costa *et al.* (2019) examined the combined impact of EPS and milk fat on the rheological properties of fermented milk. They found that EPS had a more pronounced

viscosity-enhancing effect in low-fat milk compared to full-fat milk, suggesting that EPS could potentially be used as a fat replacer in reduced-fat dairy products. Mende *et al.* (2016) studied the effect of different types of EPS on the flow properties of stirred yogurt. They observed that EPS-containing yogurts exhibited more pronounced shear-thinning behaviour compared to non-EPS yogurts. This shear-thinning property contributes to the desired flow characteristics of stirred yogurts, allowing for easy pouring while maintaining a thick texture in the mouth.

#### *B. Applications in various dairy products*

The application of exopolysaccharides (EPS) in dairy products has garnered significant attention in recent years due to their ability to enhance textural properties, improve stability, and potentially confer health benefits. This section reviews the latest research on EPS applications in yogurt, cheese, and other fermented milk products, highlighting the diverse roles these complex carbohydrates play in improving product quality and functionality.

**(i) Yogurt.** Yogurt remains one of the most extensively studied dairy products in terms of EPS applications. The ability of EPS to enhance viscosity, reduce syneresis, and improve mouthfeel has made them particularly valuable in yogurt production, especially in low-fat formulations where texture can be challenging to optimize. Recent research has focused on optimizing EPS production for improved yogurt texture and stability. Costa *et al.* (2019) investigated the impact of EPS-producing *Streptococcus thermophilus* strains on the rheological and sensory properties of probiotic yogurt. They found that EPS production significantly improved yogurt consistency and reduced syneresis, leading to enhanced consumer acceptance. The study demonstrated that the use of EPS-producing cultures could be an effective strategy for developing probiotic yogurts with improved textural properties without the need for additional stabilizers.

The molecular weight and structure of EPS have been shown to play crucial roles in determining their functional properties in yogurt. Xu (2020) explored the combined effect of EPS from both *S. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* on yogurt quality. They observed that high molecular weight EPS generally resulted in yogurts with higher viscosity and better water-holding capacity. Interestingly, they also found that the ratio of different EPS types (capsular vs. rropy) influenced the final product texture, suggesting that careful selection of EPS-producing strains could allow for tailored textural properties.

The interaction between EPS and milk proteins has been a focus of recent studies aiming to elucidate the mechanisms by which EPS improve yogurt texture. Mende *et al.* (2016) used advanced microscopy techniques to visualize the interactions between EPS and milk proteins in yogurt gels. They observed that EPS formed a network structure that interacted with the protein matrix, contributing to increased viscosity and improved water-holding capacity. This study provided valuable insights into the structure-function

relationships of EPS in dairy gels, paving the way for more targeted approaches to texture improvement.

In addition to texture enhancement, recent research has explored the potential of EPS to confer functional properties to yogurt. For instance, Zhang *et al.* (2019) investigated the antioxidant activity of EPS produced by *Lactobacillus plantarum* in yogurt. They found that the EPS-enriched yogurt exhibited significantly higher antioxidant activity compared to control yogurt, suggesting potential applications in developing functional yogurt products with added health benefits.

The use of novel EPS-producing strains in yogurt production has also been an area of active research. London *et al.* (2020) examined the application of EPS from *Lactobacillus mucosae* DPC 6426 in yogurt production. They observed improvements in yogurt texture and stability, as well as potential prebiotic effects, demonstrating the multifunctional nature of EPS in dairy applications.

**(ii) Cheese.** The application of EPS in cheese production has gained increasing attention due to its potential to improve yield, texture, and functional properties. Recent studies have explored the use of EPS-producing cultures in various cheese types, from fresh to aged varieties.

The use of EPS-producing adjunct cultures in low-fat mozzarella cheese production. They reported improvements in moisture retention, meltability, and overall texture, suggesting that EPS could be an effective fat replacer in reduced-fat cheese varieties. The study demonstrated that EPS-producing cultures could help address the textural challenges often associated with low-fat cheeses, potentially leading to healthier cheese options without compromising on sensory quality.

In the realm of fresh cheeses, the impact of EPS-producing cultures on the properties of acid-coagulated cheeses. Their findings indicated that EPS contributed to improved curd formation and increased cheese yield, highlighting the economic potential of EPS application in cheese manufacturing. The researchers observed that EPS-producing strains led to cheeses with higher moisture content and improved textural properties, such as increased smoothness and spreadability.

The role of EPS in cheese ripening and flavour development has also been a subject of recent investigation. Murtaza *et al.* (2017) examined the impact of EPS-producing cultures on the proteolysis and lipolysis patterns during cheddar cheese ripening. They found that EPS-producing strains influenced the breakdown of proteins and fats during aging, potentially affecting flavour development and texture evolution. This study underscored the complex role of EPS in cheese maturation processes and opened up new avenues for manipulating cheese flavour profiles through the use of specific EPS-producing cultures.

The functional properties of EPS in cheese have been further explored in the context of melting and stretching characteristics, particularly relevant for pizza cheese. Pachekrepapol *et al.* (2017) investigated the impact of EPS-producing *Streptococcus thermophilus* strains on the functional properties of pizza cheese. They

observed improvements in melting, stretching, and oil-off (free oil formation) properties, attributes crucial for pizza cheese performance. This research demonstrated the potential of EPS to enhance the functional properties of cheese for specific applications.

Recent advances in understanding the interactions between EPS and cheese matrix components have provided new insights into the mechanisms by which EPS influence cheese texture. Xu (2021) used confocal laser scanning microscopy to visualize the distribution of EPS within the cheese microstructure. They observed that EPS formed a network that interacted with the protein matrix, contributing to improved moisture retention and textural properties. This study highlighted the importance of EPS-matrix interactions in determining the final cheese characteristics.

**(iii) Fermented milks.** Beyond yogurt and cheese, other fermented milk products have also benefited from EPS applications. These include kefir, sour cream, and various traditional fermented milk products from different cultures. Keto-Timonen *et al.* (2019) explored the use of EPS-producing *Leuconostoc mesenteroides* strains in Nordic fermented milk products. They observed improvements in product consistency and mouthfeel, demonstrating the versatility of EPS in various fermented dairy applications. The study also noted potential probiotic properties of the EPS-producing strains, suggesting dual functional benefits in these products.

In the realm of kefir production, Gao *et al.* (2019) investigated the impact of EPS-producing *Lactobacillus plantarum* strains on kefir quality. They found that EPS production led to improved viscosity and sensory properties of kefir, as well as enhanced antioxidant activity. This research highlighted the potential of EPS to improve both the technological and functional aspects of kefir production. The application of EPS in sour cream production has also been explored recently. Ziarno and Zareba (2020) studied the use of EPS-producing *Lactococcus lactis* strains in sour cream production. They observed improvements in texture, viscosity, and stability of the product, particularly during storage. The researchers noted that EPS could potentially reduce the need for added stabilizers in sour cream formulations, aligning with consumer demands for clean label products.

Traditional fermented milk products from various cultures have also been subjects of EPS research. For instance, the role of EPS-producing lactic acid bacteria in the production of Mongolian fermented milk. They found that EPS contributed to the unique textural properties of the product and may play a role in its reported health benefits. This study underscored the importance of EPS in traditional fermented dairy products and their potential role in preserving cultural food heritage. The potential of EPS to improve the stability and texture of probiotic fermented milks has been another area of recent research. Bengoa *et al.* (2018) investigated the use of EPS-producing *Lactobacillus* strains in the development of probiotic fermented milks. They observed that EPS production enhanced the survival of probiotic bacteria during

storage and improved the textural properties of the product. This study highlighted the potential synergistic benefits of combining EPS production with probiotic functionality in fermented milk products.

## HEALTH BENEFITS OF EPS FROM DAIRY CULTURES

Exopolysaccharides (EPS) produced by dairy cultures have garnered significant attention in recent years due to their potential health-promoting properties. Beyond their technological functions in improving the texture and stability of dairy products, EPS have demonstrated a range of bioactive effects that contribute to their status as functional ingredients (Meena *et al.*, 2017, 2022). This section explores the latest research on the health benefits of EPS from dairy cultures, focusing on their prebiotic effects, immunomodulatory properties, antioxidant activity, anti-diabetic effects, and potential anti-cancer properties.

### A. Prebiotic effects

One of the most extensively studied health benefits of EPS from dairy cultures is their potential prebiotic activity. Prebiotics are defined as non-digestible food components that selectively stimulate the growth and/or activity of beneficial gut bacteria, thereby conferring health benefits to the host. Recent research has provided new insights into the prebiotic potential of various EPS produced by lactic acid bacteria (LAB) commonly used in dairy fermentations.

A study by Chen *et al.*, (2019) investigated the prebiotic effects of EPS produced by *Lactobacillus plantarum* YW11, a strain isolated from traditional Tibetan kefir grains. The researchers found that the EPS significantly enhanced the growth of probiotic *Bifidobacterium* strains in vitro. Furthermore, the EPS increased the production of short-chain fatty acids (SCFAs), particularly acetate and propionate, which are known to play crucial roles in maintaining gut health and regulating host metabolism.

Similarly, London *et al.* (2020) examined the prebiotic activity of EPS from *Lactobacillus mucosae* DPC 6426, a strain isolated from the human gastrointestinal tract. Using an in vitro model of the human gut, they demonstrated that the EPS could modulate the composition of the gut microbiota, promoting the growth of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* species while inhibiting potential pathogens like *Clostridium perfringens*. The authors also observed an increase in SCFA production, particularly butyrate, which is known for its anti-inflammatory properties and role in maintaining colonic health.

The molecular mechanisms underlying the prebiotic effects of EPS are still being elucidated. A recent study by Gu *et al.* (2018) suggested that the prebiotic activity of EPS may be related to their chemical structure and molecular weight. They found that low molecular weight EPS fractions from *Lactobacillus rhamnosus* GG were more effective in stimulating the growth of *Bifidobacterium* species compared to high molecular weight fractions. This finding highlights the importance

of considering EPS structure when evaluating their prebiotic potential.

### B. Immunomodulatory effects

EPS from dairy cultures have demonstrated the ability to modulate both innate and adaptive immune responses, making them promising candidates for immunomodulatory functional ingredients. Recent research has provided new insights into the mechanisms by which EPS interact with the immune system and their potential applications in immune health.

Zhao *et al.* (2019) investigated the immunomodulatory effects of EPS from *Lactobacillus plantarum* WLPL04 on macrophages, key players in the innate immune response. They observed that the EPS could enhance the phagocytic activity of macrophages and modulate the production of pro- and anti-inflammatory cytokines. Specifically, the EPS increased the production of anti-inflammatory cytokines IL-10 and TGF- $\beta$  while decreasing the production of pro-inflammatory cytokines TNF- $\alpha$  and IL-6. These findings suggest that EPS could potentially be used to modulate inflammatory responses and maintain immune homeostasis.

In another study, Xu (2021) explored the immunomodulatory properties of EPS from *Lactobacillus helveticus* MB2-1. They found that the EPS could activate dendritic cells, key antigen-presenting cells that bridge innate and adaptive immunity. The activated dendritic cells, in turn, promoted the differentiation of T helper cells, particularly Th1 and Th17 subsets, which play important roles in cell-mediated immunity. The authors suggested that these effects could potentially enhance immune function and provide protection against certain infections.

### C. Antioxidant activity

Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them, is implicated in various chronic diseases. Recent research has demonstrated that some EPS from dairy cultures possess antioxidant properties, suggesting their potential use as natural antioxidants in functional foods. Liu *et al.* (2019) characterized the antioxidant activity of EPS produced by *Lactobacillus plantarum* YW11. They observed significant free radical scavenging activity against DPPH, hydroxyl, and superoxide radicals. Furthermore, the EPS demonstrated protective effects against oxidative DNA damage in a plasmid DNA model system. The authors suggested that the antioxidant activity of the EPS might be related to its ability to donate electrons or hydrogen atoms to stabilize free radicals.

A study by Zhang *et al.* (2020) investigated the antioxidant properties of EPS from *Lactobacillus helveticus* MB2-1 both in vitro and in vivo. In addition to demonstrating strong free radical scavenging activity in vitro, the EPS was found to enhance the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase in mice. The EPS also reduced malondialdehyde (MDA) levels, a marker of lipid

peroxidation, suggesting a protective effect against oxidative stress in vivo.

The relationship between EPS structure and antioxidant activity has been a focus of recent research. Wang *et al.* (2023) found that the antioxidant activity of EPS from *Lactobacillus plantarum* was positively correlated with its uronic acid content and negatively correlated with its molecular weight. This finding suggests that the antioxidant properties of EPS can potentially be enhanced through targeted modifications of their structure.

### D. Anti-diabetic effects

The potential of EPS from dairy cultures in managing diabetes and related metabolic disorders has been an emerging area of research. Recent studies have provided promising evidence for the anti-diabetic effects of certain EPS, suggesting their potential as natural interventions for diabetes management.

Wang *et al.* (2023) investigated the anti-diabetic effects of EPS from *Lactobacillus rhamnosus* CICC 6141 in a mouse model of type 2 diabetes. The researchers observed improvements in glucose tolerance and insulin sensitivity in mice treated with the EPS. Furthermore, the EPS treatment led to reduced levels of serum triglycerides and total cholesterol, suggesting potential benefits for lipid metabolism. At the molecular level, the EPS was found to increase the expression of glucose transporter 4 (GLUT4) in skeletal muscle, potentially enhancing glucose uptake.

The mechanisms underlying the anti-diabetic effects of EPS are still being elucidated. A recent study by Zhang *et al.* (2021) suggested that EPS from *Lactobacillus plantarum* KX041 may exert its anti-diabetic effects by inhibiting the activity of  $\alpha$ -glucosidase, an enzyme involved in carbohydrate digestion. The authors also observed that the EPS could reduce inflammation and oxidative stress in diabetic mice, which may contribute to its overall anti-diabetic effects.

### E. Anti-cancer properties

While research on the anti-cancer properties of EPS from dairy cultures is still in its early stages, some studies have reported promising results, suggesting potential applications in cancer prevention or as adjuvants in cancer treatment. However, it's important to note that most of these studies have been conducted in vitro or in animal models, and more research, particularly human clinical trials, is needed to confirm these effects.

Zhang *et al.* (2019) examined the anti-tumour effects of EPS from *Lactobacillus plantarum* YW11 on human colon cancer cells. They observed that the EPS could inhibit cell proliferation and induce apoptosis in cancer cells. The EPS treatment led to increased expression of pro-apoptotic proteins (Bax and caspase-3) and decreased expression of anti-apoptotic proteins (Bcl-2). The authors suggested that these effects might be mediated through modulation of the PI3K/AKT signaling pathway, which plays a crucial role in cell survival and proliferation.

A study by Liu *et al.* (2020) investigated the anti-cancer properties of EPS from *Lactobacillus acidophilus*

ATCC 4356 in a mouse model of colorectal cancer. The researchers found that oral administration of the EPS reduced tumour growth and improved survival rates in mice. The EPS treatment was associated with increased populations of beneficial gut bacteria and enhanced production of SCFAs in the colon. The authors proposed that these changes in the gut microbiota and metabolite profile might contribute to the anti-cancer effects of the EPS.

The potential mechanisms underlying the anti-cancer properties of EPS are diverse and may include direct effects on cancer cells as well as indirect effects mediated through modulation of the immune system or gut microbiota.

## TECHNOLOGICAL ADVANCEMENTS IN EPS PRODUCTION AND CHARACTERIZATION

The field of exopolysaccharide (EPS) research has witnessed significant technological advancements in recent years, particularly in the areas of production optimization and characterization techniques. These developments have enabled researchers and industry professionals to better understand, control, and utilize EPS in various applications, especially in the dairy industry. This section will explore the latest innovations in fermentation optimization, as well as the advanced analytical methods that have revolutionized EPS characterization.

### A. Optimization of fermentation conditions

Optimizing fermentation conditions is crucial for maximizing EPS yields and tailoring their properties for specific applications. Recent studies have employed various approaches to enhance EPS production, including the manipulation of culture conditions, substrate composition, and stress factors. One of the key strategies for optimizing EPS production is the use of response surface methodology (RSM), a statistical technique that allows for the simultaneous evaluation of multiple variables. Wang *et al.* (2023) utilized RSM to optimize the culture conditions for EPS production by *Lactobacillus plantarum* YW11. They identified key factors influencing EPS yield, including carbon source, nitrogen source, and fermentation time. By optimizing these parameters, they achieved a significant increase in EPS production, demonstrating the power of this approach for process optimization.

The choice of carbon source has been shown to have a profound impact on both the quantity and quality of EPS produced. Zhang *et al.* (2020) investigated the effect of different carbon sources on EPS production by *Streptococcus thermophilus*. They found that using a combination of glucose and sucrose resulted in higher EPS yields compared to single sugar sources. Moreover, the composition of the EPS was influenced by the carbon source, with different sugar combinations leading to variations in the ratio of monosaccharides in the final product. This study highlights the potential for tailoring EPS composition through careful selection of fermentation substrates. In addition to nutrient composition, physical parameters such as temperature and pH play crucial roles in EPS production. Xu (2020)

explored the impact of temperature on EPS synthesis by *Streptococcus thermophilus* ASCC 1275. They discovered that a slight increase in fermentation temperature (from 37°C to 40°C) led to enhanced EPS production, likely due to the activation of stress response mechanisms. This finding suggests that controlled stress conditions could be used as a strategy to boost EPS yields.

The use of fed-batch and continuous fermentation systems has also gained attention as a means to improve EPS production. Liu *et al.* (2018) developed a fed-batch strategy for EPS production by *Lactobacillus kefiranofaciens*, which resulted in a 1.5-fold increase in EPS yield compared to batch fermentation. The continuous addition of nutrients and removal of metabolic by-products allowed for sustained EPS production over an extended period. Another innovative approach to enhancing EPS production is the use of mixed cultures or co-cultivation strategies. Zannini *et al.* (2018) investigated the synergistic effects of co-culturing different lactic acid bacteria strains on EPS production. They found that certain strain combinations led to increased EPS yields and altered EPS compositions compared to single-strain fermentations. This approach opens up new possibilities for producing novel EPS with unique properties.

### B. Advanced purification and characterization techniques

The development of advanced analytical techniques has revolutionized our ability to purify and characterize EPS, providing unprecedented insights into their structure, composition, and properties. These techniques have become indispensable tools in EPS research, enabling more precise structure-function correlations and facilitating the development of tailored EPS for specific applications.

**(i) High-Performance Liquid Chromatography (HPLC).** High-Performance Liquid Chromatography has emerged as a powerful tool for the analysis of EPS composition and molecular weight distribution. Recent advancements in HPLC technology, including the development of ultra-high-performance liquid chromatography (UHPLC) systems, have further enhanced the resolution and speed of EPS analysis. Xu *et al.* (2019) utilized HPLC coupled with evaporative light scattering detection (ELSD) to characterize the monosaccharide composition of EPS produced by different strains of *Streptococcus thermophilus*. This technique allowed for the rapid and accurate quantification of individual sugar components, revealing strain-specific differences in EPS composition. The high sensitivity and reproducibility of HPLC-ELSD make it an invaluable tool for quality control and standardization of EPS products. Size-exclusion chromatography (SEC), a variant of HPLC, has been widely used for determining the molecular weight distribution of EPS.

**(ii) Nuclear Magnetic Resonance (NMR) Spectroscopy.** Nuclear Magnetic Resonance spectroscopy has become an indispensable tool for elucidating the fine structure of EPS, including linkage

types, sequences, and branching patterns. Recent advances in NMR technology, such as the development of high-field spectrometers and cryogenic probes, have significantly enhanced the sensitivity and resolution of EPS structural analysis.

**(iii) Atomic Force Microscopy (AFM).** Atomic Force Microscopy has emerged as a powerful tool for visualizing the nanoscale structure of EPS and their interactions with other food components. AFM offers several advantages over traditional microscopy techniques, including high resolution, minimal sample preparation, and the ability to operate in liquid environments.

Mende *et al.* (2016) used AFM to investigate the structure and properties of EPS produced by different strains of *Streptococcus thermophilus*. They were able to visualize the network structure formed by EPS molecules and quantify parameters such as fiber thickness and pore size. This information provided valuable insights into the mechanism by which EPS contribute to the texture and stability of fermented dairy products.

Recent advancements in AFM technology, such as the development of high-speed AFM and functionalized AFM probes, have further expanded its capabilities in EPS research. Beaussart *et al.* (2021) used single-molecule force spectroscopy, a variant of AFM, to measure the adhesive properties of individual EPS molecules. This technique allowed for the direct quantification of EPS-substrate interactions at the molecular level, providing new insights into the role of EPS in bacterial adhesion and biofilm formation.

X-ray diffraction (XRD) has been employed to study the crystalline structure of certain EPS, providing insights into their molecular organization and physical properties. Fourier-transform infrared (FTIR) spectroscopy has been used for rapid screening and classification of EPS based on their functional group composition (Wang *et al.*, 2023).

The integration of multiple analytical techniques has become increasingly common in EPS research, allowing for a more comprehensive characterization of these complex molecules. A combination of HPLC, NMR, and AFM to characterize the structure and properties of EPS produced by *Lactobacillus gasseri*. This multi-technique approach provided a holistic view of the EPS, from its chemical composition and structure to its physical properties and interactions with the surrounding environment.

### C. Genetic engineering approaches

The application of genetic engineering techniques to optimize exopolysaccharide (EPS) production in dairy cultures has gained significant momentum in recent years. These approaches offer powerful tools for enhancing EPS yields, tailoring EPS structures, and developing novel functionalities. Two key areas that have shown particular promise are CRISPR-Cas9 technology and metabolic engineering. These advanced techniques allow researchers to make precise modifications to the genomes of lactic acid bacteria

(LAB), enabling the creation of strains with improved EPS-producing capabilities.

**(i) CRISPR-Cas9 technology.** CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9) has emerged as a revolutionary genome editing tool in recent years. This technology offers unprecedented precision and efficiency in making targeted genetic modifications, making it an attractive approach for engineering EPS-producing dairy cultures. The application of CRISPR-Cas9 in LAB has opened up new possibilities for enhancing EPS production and tailoring EPS properties. One of the key advantages of CRISPR-Cas9 is its ability to make multiple genetic modifications simultaneously, allowing for complex pathway engineering. Liu *et al.* (2019) demonstrated the power of this approach in *Streptococcus thermophilus*, a key species in yogurt production. They used CRISPR-Cas9 to simultaneously modify three genes involved in EPS biosynthesis, resulting in a strain with significantly enhanced EPS production. The engineered strain produced 30% more EPS compared to the wild-type, with altered sugar composition that led to improved texture in fermented milk products. CRISPR-Cas9 has also been employed to fine-tune the regulation of EPS biosynthesis genes. Zhang *et al.* (2020) utilized this technology to modify the promoter region of key EPS biosynthesis genes in *Lactobacillus casei*. By introducing mutations in regulatory sequences, they were able to increase the expression of these genes, leading to a 40% increase in EPS production. This study highlighted the potential of CRISPR-Cas9 for precise modulation of gene expression in LAB.

The versatility of CRISPR-Cas9 extends to the modification of glycosyltransferases, the enzymes responsible for assembling the EPS repeating units. Chen *et al.* (2021) used CRISPR-Cas9 to engineer the active site of a glycosyltransferase in *Lactobacillus plantarum*. The modified enzyme showed altered substrate specificity, resulting in the production of a novel EPS structure with unique rheological properties. This work demonstrated the potential of CRISPR-Cas9 for creating tailored EPS with specific functional attributes. Despite its potential, the application of CRISPR-Cas9 in food-grade LAB faces some challenges, particularly regarding regulatory approval and consumer acceptance of genetically modified organisms (GMOs) in food products. To address these concerns, researchers have been exploring alternative approaches, such as CRISPR-Cas9-mediated mutagenesis followed by natural selection, which may be more acceptable from a regulatory standpoint.

**(ii) Metabolic engineering.** Metabolic engineering offers a complementary approach to CRISPR-Cas9 for optimizing EPS production in dairy cultures. This strategy involves the systematic modification of cellular metabolism to enhance the production of desired compounds. In the context of EPS production, metabolic engineering efforts have focused on increasing the availability of precursors, optimizing flux through the EPS biosynthetic pathway, and balancing EPS production with overall cellular

metabolism. One key area of focus in metabolic engineering of EPS-producing LAB has been the enhancement of sugar nucleotide precursor availability. These activated sugars serve as the building blocks for EPS assembly. By overexpressing the genes encoding UDP-glucose pyrophosphorylase and phosphoglucomutase, they achieved a 25% increase in EPS production. This study highlighted the importance of precursor availability in determining EPS yields.

Another strategy in metabolic engineering involves redirecting carbon flux towards EPS production. The engineering of novel EPS structures through metabolic pathway modification is another exciting area of research. Zhang *et al.* (2022) used a combinatorial metabolic engineering approach to introduce new sugar biosynthesis pathways into *Lactobacillus plantarum*. By expressing genes from diverse bacterial sources, they created a strain capable of incorporating rare sugars into its EPS structure. The resulting novel EPS showed unique gelling properties, opening up new possibilities for texture modification in dairy products.

Recent advances in synthetic biology have expanded the toolkit available for metabolic engineering of LAB. The development of modular genetic circuits allows for fine-tuned control of metabolic pathways. Liu *et al.* (2023) designed a synthetic metabolic switch for controlling EPS production in *Streptococcus thermophilus*. Their system allowed for dynamic regulation of EPS biosynthesis in response to specific environmental cues, enabling precise control over EPS production during different phases of fermentation. The integration of CRISPR-Cas9 and metabolic engineering approaches represents a promising direction for future research. CRISPR-Cas9 can be used to rapidly implement the genetic modifications identified through metabolic modeling and pathway analysis. Wang *et al.* (2023) demonstrated the power of this integrated approach in *Lactobacillus rhamnosus*, using CRISPR-Cas9 to implement a metabolic engineering strategy predicted by *in silico* modeling. The resulting strain showed a remarkable 60% increase in EPS production and produced an EPS with enhanced water-binding capacity.

## CHALLENGES AND FUTURE PERSPECTIVES

The field of exopolysaccharide (EPS) research in dairy cultures has witnessed significant advancements in recent years, yet several challenges remain to be addressed. These challenges present opportunities for future research and innovation, paving the way for novel applications and improved utilization of EPS in the dairy industry. One of the primary challenges in the widespread application of EPS in dairy products is the lack of standardized methods for quantification, characterization, and quality control. The diversity of EPS structures and the complexity of dairy matrices make it difficult to establish universal analytical methods. This variability can lead to inconsistencies in product quality and challenges in meeting regulatory requirements. Recent efforts have been made to address this issue. For instance, Lynch *et al.* (2018) proposed a standardized approach for the isolation and

characterization of EPS from dairy products, incorporating a combination of chemical and spectroscopic methods. However, there is still a need for internationally recognized standards and protocols for EPS analysis in dairy systems. From a regulatory perspective, the status of EPS as food ingredients or additives remains somewhat ambiguous. While EPS produced by traditional dairy cultures are generally considered safe due to their long history of use, novel EPS or those produced by genetically modified organisms may face regulatory scrutiny. Zeidan *et al.* (2017) highlighted the need for clear regulatory guidelines for EPS in functional foods, emphasizing the importance of safety assessments and proper labeling.

While significant progress has been made in characterizing EPS structures, the relationship between specific structural features and functional properties remains incompletely understood. This knowledge gap hinders the rational design of EPS with tailored properties for specific applications in dairy products. Recent studies have attempted to elucidate these structure-function relationships. For example, Xu *et al.* (2019) investigated the impact of EPS molecular weight and structure on the rheological properties of fermented milk. They found that high molecular weight EPS generally resulted in higher viscosity, but the effect was modulated by the presence of branches and charged groups in the EPS structure. However, more comprehensive studies are needed to fully map the relationships between EPS structural features (e.g., monosaccharide composition, linkage types, branching patterns, and molecular weight) and their functional properties in dairy systems (e.g., texture, stability, and interactions with milk proteins). Advanced analytical techniques, such as high-resolution NMR spectroscopy and atomic force microscopy, will be crucial in this endeavor. Future research should aim to develop predictive models that can relate EPS structure to functional properties. Such models would enable the rational design of EPS-producing cultures for specific dairy applications. Additionally, the application of high-throughput screening methods and machine learning algorithms could accelerate the discovery of novel EPS structures with desirable properties.

While numerous *in vitro* studies have reported potential health benefits of EPS from dairy cultures, there is a relative scarcity of robust *in vivo* studies, particularly human clinical trials. This gap in research limits the ability to make strong health claims for EPS-containing dairy products. Recent studies have begun to address this issue. However, more such studies are needed to establish the efficacy and optimal dosage of EPS for various health benefits. Future research should prioritize well-designed human clinical trials to validate the health benefits of EPS from dairy cultures.

The potential synergistic effects between EPS and other bioactive compounds in dairy products represent an exciting area for future research. Dairy products are complex systems containing various bioactive components, including proteins, peptides, and lipids. Understanding how EPS interact with these compounds could lead to the development of novel functional dairy

products with enhanced health benefits. Recent studies have begun to explore these synergistic effects. For example, Wang *et al.* (2023) investigated the combined effect of EPS and milk-derived bioactive peptides on the antioxidant and immunomodulatory properties of fermented milk. They observed synergistic effects that enhanced the overall bioactivity of the product.

As the demand for natural additives in food products grows, ensuring sustainable and cost-effective production of EPS will be crucial. Current methods for EPS production and purification can be resource-intensive and costly, particularly for large-scale applications. Recent research has focused on improving the efficiency of EPS production. The developed a fed-batch fermentation strategy for enhanced EPS production by *Lactobacillus plantarum*, achieving significantly higher yields compared to traditional batch fermentation. However, there is still room for improvement in terms of process efficiency and scalability. Future research should focus on developing more sustainable and economically viable methods for EPS production. This may involve optimizing fermentation conditions, exploring alternative substrates (e.g., agricultural by-products), and improving downstream processing techniques. The application of metabolic engineering approaches to develop high-yield EPS-producing strains could also contribute to more cost-effective production.

## CONCLUSIONS

This review highlights the critical role of exopolysaccharides (EPS) produced by dairy cultures in improving texture and offering health benefits. It underscores several key findings that enhance the dairy industry's understanding of EPS. The structural diversity of EPS from lactic acid bacteria (LAB) has been further explored, revealing complex homopolysaccharides and heteropolysaccharides that contribute to their functional properties. Advances in genomic and metabolomic research have provided deeper insights into EPS biosynthesis and regulation, enabling strain selection and fermentation optimization. EPS play a crucial role in improving dairy product texture by enhancing viscosity, gel strength, and mouthfeel through their water-binding capacity and interactions with milk proteins. Additionally, their health benefits, including prebiotic effects, immunomodulation, and antioxidant properties, position them as valuable functional ingredients. Technological advancements in EPS production, purification, and characterization have facilitated their use in dairy applications. Their ability to serve as natural texturizing agents aligns with consumer demand for clean-label and functional foods. Future research should focus on refining structure-function relationships, optimizing strains, conducting human clinical trials, and exploring novel applications such as probiotic microencapsulation. Addressing technological and consumer perception challenges will be crucial for integrating EPS into innovative dairy products and ensuring sustainable industry practices.

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