



A review on exopolysaccharide production by *Lactobacillus acidophilus* and their techno-functional applications in food and pharmaceutical industry

Daniel Joe Dailin^{1,2*}, Loo Yoo Bing¹, Siti Zulaiha Hanapi³, Siti Alyani Mat¹, Tong Woei Yenn⁴, Siti Nazrah Zailani⁵, Ilker Çamkerten⁶, Dalia Sukmawati⁷ and Hesham Ali El Enshasy^{1,2,8}

¹Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310, Skudai, Johor, **Malaysia**

²Department of Bioprocess and Polymer Engineering, Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, 81310, Skudai, Johor, **Malaysia**

³Research Alliances, Universiti Teknologi Malaysia, 81310, Skudai, Johor, **Malaysia**

⁴Universiti Kuala Lumpur, Institute of Medical Science Technology (UniKL MESTECH), Clinical Laboratory Section, A1, 1, Jalan TKS 1, Taman Kajang Sentral, 43000 Kajang, Selangor, **Malaysia**

⁵Faculty of Chemical Engineering & Technology, Universiti Malaysia Perlis, 02600 Arau, Perlis, **Malaysia**

⁶Department of Internal Medicine, Faculty of Veterinary Medicine, Aksaray University, Aksaray, **Turkiye**

⁷Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta, Rawamangun Jakarta Timur, **Indonesia**

⁸City of Scientific Research and Technology Applications (CSAT), New Burg Al Arab Alexandria, **Egypt**

*Correspondence: jddaniel@utm.my Received 04-02-2023, Revised: 24-03-2023, Accepted: 27-03-2023 e-Published: 31-03-2023

Bacterial exopolysaccharides (EPS) are renowned for their technical and functional applications in the food and pharmaceutical industry. EPS retrieved from *Lactobacillus* sp. is one of the bacterial groups that have garnered a great deal of attention by showing its unique behaviors by expressing their ability to promote health benefits. The EPS from *Lactobacillus acidophilus* finds application in many areas like food, pharmaceuticals, bioremediation, cosmetics, and others. As EPS produced by *Lactobacilli* has conferred a range of local and systemic health benefits on the host, synthesis of these metabolites through fermentation should be emphasized. An optimal and cost-effective fermentation process by understanding the parameters and conditions is crucial in optimising the EPS yields. This review gives an overview of the biosynthesis of the EPS and the factors influencing the production of the EPS. Finally, techno-functional applications in food and pharmaceutical products associated with EPS are discussed.

Keywords: exopolysaccharide, *Lactobacillus acidophilus*, pharmaceutical, food

INTRODUCTION

Today, the human diet has evolved into a new trend, as it contains a bioactive compound that can potentially reduce the risk of chronic disease. Of different classes of bioactive compounds microbial polysaccharides of microbial origin such as mushrooms and bacterial polysaccharides received more attention. This is because of their immunomodulatory and anticancer properties with high potential applications in both the nutraceutical and pharmaceutical industries.

One of the bio-active molecules that gained much attention to human health benefits is the exopolysaccharide (EPS) produced by probiotic bacteria (Dailin et al. 2016; Angelin and Kavita, 2020). The EPS contains naturally occurring macromolecules that confer distinctive rheological and physico-chemical properties on the food matrix (Tao et al. 2022). With a high

molecular weight composed of sugar residues, substituted by proteins, DNA, phospholipid and other non-carbohydrates, EPS is regarded as safe (GRAS) to be used in various food and biomedical applications (Mohd Nadzir et al. 2021).

EPS produced by bacteria is experiencing significant growth in the field of functional food engineering and gut health management, as it can reproduce quickly, is easily renewed, and is compatible with the almost EPS isolation method (Mohd Nadzir et al. 2021). Commercially important EPS like xanthan and pullulan were mass-manufactured, but novel EPS features from other sources are required (Dailin et al. 2019; Nordin et al. 2020). Some of the well-known probiotic bacteria have been addressed to produce EPS such as *Lactobacillus acidophilus* LA5 and *Bifidobacterium animalis* subsp. *lactis* BB12 (Amiri et al. 2019), *Lactobacillus fermentum* (Jurášková et al.

2022), *Lactobacillus plantarum* (Silva et al. 2019), *Lactobacillus reuteri* (Dailin et al. 2022) *Lactobacillus lactis* (A-AlMalki, M., 2020) *Lactobacillus rhamnosus* (Oleksy-Sobczak et al. 2020a), *Lactobacillus delbreueckii subsp. Bulgaricus* (Nishimura et al. 2014) and *Lactobacillus kefiranoformis* (Dailin et al., 2021). To enhance EPS production and make it practical for industrial uses, various initiatives including medium and process optimization have been made (Dailin et al. 2015; Dailin et al. 2020).

Lactobacillus acidophilus is one of the most noticeable *Lactobacillus* species that produce EPS for various applications. EPS from *L. acidophilus* is reported to have inhibitory effects against cancer cells (El-Deeb et al. 2018; Deepak et al. 2016), antioxidant properties and antitumor effect (El Ghany et al. 2014) and a pivotal role in maintaining human health (Burakova et al. 2022). *L. acidophilus* is included among the LAB species listed on the Qualified Presumption of Safety (QPS) issued by European Food Safety Authority (EFSA) and generally regarded as safe (GRAS) to be used as probiotic in food and feed ingredients (EFSA, 2022). In particular, US Food and Administration has profoundly recognized *L. acidophilus* NCFM as the approved ingredient of dairy products, functional beverages and nutritional powders, juice bars, ready-to-eat breakfast cereals, chewing gum and confections (FDA, 2019).

Compared to other EPS-producing strains, the yields obtained from LAB are rather low (Sørensen et al. 2022). In accordance with Mozzi et al. (2006), a yield range of 10-15 g/L EPS production is needed from lactic acid bacteria for effective use as food ingredients. Due to this many studies have been carried out to optimize the process in achieving the highest possible yield of EPS at an economical cost. This present review will cover be focusing on the biosynthesis of EPS and analyze the impact of several factors that contribute to the production of EPS as well as its isolation and characterization methods. In this paper, the techno-functional application of EPS in food and pharmaceutical products has also been discussed.

Characteristics of *Lactobacillus acidophilus*

Lactobacillus acidophilus is a highly heterogeneous genus bacteria with a wide range of biochemical and physiological properties (Felis and Dellaglio, 2007). Lactic acid bacteria have the most important gene for *Lactobacillus*, with 145 species in 2008 and increasing progressively to 185 and it causes the reclassification of several species (Carr et al. 2002). Known as an intestinal probiotic, *L. acidophilus* was first isolated by Moro (1900) from infant feces with the initial designation name as *Bacillus acidophilus*. Morphologically with rod rounded ends that occur singly, in pairs, and in short chains that are usually 0.6–0.9 × 1.5–6 µm dimension (Ozogul and Hamed, 2016) and bacteriocins belonging to class II a (Anjum et al. 2014), this species are gram-positive, non-

spore-forming, nonflagellated, nonmotile and is intolerant to salt (William, 1999). By 1920 (Holland) had identified species of *L. acidophilus* from mucous surfaces and this evolved many studies later which eventually came out with 14 phylogenetic subgroups, however, have some different characteristics depending on their differences in strains. Different strain will have their unique uses in different fields (Felis and Dellaglio, 2007; Hammes et al. 2006).

It is undeniable that *L. acidophilus* has become a main useful bacterial in the food industry field, such as in producing milk products, yogurts products as well as in dietary supplementary during the 20th century. This can be proved when *L. acidophilus* is used as a starter culture for milk fermentation, a preservation process developed at the beginning Neolithic era and used in the production of traditional fermented foods for more than 10 000 years (García-Lorenzo et al. 2012). In the food fermentation process, EPS from *L. acidophilus* has been added to contribute to flavour, texture and preservation (Angelin and Kavitha, 2020).

L. acidophilus can be described as probiotic bacteria that is easily found in many parts of the body, especially the human gut. Consuming probiotics may help maintain a healthy mix of the good and bad bacteria in the gut, according to research (Binns and Lee, 2010). Along with other health advantages like boosting the immune system, this helps to promote digestion. Consuming probiotics may strengthen the immune system, according to animal research. For instance, a study indicated that feeding black swordtail fish *L. acidophilus* benefited their immune system in several ways (Ljungh and Wadström, 2016).

Among the characteristics of *L. acidophilus* are that it grows in an acidic pH (<5), anaerobic conditions and undergoes fermentation only. This shows that *L. acidophilus* can be cultured to produce food fermented products. Additionally, *L. acidophilus* is incapable of respiration or oxidative phosphorylation, as it does not contain any cytochromes, porphyrins, and respiratory enzymes. In the metabolic process known as oxidative phosphorylation, cells use enzymes to oxidise nutrients, generating energy that is then used to generate adenosine triphosphate (ATP). They live in areas with high sugar abundance because they utilise sugars as their fermentation substrates, such as the intestines of humans. They have also been described as short gram-positive rods (2-10 m), homo fermentative, and with best growth at 37 to 42 °C (Gilliland et al. 2014). Homo fermentative means that *L. acidophilus* undergoes fermentation as well as producing only a single product. In terms of size and shape, *L. acidophilus* usually forms straight chains of varied lengths and may form coccobacillary shapes that have many round shapes (Altermann et al. 2005).

Exopolysaccharide

Depending on the area, EPS might take one of two types. Slime polysaccharides are loosely linked with the cell surface, whereas capsular polysaccharides have a

close relationship with the cell surface. Bacterial EPS are released extracellularly by cell wall-anchored enzymes or are secreted into the surrounding environment by bacteria. As it may provide rigorous protection against desiccation, poisonous substances, bacteriophages, and osmotic stress, it plays a significant function in lactic acid bacteria. Besides, it also permits adherence against the solid surface and biofilm formation. Based on their chemical structure, purpose, molecular weight, and linkage bonds, the various bacterial polysaccharides can be categorised into several different groups. EPS can be examined based on their monomeric structure to determine their chemical composition (Ruas-Madiedo et al. 2012). Two classes involving homopolysaccharides and heteropolysaccharides are biosynthesized in more than one type of monosaccharide unit (Patterson et al. 2014).

EPS have been categorised into seven groups based on their functional properties. These groups are, respectively, constructive or structural, surface active, active, instructive, redox-active, and nutritive EPS. Neutral EPS are included in the group of biomolecules known as structural EPS because of their architectural role in the support of water retention and cell protection. The development of biofilms may be influenced by molecules with amphiphilic behavior, such as surface-active EPS, which have a variety of chemical structures and surface characteristics. EPS is made up of charged polymers that serve as a cell surface interface with an oppositely charged surface (Nichols et al. 2015).

Biosynthesis of exopolysaccharide production

To avoid the starving conditions brought on by the environment, *L. acidophilus* uses polysaccharides as a source of carbon and energy. Polysaccharides on the surface of cells are essential for interactions between bacteria and their environment (Schmid et al. 2015). EPS aids in bacterial interactions with hosts, namely enhancing colonisation due to their capacity to cling to surfaces. Three distinct pathways such as the Wzx/Wzy-dependent pathway, the ATP-binding ABC transporter pathway, and the synthase-dependent pathway allow *L. acidophilus* to release polysaccharides either sequentially or in bulk (Tytgat and Lebeer, 2014). Glycosyltransferases (GTs) produce lipid-linked polysaccharide repeating units from nucleotide diphosphates (NDPs) or monophosphates (NMPs) in all the pathways.

The Wzx/Wzy dependent pathway is demonstrated by the many glycosyltransferases (GTs) that are transported across the cytoplasmic membrane by a Wzx protein and that are closely related to a undecaprenol diphosphate anchor at the inner membrane. Next, the Wzy protein initiates polymerization at the periplasmic region before they are generated outside the cell surface (Islam and Lam, 2014). It has been demonstrated that additional

proteins associated with the polysaccharide co-polymerase and the exported outer membrane polysaccharide are necessary for the transport of the polymerized repeat units from the periplasm to the cell surface. Since the sugar patterns in every polysaccharide created by the Wzx/Wzy pathway are so different, they are all categorised as heteropolymers. The genes for the flippase (Wzx) and the polymerase (Wzy) are present in all strains that utilise this pathway inside their extracellular polysaccharide operons (Cuthbertson et al. 2010).

Aside from that, the production of capsular polysaccharides primarily uses an ABC transporter-dependent process (Whitney and Howell, 2013). Since they do not adhere to the cell surface, these polysaccharides are not EPS. When only one GT-containing operon is involved, the CPS is constructed similarly to the Wzx/Wzy dependent EPS by the action of GTs at the cytoplasmic face of the inner membrane, resulting in homopolymers. Additionally, when it is produced through the ABC-transporter dependent pathway and multiple GTs are used for the assembly process, it also happens in hetero polymers (Whitney and Howell, 2013). The complex is made up of periplasmic proteins and ABC transporters that bridge the inner membrane. These proteins also share a relationship with those involved in the Wzx/Wzy pathway's secretion mechanism. The ABC-dependent pathway, which is still distinct from Wzx/Wzy, produces molecules that all have a conserved glycolipid at the reducing terminus made of phosphatidic glycerol and a poly-2-keto-3-deoxyoctulosonic acid linkage (Willis et al. 2013).

The third mechanism exports entire polymer strands through membranes and the cell wall and is synthase dependent. For moving repeat units, it does not require a flippase. It is common to practice assembling homo polymers using synthase-dependent routes since they only need one kind of sugar precursor (Chong et al. 2005).

Effects of cultivation conditions on EPS production

To survive in harsh settings, microorganisms like lactic acid bacteria secrete EPS, which are biological polymers. The formation of an extracellular biofilm matrix, which protects germs from hazardous factors like temperature, pH, antibiotics, and host immune systems, depends on EPS as one of the essential components. Diverse LAB has varied adaptation mechanisms that include the build-up of energy-storing solutes and compounds, regulation of the pathways that produce energy, and alteration of the cell membrane under varying growth conditions and medium compositions. The cultivation parameters for *L. acidophilus* refer to such as temperature, pH, time of incubation and agitation rate. The incubation parameter affects the growth and EPS production of *L. acidophilus*.

Table 1: EPS yield in different cultivation parameters

Strain	Processing parameters	Yield of EPSs	Reference
<i>L. acidophilus</i>	Temperature - 37°C Incubation time – 24 hrs pH value – 5.0 Rotation speed – 10 000 rpm	3.96 ± 0.08 g/L	Liu et al. 2016
<i>L. acidophilus</i> DSMZ 20079	Temperature - 37°C Incubation time – 24 – 48 hrs Rotation speed – 10 000 rpm	2 – 5 g/L	El-Deeb et al. 2018
<i>L. acidophilus</i> LA5	Temperature – 30 - 38°C Incubation time –12–48 hrs pH value – 4.5	349.82 ± 5.39 mg/L	Amiri et al. 2019
<i>L. acidophilus</i> TLAB	Temperature - 40°C Incubation time – 24 hrs Rotation speed – 10 000 rpm	0.74 g/L	Abdellah et al. 2015

Table 2: EPS production by *L. acidophilus* using different medium compositions

Strain	Medium composition	Yield (g/l)	Reference
<i>L. acidophilus</i> ATCC	Beef extract 10g, Peptone 20g, Yeast extract 7.5g, Sodium acetate 5.0g, Magnesium sulfate 150mg, Manganous sulfate 35mg, Tween 80 1.5ml, Dipotassium phosphate 3g, Glucose 30g, Trisodium citrate 3g, NaCL 1g	3.45	Liu et al. 2016
<i>L. acidophilus</i> 20079	Beef extract 15g, Peptone 15g, Yeast extract 5g, Sodium acetate 7.5 g, Magnesium sulfate 150mg, Manganous sulfate 35mg, Tween 80 1.0ml, Dipotassium phosphate 3g, Glucose 20g, Trisodium citrate 3g, NaCL 1.5g	3.25	El-Deeb et al. 2018
<i>L. acidophilus</i> LA5	Beef extract 10g, Peptone 15g, Yeast extract 5.0g, Sodium acetate 7.5g, Magnesium sulfate 150mg, Manganous sulfate 53mg, Tween 80 1.5ml, Dipotassium phosphate 2g, Glucose 20g, Trisodium citrate 3g, NaCL 1g	1.16	Amiri et al. 2019
<i>L. acidophilus</i> ATCC	Beef extract 15g, Peptone 10g, Yeast extract 5g, Sodium acetate 5.0g, Magnesium sulfate 100mg, Manganous sulfate 53mg, Tween 80 1ml, Dipotassium phosphate 3g, Glucose 20g, Trisodium citrate 2g, NaCL 1g	2.63	Liu et al. 2016
<i>L. acidophilus</i> LACVG02	Beef extract 15g, Peptone 15g, Yeast extract 7.5g, Sodium acetate 5.0g, Magnesium sulfate 100mg, Manganous sulfate 53mg, Tween 80 1.5ml, Dipotassium phosphate 2g, Glucose 30g, Trisodium citrate 3g, NaCL 1g	1.56	Vijayalakshmi et al. 2017

Fermentation temperature is among the important factors affecting EPS production as it influences the log phase of *L. acidophilus* growth. The optimum temperature for the growth curve of *L. acidophilus* LA5 strain will be at the estimation of 30- 38°C according to the research from Amiri et al. (2019). This is because the optimum temperature gives the highest kinetic energy for the growth curve of *L. acidophilus* and thus increases the rate of biofilm formation. The highest temperature of 40°C for the *L. acidophilus* TLAB strain was reported for the production of EPS (Abdellah et al. (02015). However, it shows a lower EPS production if compared to the

optimum temperature of *L. acidophilus* LA5 strain. This concludes that the optimum temperature for microbial cell growth is not the same as for EPS production. High

temperature may be appropriate for microbial growth, but it suppresses EPS synthesis. Table 1 shows the different parameters of cultivation conditions extracted from different references.

Medium compositions

Different medium compositions will affect the production of EPS by *L. acidophilus*. Different medium compositions including carbon source and nitrogen source influence the cell growth of *L. acidophilus* and EPS production. Table 2 shows the different medium compositions used for *L. acidophilus* cultivation and EPS production.

Effect of carbon sources

Carbon source that plays an important role in the growth of *L. acidophilus* are glucose, sucrose, lactose,

fructose, galactose, raffinose and maltose. According to the research by Vijayalakshmi et al. (2017), glucose provided the highest EPS yield followed by sucrose. However, fructose gives the result of lowest EPS production among the carbon element. This may be because of the molecular formula of different carbon source that determines carbon sources that determine the effect of carbon sources on the growth of *L. acidophilus*. Besides, it also shows that glucose gives higher sugar concentration to it that acts as the major nutrition source for the fermentation process. Besides, the carbon source plays a major role in the cell proliferation and metabolite biosynthesis of *L. acidophilus* via its mechanical pathway. In addition, the carbon source is very essential in the biofilm formation of the lactic acid bacteria and this will lead to EPS production. As a result, the generation of polysaccharides will rise with increased concentration of carbon sources until it hit the maximum point. When beyond this point, any increment of carbon concentration will lead to a decrease in the amount of polysaccharide generated. This is because of the impact of the osmotic effects, lower level of water activity and plasmolysis (Liu et al. 2016).

According to Chen et al. (2015), *L. acidophilus* has a greater carbon source element of whey powder compared to glucose. The research shows a higher biofilm and EPS production compared to glucose, maltose and lactose. However, whey powder shows a magnificent disadvantage in the preparation process. This is because whey powder is produced and would be very inconvenient for subsequent preparation of bacterial powder and this makes the whole process more complicated.

Effects of Nitrogen Source

Nitrogen source contributes to the production of EPS significantly and usage of nitrogen is considered a major sign of production for EPS. Nitrogen source is critical to the cell growth of *L. acidophilus* as well as the trichloroethylene degradation ability. Ammonium salts like ammonium nitrate and ammonium sulphate are more favoured by the production of EPS (Badel et al. 2011). Apart from this, research shows that the supplementation of additional nitrogen sources such as ammonium molybdate, beef extract, ammonium ferric citrate, ammonium per sulphate, peptone, yeast and gelatin in the skim milk enhanced gradually the EPS production of *L. acidophilus*. However, there is no significant increase in EPS production when the concentration of nitrogen source is increased. The change in the amount of nitrogen element does not give a big impact on the production of EPS (Vijayalakshmi et al. 2017). This is probably because supplementation of the small amount of nitrogen source is provided enough for the growth of *L. acidophilus* and EPS production.

In addition, based on research from Zisu and Shah (2013), it is found that the EPS yield of *L. acidophilus* increased with supplementation of 0.5% (w/v) in the skim

milk, and the appearance of EPS increased at higher concentrations. Besides, it also gave a significant increase in production when the medium culture is replaced with milk medium supplemented with whey protein hydrolysate. This can be concluded that nitrogen sources provide a significant impact on the increment of EPS production and growth of *L. acidophilus*.

Effect of phosphate

Polyphosphates (poly-P) are omnibus molecules made up of linear chains of hundred phosphate molecules connected by bonds in living organisms. This polymer is deposited intracellularly in typical metachromatic inclusions and can be known as a receptor for phosphate and energy storage. However, later studies showed it participates in different physiological and regulatory functions of bacteria. The responses to hunger and other pressures like osmotic, acidic, osmotic or UV tension are used. Furthermore, poly-P has been shown to perform motility, abilities, biofilm production or virulence functions. Poly-P's influencing mechanisms are still largely unclear and can involve a variety of aspects, like gene expression regulation, mRNA stability, protein turnover or ATP homeostasis. Poly-P aggregation in lactobacilli could have a significant effect on the technical methods used for these bacteria. This compound can also play a part in the action of some strains as probiotics, in addition to the possible involvement of poly-P synthesis in lactobacilla stress adaptation.

The pH of media is one of the main factors that will affect EPS production by *L. acidophilus*. The optimal initial pH value for EPS production depends on different *L. acidophilus*, growth conditions and the compositions of fermentation mediums. Different researchers have reported eps production at different pH ranges. According to Liu et al. (2016), the ideal pH for EPS production is pH 5 with the strain of *L. acidophilus* ATCC. However, it shows a different optimal pH value for the *L. acidophilus* LACVG02, LACVG06, LACVG17, LACVG36, LACVG38, LACVG75, LACVG85 with a value of pH 6.5 (Vijayalakshmi et al. 2017). Therefore, *L. acidophilus* has a different chemical characteristic from strain to strain; the same goes for the pH too. This may be due to the medium constituents influencing the critical level at a specific value of the medium and it will eventually affect more or less hydrogen ion concentration. It shows that the amount of hydrogen ions in the medium composition affects the cellular growth of *L. acidophilus* and the synthesis of EPS.

Applications of EPS in food and pharmaceutical

Using LAB capable of producing EPS can help fermented products meet the growing customer demand for food free of artificial ingredients. In addition to their positive health impacts, EPS is known for their shelf-life extending qualities by preserving food in terms of inhibiting the growth of other microbes that may contribute to foodborne illness as well as spoilage (Bernardeau et al.

2006), technologically functional enhancing capacities in the food and dairy industries. EPS also have a wide range of potential and well-established contributions to the pharmaceutical sector (Daba et al. 2020). EPS was produced by *in situ* fermentation which also contributes to food sensory experiences that is important in attributes of quality food. EPS has been reported to have therapeutic properties such as anticancer, antidiabetic, antiviral, antioxidant, antiulcer, and immunomodulator properties (Yildiz and Karatas, 2018; Li et al. 2012). Besides therapeutic properties, EPS produced by LAB contributes contemptuously to the baking industry, cereal-based products, high-fiber wheat bread, as well as in the dairy industry. EPS also plays a substantial aspect in the pharmaceutical industry such as drug delivery systems, polymer interpenetration, anticancer drug-targeting, gene delivery, recombinant macromolecular, and tissue engineering (Daba et al. 2020). Apart from that, they have also been employed in agriculture, paint, paper and petroleum industries (Fenibo et al. 2019). Figure 1 shows the applications of EPS in the food and pharmaceutical industries.



Figure 1: Applications of EPS in food and pharmaceutical industries

EPS produced by *L. acidophilus* is crucial in the creation of our fermented dairy products, which include drinking yogurt, cheese, fermented cream, and milk-based sweets. Firmness and creaminess are two key sensory factors that influence customer appetite for dairy products. EPS can be important texturizers and stabilisers because they raise the viscosity of a final product, hydrate water, and interact with other milk components like proteins and micelles to make the case in the network stiffer. EPS can thereby lessen syneresis and boost product stability (Duboc & Mollet, 2011).

When stirred yogurts are made by mildly

homogenising the milk coagulum after fermentation, it provides a crystal-clear illustration of the yogurt-producing sector. However, mechanical treatment has a significant impact on the rheology of the coagulum. Several techniques have been developed to improve the texture and stability of fermented milk products while reducing syneresis. EPS from *L. acidophilus* is used as a starter culture in fermentation to solve this problem because they have highly cost-effective texture and stability qualities. In addition to that, EPS produced by *Lactobacillus acidophilus* plays an important role in food processing with its well-recognized antioxidant activity.

EPS made by lactic acid bacteria is also useful in the manufacture of fermented milk, making them crucial for both products. The pH of the milk is lowered by the lactic acid created during the fermentation process, and as a result, micelles become unstable due to the increased solubility of calcium and phosphate. EPS increase the nutrient value of the milk products (Kojic et al. 2012). Antilisteria activity of *L. acidophilus* LA5 was studied *in vitro* and it was found that more than 50% of their residual antimicrobial properties that able to control the growth of *Listeria monocytogenes* (Moradi et al. 2019)

Another well-known microbial EPS is dextran, having a decomposable and biocompatible property. It has been used as a dietary supplement but later employed in food products as a food additive for gelling properties, viscosity, texture and emulsification (Suryawanshi et al. 2022). Dextran is also widely used in the production of ice cream, frozen foods, prebiotics, fermented dairy products, protein-dextran conjugates and reduced-fat cheese (Zhang et al. 2017; Suryawanshi et al. 2022). Dextran was a substrate from strain *Leuconostoc mesenteroides* subspmesenteroides; another LAB that is renowned for its production of EPS. The second most commercialized natural microbial polysaccharide is xanthan which is a significant industrial biopolymer. Xanthan is applied in bakeries, dairy products, beverages, sauces, gravies, dressings, relish and pet food. Several other Lab strains have been a major contribution to EPS in the food industry such as *Azobacter vinelandii* (for producing alginate), *Streptococcus paucimobilis* (producing hyaluronic acid), *Xanthomonas campestris* (producing glucose, mannose, and glucuronic acid) (Moscovici, 2015). These EPS has greatly assisted in the food industry as ingredients and facilitate the manufacturing and producing other food products (Zannini et al. 2016; Ale et al. 2020).

Under *in vitro* circumstances, EPS from several probiotic bacteria has demonstrated anti-cancer potential. EPS from *Lactobacillus kefiranofaciens* against cervical and hepatocellular cancer cells (Elsayed et al. 2017) and EPS from *Lactobacillus helveticus* against HT-29 cells (Xiao et al. 2020) are a few examples.

A study by Deepak et al. (2021) has exhibited the effect of EPS from *L. acidophilus* able to increase the level of antioxidants enzymes in colon cancer of tested rats. EPS from *L. acidophilus* against CaCo-2 (cells) from a

study by El-Deeb et al., (2018) has proven to have anti-cancer properties under in vitro conditions. However, even after many studies, the exact prevention mechanisms for cancer remain unclear (Deepak et al. 2021).

Some EPS can be categorised as traditional pharmaceutical excipients, which are originally used in medicine. Alginates are one type of polysaccharide that can be used as a matrix for tablets, a dental impression material, or an anti-reflux agent. The use of hyaluronic acid and its derivatives in surgery, the treatment of arthritis, and wound healing. Bacterial cellulose is used in tissue engineering scaffolds or as a treatment for wounds.

EPS and its derivatives are also suitable as biodegradable, potentially non-toxic medicinal carriers. The purpose of preserving stable transport in the body as well as preventing build-up and variable rate-release of a medicinal molecule helps to explain their extensive use of EPS. EPS can be used as controlled drug delivery agents for vaccination adjuvants and diagnostic imaging systems. One can categorically assert that EPS medical applications have a bright future ahead based on past and present outcomes in significant medical-pharmaceutical sectors (Nwodo et al. 2012).

CONCLUSION

The techno-functionality of EPS from lactic acid bacteria specifically *L. acidophilus* was discussed in this paper. EPS that was produced by LAB has been proven to give more benefits, particularly in health benefits, pharmaceutical function, and biochemical capacity for food additives. However, more studies on the basic and common principle of the manufacturing of distinguish EPS from different strains of LABs shall be continued and elucidated with different pathways. Extensively, EPS was proven to have great functions including drug delivery, in food industries, medicine, as well as agriculture field.

CONFLICT OF INTEREST

The authors declared that the present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

The authors would like to thank RMC UTM through grant no. R.J130000.7646.4C650 and the Ministry of Higher Education, Malaysia through FRGS Grant No (FRGS/1/2020/TK0/UTM/02/16).

AUTHOR CONTRIBUTIONS

LYB, SZH, DJD and SAM were involved in the data collection and writing the manuscript. DJD, TWY, SNZ, IC, DS and HAE reviewed the manuscript. All authors read and approved the final version.

Copyrights: © 2023 @ author (s).

This is an open access article distributed under the terms of the [Creative Commons Attribution License \(CC BY 4.0\)](https://creativecommons.org/licenses/by/4.0/), which

permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

REFERENCES

- Abdellah M, Ahcene H, Benalia Y, Saad B, Abdelmalek B. 2015. Evaluation of biofilm formation by exopolysaccharide-producer strains of thermophilic lactic acid bacteria isolated from Algerian camel milk. *Emirates Journal of Food and Agriculture*, 27(6): 513–521.
- Altermann E, Russell WM, Azcarate-Peril MA, Barrangou R, Buck BL, McAuliffe O, Souther N, Dobson A, Duong T, Callanan M, Lick S, Hamrick A, Cano R, Klaenhammer TR. 2005. Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. *Proceedings of the National Academy of Sciences of the United States of America*. 102(11): 3906–3912.
- Ale EC, Rojas MF, Reinheimer JA, Binetti AG. 2020. *Lactobacillus fermentum*: Could EPS production ability be responsible for functional properties?. *Food Microbiology*. Volume 90: 2020
- Almalki MA. 2020. Exopolysaccharide production by a new *Lactobacillus lactis* isolated from the fermented milk and its antioxidant properties, *Journal of King Saud University – Science*. 32(2): 1272-1277,
- Amiri S, Mokarram RR, Khiabani MS, Bari MR, Khaledabad MA. 2019. Exopolysaccharides production by *Lactobacillus acidophilus* LA5 and *Bifidobacterium animalis* subsp. *lactis* BB12: Optimization of fermentation variables and characterization of structure and bioactivities. *International Journal of Biological Macromolecules*. 15(123): 752–765.
- Angelin J, Kavitha M. 2020. Exopolysaccharides from probiotic bacteria and their health potential. *International Journal of Biological Macromolecules*. 2020. 1(162) :853-865.
- Anjum N, Maqsood S, Masud T, Ahmad A, Sohail A, Momin A. 2014. *Lactobacillus acidophilus*: characterization of the species and application in food production. *Critical Review in Food Science and Nutrition*. 54(9):1241-1251.
- Badel S, Bernardi T, Michaud P. 2011. New perspectives for *Lactobacilli* exopolysaccharides. *Biotechnology Advances*. 29(1): 54–66.
- Bernardeau M, Guguen M, Vernoux JP (2006). Beneficial *Lactobacilli* in food and feed: long-term use, biodiversity and proposals for specific and realistic safety assessments. *FEMS Microbiology Review*. 30(4) : 487 – 513.
- Bibi A, Xiong Y, Rajoka MSR, Mehwish HM, Radicetti E,

- Umair M, Shoukat M, Khan MKI, Aadil RM. 2021. Recent Advances in the Production of Exopolysaccharide (EPS) from *Lactobacillus* spp. and Its Application in the Food Industry: A Review. *Sustainability*. 13(2): 12429.
- Binns C, Lee MK. 2010. The use of probiotics to prevent diarrhea in young children attending child care centers: a review. *Journal of Experimental and Clinical Medicine*. 2(6): 269–273.
- Burakova I, Smirnova Y, Gryaznova M, Syromyatnikov M, Chizhkov P, Popov E, Popov V. 2022. The Effect of Short-Term Consumption of Lactic Acid Bacteria on the Gut Microbiota in Obese People. *Nutrients*. 14(16): 3384.
- Carr FJ, Chill D, Maida N. 2002. The lactic acid bacteria: a literature survey. *Critical Reviews in Microbiology*, 28(4): 281–370.
- Chen H, Niu J, Qin T, Ma Q, Wang L, Shu G. 2015. Optimization of the medium for *Lactobacillus acidophilus* by Plackett-Burman and steepest ascent experiment. *Acta Scientiarum Polonorum Technologia Alimentaria*. 14(3): 227-232.
- Chong BF, Blank LM, Mclaughlin R, Nielsen LK. 2005. Microbial hyaluronic acid production. *Applied Microbiology and Biotechnology*. 66(4): 341–351.
- Cuthbertson L, Kos V, Whitfield C. 2010. ABC Transporters involved in export of cell surface glycoconjugates. *Microbiology and Molecular Biology Reviews*. 74(3): 341-362.
- Dailin DJ, Elsayed EA, Othman NZ, Malek RA, Ramli S, Sarmidi MR., Aziz R, Wadaan MA, El-Enshasy HA. 2015. Development of cultivation medium for high yield kefir production by *Lactobacillus kefirifaciens*. *International Journal of Pharmacy and Pharmaceutical Sciences*. 7: 159-163.
- Dailin DJ, Elsayed EA, Othman NZ, Malek R, Phin HS, Aziz R, Wadaan M, El-Enshasy HA. 2016. Bioprocess development for kefir production by *Lactobacillus kefirifaciens* in semi industrial scale bioreactor. *Saudi Journal of Biological Sciences*. 23(4): 495-502.
- Dailin DJ, Low LZMI, Malek RA, Wan Azelee, NI, Abdul Manas, NH, Keat, HC, Sukmawati D, El Enshasy, HA (2019). Pullulan, a biopolymer with potential applications in pharmaceutical and cosmeceutical: A review. *Bioscience Research*. 16(3): 2604-2616.
- Dailin DJ, Elsayed EA, Malek RA, Hanapi SZ, Selvamani S, Ramli S, Sukmawati D, Sayyed RZ, El-Enshasy HA. 2020. Efficient kefir production by *Lactobacillus kefirifaciens* ATCC 43761 in submerged cultivation: Influence of osmotic stress and nonionic surfactants, and potential bioactivities. *Arabian Journal of Chemistry*. 13(12): 8513-8523.
- Dailin DJ, Malek RA, Ting TL, Wehbe, R, Ramli S, Elsayed AA, Leng, OM, Ho T, and El-Enshasy H (2021). Biosynthesis, Production and Application of Kefiran In Food Industry: A Review. *Bioscience Research*. 18(1): 102-119.
- Dailin DJ, Selvamani S, Michelle K, Jusoh YMM, Chuah, LF, Bokhari A, El Enshasy HA & Show, PL (2022). Production of high-value added exopolysaccharide by biotherapeutic potential *Lactobacillus reuteri* strain. *Biochemical Engineering Journal*. 188: 108691.
- Daba GM, Elnahas MO, Elkhateeb WA. (2020). Contributions of exopolysaccharides from lactic acid bacteria as biotechnological tools in food, pharmaceutical and medical applications. *International Journal of Biological Macromolecules*. 173: 79-89.
- Deepak V, Ramachandran S, Balahmar RM, Pandian SRK, Sivasubramaniam SD, Nellaiah H, Sundar, K. 2016. In vitro evaluation of anticancer properties of exopolysaccharides from *Lactobacillus acidophilus* in colon cancer cell lines. *In Vitro Cellular & Developmental Biology Animal*. 52(2): 163-173.
- Deepak V, Sundar WA, Pandian SRK, Sivasubramaniam SD, Hariharan N, Sundar K. 2021. Exopolysaccharides from *Lactobacillus acidophilus* modulates the antioxidant status of 1,2-dimethyl hydrazine-induced colon cancer rat model. *3 Biotech*. 11: 225.
- Duboc P, Mollet B. 2011. Applications of exopolysaccharides in the dairy industry. *International Dairy Journal*. 11(9): 759–768.
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V, Azimonti, G, Bastos, ML, Christensen, H, Dusemund, B, Durjava, MF, Kouba, M, López-Alonso, M, Puente, SL, Marcon, F, Mayo, B, Pechová, A, Petkova, M, Ramos, F, Sanz, Y, Villa, RE, Woutersen, R, Saarela, M, Anguita, M, Galobart, J, Pettenati, E, Revez, J, Ortuño, J, Tarrés, J and Brozzi, R, 2022. Scientific Opinion on the safety and efficacy of a feed additive consisting of *Enterococcus faecium* NBIMCC 8270, *Lactobacillus acidophilus* NBIMCC 8242, *Lactobacillus helveticus* NBIMCC 8269, *Lactobacillus delbrueckii* ssp. *lactis* NBIMCC 8250, *L. delbrueckii* ssp. *bulgaricus* NBIMCC 8244 and *Streptococcus thermophilus* NBIMCC 8253 (Probiotic Lactina®) for cats and dogs (Lactina Ltd.). *EFSA Journal* 2022.20(9):7423, 10 pp. <https://doi.org/10.2903/j.efsa.2022.7423>
- El-Deeb NM, Yassin AM, Al-Madboly LA, El-Hawiet A. 2018. A novel purified *Lactobacillus acidophilus* 20079 exopolysaccharide, LA-EPS-20079, molecularly regulates both apoptotic and NF-κB inflammatory pathways in human colon cancer. *Microbial Cell Factories*, 17(1): 29.
- El Ghany KA, Elhafez EA, Hamouda RA, Mahrous H, Ahmed FAH, Hamza HA, 2014. Evaluation of Antioxidant and Antitumor Activities of *Lactobacillus acidophilus* Bacteria Isolated from Egyptian Infants.

- International Journal of Pharmacology. 10(5): 282-288
- Elsayed AE, Farooq M, Dailin DJ, El-Enshasy HA, Othman NZ, Malek R, Danial E, Wadaan M (2017). In vitro and in vivo biological screening of kefiran polysaccharide produced by *Lactobacillus kefiranofaciens*. Biomedical Research, 28 (2).
- Felis GE, Dellaglio F. 2007. Taxonomy of lactobacilli and bifidobacteria. Current Issues in Intestinal Microbiology 8(2): 44–61.
- Fenibo EO, Ijoma GN, Selvarajan R, Chikere CB. 2019. Microbial surfactants: The next generation multifunctional biomolecules for applications in the petroleum industry and its associated environmental remediation. Microorganisms. 7(11): 7110581.
- Food and Drug Administration (FDA). 2019. GRAS Notice (GRN) No. 865. <https://www.fda.gov/food/generally-recognized-safe-gras/gras-notice-inventory>.
- García-Lorenzo A, Rodríguez-Piñeiro A, Rodríguez-Berrolcal F, Cadena M, Martínez-Zorzano V. 2012. Changes on the Caco-2 Secretome through Differentiation Analyzed by 2-D Differential In-Gel Electrophoresis (DIGE). International Journal of Molecular Sciences, 13(11): 14401–14420.
- Gilliland SE, Speck ML, Morgan CG. 2014. Detection of *Lactobacillus acidophilus* in Feces of Humans, Pigs and Chickens. Applied Microbiology. 30(4): 541–545.
- Hammes WP, Hertel C. 2006. The Genera *Lactobacillus* and *Carnobacterium*. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, KH., Stackebrandt, E. (eds) The Prokaryotes. Springer, New York, NY. https://doi.org/10.1007/0-387-30744-3_10
- Holland DF. 1920. Generic index of the commoner forms of bacteria. Journal of Bacteriology. 5: 215-229
- Islam ST, Lam JS. 2014. Synthesis of bacterial polysaccharides via the Wzx/Wzy-dependent pathway. Canadian Journal of Microbiology. 60(11): 697–716.
- Jurášková D, Ribeiro SC, Silva CCG. 2022. Exopolysaccharides, Produced by Lactic Acid Bacteria: From Biosynthesis to Health-Promoting Properties. Foods. 11(2): 156.
- Kojic M, Vujcic M, Bania A, Cocconcelli P, Cerning J, Topisirovic L. 2012. Analysis of exopolysaccharide production by *Lactobacillus casei* CG11, isolated from cheese. Applied and Environmental Microbiology. 58(12): 4086–4088.
- Li S, Zhao Y, Zhang L, Zhang X, Huang L, Li D, Niu C, Yang Z, Wang Q. 2012. Antioxidant activity of *Lactobacillus plantarum* strains isolated from traditional Chinese fermented foods. Food Chemistry. 135(3): 1914–1919.
- Liu Q, Huang X, Yang D, Si T, Pan S, Yang F. 2016. Yield Improvement of Exopolysaccharides by Screening of the *Lactobacillus Acidophilus* Atcc and Optimization of the Fermentation and Extraction Conditions. EXCLI journal. 15: 119–133.
- Ljungh A, Wadström T. 2016. Lactic acid bacteria as probiotics. Current Issues in Intestinal Microbiology. 7(2): 73–89.
- Mohd Nadzir M, Nurhayati RW, Idris FN, Nguyen MH. 2021. Biomedical Applications of Bacterial Exopolysaccharides: A Review. Polymers (Basel).13(4):530.
- Moscovici M (2015). Present and future medical applications of microbial exopolysaccharides. Frontiers in Microbiology. 6: 1012.
- Moro E (1900). Ueber die nach Gram farbbaeren bacillen des säuglingsstuhles. Wien Klin Wochenschr 13: 114 – 115.
- Moradi M, Mardani K, Tajik H. 2019. Characterization and application of postbiotics to *Lactobacillus* spp. on *Listeria monocytogenes* *in vitro* and in food models. LTW-Food Science and Technology. 111: 457-464.
- Mozzi F, Vaningelgem F, Hébert EM, Van Der Meulen R, Moreno MRF, Font De Valdez G, De Vuyst L. 2006. Diversity of Heteropolysaccharide-Producing Lactic Acid Bacterium Strains and Their Biopolymers. Applied and Environmental Microbiology. 72(6): 4431–4435.
- Nishimura J. 2014. Exopolysaccharides Produced from *Lactobacillus delbrueckii* subsp. *bulgaricus*. Advances in Microbiology. 4: 1017-1023.
- Nichols CM, Lardiere SG, Bowman JP, Nichols PD, Gibson JAE, Guezennec J. 2015. Chemical characterization of exopolysaccharides from Antarctic marine bacteria. Microbial Ecology, 49(4): 578–589.
- Nordin NZ, Rashidi AR, Dailin DJ, Malek R, Azelee NIW, Manas NH., Selvamani S, Zaidel DNA, Alsaheb RAA, Sukmawati D, El Enshasy, H (2020). Xanthan biopolymer in pharmaceutical and cosmeceutical applications: critical review. Bioscience Research.17 (1): 205-220.
- Nwodo U, Green E, Okoh A. 2012. Bacterial Exopolysaccharides: Functionality and Prospects. International Journal of Molecular Sciences. 13(12): 14002–14015.
- Ozogul F, Hamed I2016. Lactic Acid Bacteria: *Lactobacillus* spp.: *Lactobacillus acidophilus*, Reference Module in Food Science, Elsevier. 2016.ISBN 9780081005965.
- Oleksy-Sobczak M, Klewicka E, 2020a. Optimization of Media Composition to Maximize the Yield of Exopolysaccharides Production by *Lactobacillus rhamnosus* Strains. Probiotics Antimicrob Proteins. 12(2): 772-783
- Oleksy-Sobczak M, Klewicka E, Piekarska-Radzik L. 2020b. Exopolysaccharides production by *Lactobacillus rhamnosus* strains – Optimization of synthesis and extraction conditions. LWT. 122.
- Patterson E, Cryan JF, Fitzgerald GF, Ross RP, Dinan TG, Stanton C. (2014). Gut microbiota, the pharmabiotics they produce and host health.

- Proceedings of the Nutrition Society. 73(4): 477–489.
- Rohm H, Kovac A. 2006. Effects of starter cultures on linear viscoelastic and physical properties of yogurt gels. *Journal of Texture Studies*. 25(3): 311–329.
- Ruas-Madiedo P, Hugenholtz J, Zoon P. 2012. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. *International Dairy Journal*. 12(2): 163–171.
- Schmid J, Sieber V, Rehm B. 2015. Bacterial exopolysaccharides: biosynthesis pathways and engineering strategies. *Frontiers in Microbiology*. 6: 496.
- Silva LA, Lopes Neto JHP, Cardarelli HR. 2019. Exopolysaccharides produced by *Lactobacillus plantarum*: technological properties, biological activity, and potential application in the food industry. *Annals of Microbiology*. 69: 321–328
- Sørensen HM, Rochfort KD, Maye S, MacLeod G, Brabazon D, Loscher C, Freeland B. 2022. Exopolysaccharides of Lactic Acid Bacteria: Production, Purification and Health Benefits towards Functional Food. *Nutrients*. 14(14):2938.
- Suryawanshi N, Naik S, Eswari JS. 2022. Exopolysaccharides and their applications in food processing industries. *Food Science and Applied Biotechnology*. 5(1): 22-24.
- Tao L, Song S, Liu C, Huang W, Bi Y, Yu L. 2022. Fermentation reduced the *in vitro* glycemic index values of probiotic-rich bean powders. *International Journal of Food Science and Technology*. 57(5): 3038-3045
- Tytgat HL, Lebeer S. 2014. The sweet tooth of bacteria: common themes in bacterial glycoconjugates. *Microbiology and Molecular Biology Review* 78(3): 372–417.
- Vijayalakshmi S, Rajasekar S, Mohankumar A. 2017. Screening and Characterization of Exopolysaccharide Producing Mesophilic *Lactobacillus acidophilus* Isolated from Human Dental Caries. *Journal of Dental and Medical Sciences*. 16(12): 54–72.
- Whitney JC, Howell PL. 2013. Synthase-dependent exopolysaccharide secretion in Gram-negative bacteria. *Trends in Microbiology*. 21(2): 63–72.
- William RA, Microflora of the Intestine. *Biology of Lactobacillus acidophilus*, Editor(s): Richard K. Robinson, *Encyclopedia of Food Microbiology*, Elsevier, 1999, Pages 1361-1365
- Willis LM, Stupak J, Richards MR, Lowary TL, Li J, Whitfield C. 2013. Conserved glycolipid termini in capsular polysaccharides synthesized by ATP-binding cassette transporter-dependent pathways in Gram-negative pathogens. *Proceedings of the National Academy of Sciences*. 110(19): 7868–7873.
- Xiao L, Ge X, Yang L, Chen X, Xu Q, Rui X, Li W (2020). Anticancer potential of an exopolysaccharide from *Lactobacillus helveticus* MB2-1 on human colon cancer HT-29 cells via apoptosis induction. *Food & Function*, 11(11): 10170-10181.
- Yildiz H, Karatas N. 2018. Microbial exopolysaccharides: Resources and bioactive properties. *Process Biochemistry*. 72: 41-46.
- Zannini E, Waters DM, Coffey A, Arendt EK. 2016. Production, properties, and industrial food application of lactic acid bacteria-derived exopolysaccharides. *Applied Microbiology and Biotechnology*. 100(3): 1121–1135.
- Zhang J, Zhao W, Guo X, Guo T, Zheng Y, Wang Y, Hao Y, Yang Z. 2017. Survival and Effect of Exopolysaccharide-Producing *Lactobacillus plantarum* YW11 on the Physicochemical Properties of Ice Cream. *Polish Journal of Food and Nutrition Sciences*. 7(3): 191–200.
- Zisu B, Shah NP. 2013. Effects of pH, Temperature, Supplementation with Whey Protein Concentrate, and Adjunct Cultures on the Production of Exopolysaccharides by *Streptococcus thermophilus* 1275. *Journal of Dairy Science*. 86(11): 3405–3415.