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Bacterial Exopolysaccharides: From Production to Functional Features

Chouhra Talbi[1], Salma Elmarrakchy[2], Mourad Youssfi[3], Sarah Bouzroud[1], Meryem Belfquih[1], Aicha Sifou[4], Nezha Bouhaddou[5], Bouabid Badaoui[6], Abdelaali Balahbib[7], Abdelhakim Bouyahya[2*], Ilhame Bourais[2]

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[1]Microbiology and Molecular Biology team, Plant and Microbial Biotechnology, Biodiversity and Environment Center, Faculty of Sciences, Mohammed V University in Rabat, BP 1014, 10000, Morocco; c.talbi@um5r.ac.ma (CT); s.bouzroud@um5r.ac.ma (SB); belfquih.meryem@gmail.com (MB)

[2]Laboratory of Human Pathologies Biology, Faculty of Sciences, Mohammed V University in Rabat, BP 1014, 10000, Morocco; salma.elmarrakechy@um5r.ac.ma (SE); i.bourais@um5r.ac.ma (IB)

[3]Laboratory of Applied Chemistry of Materials, Faculty of Sciences, Mohammed V University in Rabat, BP 1014, 10000, Morocco; mourad.elyoussfi@um5r.ac.ma (ME)

[4]Laboratory of Nanomaterials, Nanotechnologies, and Environment. Faculty of Sciences, Mohammed V University in Rabat, BP 1014, 10000, Morocco; a.sifou@um5r.ac.ma (AS)

[5]Physiology and physiopathology research team, Faculty of Sciences, Mohammed V University in Rabat, BP 1014, 10000, Morocco; n.bouhaddou@um5r.ac.ma (NB)

[6]Mohammed V University in Rabat, BP 1014, 10000, Morocco; b.badaoui@um5r.ac.ma (BB)

[7]High Institute of Nursing Professions and Health Techniques of Errachidia, Errachidia, Morocco; balahbib.abdo@gmail.com (AB)

[*]Corresponding author: Abdelhakim Bouyahya, Laboratory of Human Pathologies Biology, Faculty of Sciences, Mohammed V University in Rabat, BP 1014, 10000, Morocco; a.bouyahya@um5r.ac.ma (AB)

Abstract: Exopolysaccharides, known as bacterial EPS, are complex sugar polymers that bacteria secrete into their environment. EPSs play a crucial role in bacterial survival and proliferation by protecting cells from environmental threats. They also contribute to proper adhesion to numerous surfaces through biofilm production. Bacterial EPS display a wide range of biological activities conferred by their outstanding physical and chemical properties, which make them great candidates for medical and industrial applications. The main biological activities recorded up-to-day include antioxidant, anticancer, antiviral, anti-inflammatory, anticancer, antibacterial, immune-modulatory and chelating properties. They also can be used as thickening agents in the industry or as additives to improve soil quality in agriculture. The current review offers a thorough overview of bacterial EPS, their

biosynthesis processes and regulation, and biotechnological strategies to increase their production. The review also unravels the main extraction, purification, and identification techniques and highlights the key functional features of these complex molecules.

Keywords: Exopolysaccharides; bacterial; bioactivities; production; applications.

1. Introduction

Natural products from different organisms, including microorganisms, have several biological, biochemical, and pharmacological properties[1–4]. These molecules include numerous macromolecules such as sucrose, lipids, proteins, flavonoids, alkaloids, and phenolic acids, exhibiting various bioactivities such as antioxidant, antimicrobial[5–11] and anticancer activities[12,13].

Polysaccharides or glycans are complex carbohydrates found in various organisms, including plants, animals, bacteria, fungi, and algae. These compounds play an essential role in biological systems and exhibit diverse chemical structures and physical properties[14,15].

Bacterial exopolysaccharides (EPS) are indeed a diverse group of polysaccharides biopolymers produced by various bacterial species. EPSs are high molecular weight biopolymers ranging from 10 to 1000 kDa[16] with repeating units of azures at different proportions outside of the cell and have a protracted lifespan[17,18]. EPSs can be present in two different forms. They can either be excreted into the surrounding environment or remain bound to the cell surface. Extremophiles, thermophiles, halophiles, psychrophiles, acidophiles, and alkaliphiles are only a few of the bacterial types that can develop exopolysaccharides (EPS)[19].

EPS are classified into two major groups: homopolysaccharides and heteropolysaccharides. Homopolysaccharides consist of repeating units of a single type of monosaccharide, such as levan, produced by *Streptococcus salivarius*, glucan, which is synthesized by *Streptococcus mutans* and *Streptococcus sobrinus*, while dextran, produced by *Leuconostoc mesenteroides*. Heteropolysaccharides are composed of repeating units of various monosaccharides. They are widely produced by microorganisms that are relevant to medicine, including lactic acid bacteria, *Salmonella* spp., *Escherichia coli*, and *Enterobacter* spp.[20].

EPSs are key components in biofilm architecture. This latter is composed of different biopolymers, such as polysaccharides, lipids, nucleic acids and proteins, which confer mechanical strength and adhesion properties to the biofilm matrix[21–25]. EPSs help to regulate

the microenvironment within the biofilm by trapping nutrients and metabolites, protecting the biofilm from environmental stress, and facilitating intercellular communication between bacterial cells[26]. This communication can lead to the establishment of complex signaling networks, which in turn can regulate the growth, differentiation, and dispersal of the biofilm community[27–30]. Overall, EPSs play a vital role in biofilm architecture by providing an essential physical framework for microbial attachment, growth, and safeguarding from harmful environmental elements, and ensuring nutrition and survival.

Bacterial EPSs have gained significant attention among researchers in recent years[31–33]. They play important roles in numerous biological processes, including biofilm formation, cell-cell communication, and protection against environmental stress, which offer tremendous opportunities for applications in food, medicine, and even pharmaceuticals[34,35].

Given the outstanding potential of EPS in different applications listed above, the present review aims to provide a concise overview of (i) EPS biosynthesis pathways and regulation, (ii) biotechnological approaches to improve their production in bacteria, (iii) the different extraction methods applicable, (iv) the main functional properties and (v) the different applications described up-to-date (Figure 1).

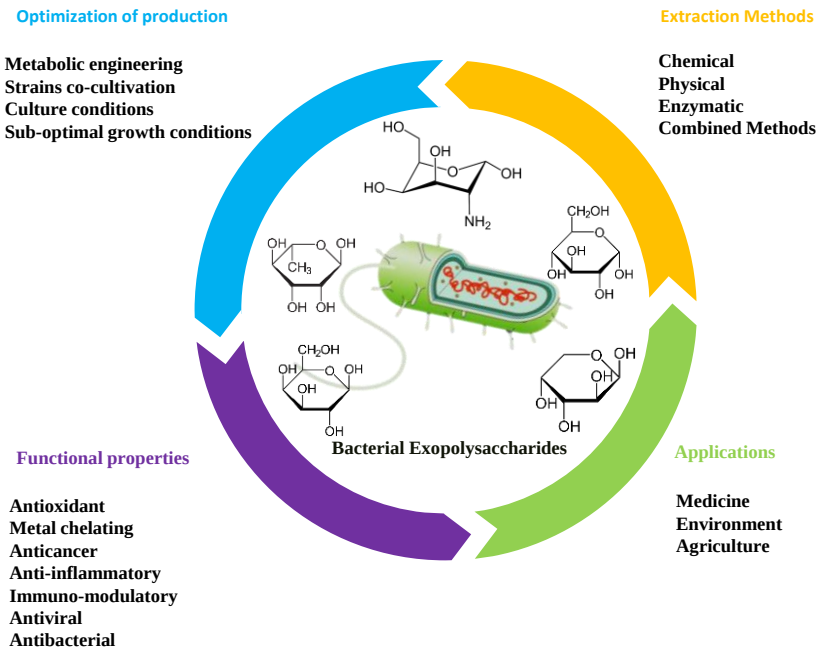

Figure 1. General overview of bacterial exopolysaccharides, optimization of production, extraction methods, function properties and applications.

2. Processes for exopolysaccharides synthesis

2.1. EPS Biosynthesis and Regulation

Bacterial EPS biosynthesis is a complex process that depends on many genes involved in polysaccharide biosynthesis, export, and modification. EPS biosynthesis has been intensively studied in several bacterial strains, as underlined by Kim *et al*.[36], Li *et al*.[37], Xie *et al*.[38] and Yang *et al*.[39]. A complex process that controls bacterial EPS biosynthesis allows bacteria to manufacture EPS in response to certain environmental constraints. EPS synthesis pathways can be classified into two categories: phosphorus-dependent and phosphorus-independent pathways[40,41].

The first one includes the alginate-type pathway as in *Pseudomonas aeruginosa* and the Colan-type pathway as in *E. coli*. Phosphorus-independent pathway of EPS synthesis including the cellulose-type pathway as in *Gluconatobacter xylinus*, as well as curdlan, xanthan and levan-type pathways in various bacterial species[42–44]. Genes in the EPS gene cluster frequently encode the enzymes necessary for this production. EPS production can be regulated at transcriptional, post-transcriptional, and secretion levels. A complex system that usually involves numerous signaling and gene regulatory networks regulates the production of bacterial EPS. One of the most common regulatory mechanisms for EPS production is the two-component regulatory mechanism. This regulatory system activates or represses the transcription of genes involved in the generation of EPS by receiving signals from the sensor, which detects environmental constraints such as nutrient deprivation, stress levels, and surface signals[22,35]. Transcriptional and post-transcriptional regulatory network of genes involved in exopolysaccharides synthesis and secretion is composed of the regulatory genes *cbrA*, and *mucR*, as well as the genes involved in ExpR / Sin Quorum sensing type ExoR / ExoS / ChvI systems. ExoR is an environmental signal receptor that controls the genes responsible for EPS synthesis. ExoS is an exported protein that controls ExoR's phosphorylation, whereas Chyl is a regulatory protein that interacts with ExoR and ExoS to facilitate their function. These regulators control the biosynthesis, segregation, and synthesis of type I and type II exopolysaccharides, as well as flagella synthesis, mobility, and also regulate genes involved in symbiotic signaling pathways[40,41].

In *E. meliloti* and *E. aridi* LMR001T, the MucR protein, which is controlled by the exopolysaccharide production regulatory system, has been demonstrated to be involved in the motility control network and flagellum biosynthesis[45,46]. It was also established that the gene regulator *nes*R, which encodes for the NesR and is an inductor of the ExpR family of type LuxR operon, regulates the genes involved in the production of the symbiotic

exopolysaccharide EPS II, the regulation of motility, chemotaxis, and production of low-molecular-weight succinoglycans. This latter represents another form of exopolysaccharide with significant symbiotic relevance[46–51]. It's noteworthy to mention that the control of motility occurs after the activity of ExpR regulator, which regulates two LuxR-like proteins called VisN and VisR[50,52,53]. Despite the fact that both proteins are regarded as LuxR family members, their autoinducer binding domains differ significantly from those of conventional LuxR proteins[52]. The VisN/VisR system increases the transcription of flagellar synthesis, flagellar motor, and chemotaxis genes in the presence of low population density[50,52]. Additionally, the ExpR regulator binds with the self-inducing chemical AHL (acyl-homoserine lactone) in high bacterial density conditions. This compound then acts as a promoter for the *vis*N gene and inhibits the expression of all the genes involved in the development of mobility and chemotaxis[53,54]. Moreover, the ExoR/ ExoS/ ChvI polysaccharide biosynthesis pathway is also activated.

The two systems ExpR/luxRs/luxI, SinI and ExoR/ExoS/ChvI and the protein MucR interact to regulate mobility as well as to activate the biosynthesis of EPS I and inhibit the synthesis of EPS II[41]. The *rem* gene, which encodes an activator of motility gene expression with targets like *fla*F (a regulatory protein of flagellar biosynthesis) and *flg*G (a component of the basal corposal stem), is suppressed by the activity of MucR protein[41]. Chemical experiments, according to Dilanji *et al*.[55], have shown that EPS II secretion is a high energy-consuming activity that uses the same quorum sensing control system. The *muc*R, *exo*R, *exo*S, *exo*D, *exp*R, *syr*M, and *pho*B are examples of plasmidic regulatory genes that govern the EPS I and EPS II production machinery in *E. meliloti*. The *exs*B, *exo*X, and *wgg*R are examples of regulatory genes found on the megaplasmid pSymB[56]. In fact, the ExoR/ExoS/ChvI system and the ExpR/Sin quorum sensing systems control EPS synthesis and motility more than other processes, depending on the stage of bacterial growth. It is assumed that MucR plays a crucial role in the coordination of bacterial processes in *E. meliloti*. The ExpR / Sin system abolishes the effect of MucR repression on galactoglucan biosynthesis but does not affect the role of MucR on a number of functions that promote symbiosis, such as motility repression, the increase of EPS I production and Nod factors[56].

The survival and development of symbiotic bacteria in their host environment depend on these gene regulatory networks. It is accurate that the Exp/Sin system is found in various symbiotic bacteria, including *R. etli* and *E. meliloti*, and that it regulates biofilm formation and colonization of the host plant root by modulating EPS expression. According to Marie *et al*.[57] and Acosta-jurado *et al*.[40], the ExoR/ExoS/Chyl system found in *E. meliloti* and *E. fredii* is involved in the control of nodules' development. In fact, Geiger *et al*.[58] 's findings

suggest that the lack of bacterial phosphatidylcholine in *E. meliloti* activates the two-component system of regulation ExoS/ChvI. This latter constitutes a molecular switch for changing from a free-living to a symbiotic lifestyle.

The mechanics of bacterial-plant symbiosis have been better understood thanks to research on these systems, which may also have benefits in sustainable agriculture. Some bacteria lack a standard genome-encoded quorum detecting system. However, they still have three genes (CC_0933, CC_0949, and CC_1356) that are identical to the *mucR* gene as in the case of *C. crescentus*. This strongly suggests the existence of a complex *MucR*-regulated mechanism involved in polysaccharides production and flagella mobility in *C. crescentus*[59]. Besides, it has been demonstrated that a reduction in the attachment level to the colonization surface as well as a loss of the capacity to form a biofilm on roots resulted from the suppression of the global receptor RosR (80% homologue mucR), which also regulates the biosynthesis of EPS[60].

Overall, numerous signaling pathways and gene regulation are involved in the regulation of EPS synthesis in bacteria. However, despite the various biosynthesis pathways, bacterial EPS production remains very low, which can limit their use. To overcome this limitation, several biotechnological approaches have been implemented. The broad positive impact of these technologies on EPS biosynthesis has been underlined in the following section.

2.2. Strategies for Exopolysaccharides Production Improvement

Given the great demand for bacteria with the ability to produce extracellular polysaccharides with desired properties, various strategies have recently emerged to improve the yield of production or even to trigger changes in the composition and structure of desired EPS[61]. Metabolic engineering, culture conditions optimization or even the application of growth-limiting conditions (abiotic stresses) can efficiently improve EPSs production by several bacterial strains, as discussed in the following section.

2.2.1. Metabolic engineering

Metabolic engineering can be easily defined as the production of specific substances or molecules, such as chemicals, fuels and drugs, through the disruption of the metabolic pathways in cells[62]. Metabolic engineering has been commonly applied to enhance EPS biosynthesis, despite the fact that EPSs are naturally produced at low levels. One of the strategies is to overexpress genes from the central metabolic pathway, known for providing sugar precursors required for EPS biosynthesis[61]. This ultimately increases the availability

of sugar precursors, thereby enhancing EPS production. In *G.lucidum*, the overexpression *pgm* gene, encoding for a phosphoglycomutase, that catalyzes the interconversion of glucose-6-phosphate into glucose-1- phosphate, resulted in a significant increase in EPS production. The increase was estimated to be around 45%[63]. The knock-out of pep (*pepF*, *pepJ* and *pepC*) genes in *Paenibacillus polymyxa* severely altered the production of EPS, which clearly underlines the putative role of these genes in EPS biosynthesis process[64]. Indeed, as reported by Rütering *et al*.[64], these genes encode for putative glycosyltransferase catalyzing glycan polymerization reaction. Moreover, the deletion in *pigA* gene, known to be involved in xanthomonadin synthesis resulted in an increase xanthan gum. Heterologous expression of *Pasteurella multocida* heparosan synthase enzymes in *Bacillus megaterium* resulted in an increase in heparosan through fed-batch fermentation to reach 2.74g/L[65]. Similarly, the introduction of glucose phosphate uridyltransferase and heparosan synthase in the genome of *Synechococcus* allowed the production of higher amounts of heparosan[66]. Hyaluronic acid production by *Corynebacterium glutamicum* strain has been improved through the application of genome-scale metabolic modeling. This approach consisted of the constitutive expression of hyaluronic acid pathway genes in a *C.glutamicum* strain, characterized by an altered pentose phosphate pathway and reduced glycolysis. By this approach, the authors were able to enhance the production of hyaluronic acid titers from 1.3g/L to 28.7g/L[67].

Metabolic optimization of the regulatory elements has also been proposed as an efficient strategy to increase EPS biosynthesis in bacterial strains. The inducible promoters PxylA (xylose inducible); Pgrac (IPTG inducible) and PsacB (sucrose inducible) have been successfully employed for the expression of protein of interest, allowing the production of target proteins[68]. For *Bacillus amyloliquefaciens*, the substitution of the native promoter of the levansucrase SacB with a strong artificial promoter Pgrac enhanced the production of a specific homopolysaccharide; levan[69].

Overall, metabolic engineering offers a new path for producing high-value EPSs at sufficient amounts and possibilities for scale-up production. However, one of the challenges associated with this approach is linked to the complexity and diversity of the EPS biosynthesis pathways, which can be highly dependent on the bacterial strains. However, a thorough knowledge of the key actors in EPSs biosynthesis and regulatory pathways can be determinants for the success of metabolic engineering.

2.2.2. Strains co-cultivation

It is well established that microorganisms co-culture enhanced their resistance to environmental fluctuations, thereby performing complex metabolic activities. EPS

production is among the complex metabolic processes triggered by co-culture[70]. The effect of bacterial co-cultivation has been well studied in the association of lactic acid bacteria - *Saccharomyces cerevisiae*. One of the great examples of the success of this approach is related to the production of kefiran, an exopolysaccharide commonly used in the agroindustry. For instance, *Lactobacillus kefiranofaciens* JCM 6985 co-culture with *Saccharomyces cerevisiae* enhanced kefiran production in a fed-batch system[71]. Likewise, *Lactobacillus rhamnosus* (ATCC 9595, RW-9595M and R0011) strains co-culture with *Saccharomyces cerevisiae* enhanced EPS production by 39%, 42% and 49%, respectively, due to the activation of EPS operon transcription[72]. Similarly, *Lactobacillus paracasei* co-culture with *S. cerevisiae* increased EPS culture compared to monoculture. This increase in EPS was linked to the over-expression of polyprenylglycosylphosphotransferase encoding gene[73]. The same authors also proposed that EPS production can be induced by the direct interaction between yeast and bacteria, which triggers EPS synthesis in lactic acid bacteria cells, allowing a better cell adhesion and thus a superior lactic acid consumption by yeast cells[73].

Bacterial-microalgal consortium has recently emerged as a promising way to enhance EPS production[74]. Bacterial strains co-cultivation (*Pseudomonas* sp., *Brevundimonas* sp. and *Proteiniphilum* sp.) with *Chlorella* sp. had a positive impact on EPS production in batch-culture system. Indeed, Liu *et al*.[75] had associated the increase in EPS amounts with the maintenance of consortium stability.

As mentioned above, EPS biosynthesis can notably be enhanced by bacterial co-culture with yeast (*Saccharomyces cerevisiae*) or microalgae (*Chlorella sp*.). This can be achieved through a good knowledge of the optimum culture conditions.

2.2.3. Culture conditions optimization

It is well established that EPS production, structure and properties are highly dependent on several factors, including strain type, culture medium composition and fermentation conditions[76,77]. A large number of studies focused on optimizing fermentation conditions to improve EPS yield of production. The use of different carbohydrate sources as substrates had significant effects on bacterial growth and EPS production[78]. For instance, Li *et al*.[79] reported that the use of sucrose at 80g/L increased the EPS amounts produced by *Streptococcus thermophilus*. Similarly, gellan exopolysaccharide production by *Sphingomonas paucimobilis* growing strains was highly increased in sucrose-containing medium. In addition to sucrose, the use of glucose as a carbon source yielded the highest EPS amounts of 8.87g/L by *Chryseobacterium indologenes* MUT.2. Meanwhile, the lowest EPS

values of 2.55, 2.39 and 2.11 were recorded with the use of dextrin, mannose and starch respectively[80]. Fructose can also prompt EPS production in some bacterial strains. This is the case of *Pantoea* BM39 strain, which produced up to 11.05 g of glucan/L when cultured in the presence of 80g/L of fructose[81]. Besides carbon, other studies showed that carbon/ nitrogen ratio is also important to achieve a better EPS yield. Several studies clearly demonstrated that a higher carbon/nitrogen ratio along with sufficient nitrogen and carbon amounts can yield a higher EPS amounts[79,82]. Indeed, the marine bacteria; *Saccharophagus degradans* produced up to 2.1g of EPS/L when glucose/ammonium ratio (C/N ratio) reached a value of 100 [82]. For *Klebsiella pneumonia*, the maximum EPS production was obtained with the use of 10g/L of glucose as a carbon source and potassium nitrate amended at the concentration of 2g/L as a nitrogen source[83].

Besides carbon and nitrogen, other nutrients like phosphate, magnesium, calcium, iron and zinc can influence EPS biosynthesis; since they can affect the conversion of carbon sources into polysaccharides[84]. Phosphate variation can stimulate the production of EPS by some bacterial strains, such as *Azotobacter vinelandii* and *Klebsiella* spp. Maximum EPS amounts were obtained in the absence of phosphate or in phosphate limitation conditions[82]. The effect of the other nutrients on EPS production is a subject of controversy. Some researchers reported a beneficial effect of iron, calcium, zinc and manganese privation on microbial growth and EPS biosynthesis, as observed with *Saccharophagus degradans*. However, other studies showed that the presence of these elements triggers EPS synthesis[17,82].

pH can also influence EPS production. A neutral pH level seems to be beneficial for EPS production. For instance, *Streptococcus thermophilus* produced EPSs were barely undetectable when the bacterial strain was grown at pH adjusted to 4.0 and 4.5. Meanwhile, bacteria cultivation at a pH of 6.5 yielded the highest EPS amounts of 338 mg of EPS/L under microaerobic conditions[85]. Similar findings have been reported for *Lactobacillus fermentum* strain by Shi *et al*.[77]. The authors found that *L.fermentum* achieved 3-fold higher EPS biosynthesis in a defined medium (CDM) with a pH adjusted to 6.5. For *S.thermophilus*, the maximum EPS amount was reached at a pH of 7.0[78].

It is well-established that temperature is one of the most important parameters influencing EPS synthesis[86]. An increase/ decrease in the culture temperature from the optimum values resulted in a decrease in EPS production[87]. As a way to improve EPS production by *S.thermophilus*, Li *et al*.[79] have tested different temperatures ranging between 27°C and 40°C. They found that the highest EPS production of 250mg/L was achieved when

the culture was maintained at 37°C. It is worth noting that EPS from *S.thermophilus* strains is produced at relatively low levels[88]. For bacteria belonging to the genus *Lactobacillus*, maximum EPS amounts were recorded when the bacterial strains were cultivated at 30°C, which corresponds to the optimum temperature, yielding the highest EPS values of 433.61, 408.53 and 377.50mg/L, respectively, for *L. plantarum* MF460, *L.plantarum* MF303 and *L.plantarum* MF176[86].

Globally, EPS production can notably be improved by the optimization of bacterial strain growth and culture conditions by varying media culture composition, temperature or pH. The challenge is to identify the best culture conditions, which are highly dependent on the bacterial strain.

2.2.4. Sub-optimal growth conditions — abiotic stresses

It is well-recognized that abiotic stresses trigger the production of EPSs. Indeed, bacterial strains tend to produce EPSs as a cellular defence response to minimize the harsh effects of abiotic stresses[89]. Thus, the ability of bacterial strains to produce high amounts of EPSs in response to stress can be explored. Several examples of the success of this approach have been reported in the literature. EPS production by *Lactobacillus confuses* TISTR 1498 was notably enhanced with media supplementation with moderate salt stress conditions[90]. The maximum yield of 86.36g/L was produced by the salt-amended medium with 5% of NaCl[90]. Similar results have been reported for *Leuconostoc pseudomesenteroides* 406 when grown in the presence of 5% NaCl[91]. For *Pseudomonas putida* GAP-P45, Sandhya and Ali[92] evaluated the effects of various abiotic stress conditions on EPS biosynthesis. They found that bacteria exposure to these stresses (high temperature, drought and salt stress) triggered the production of EPSs. They were able to record the highest amounts due to drought stress. Besides salt and drought stresses, excessive temperature was efficient for some bacterial strains, as is the case of *Bifidobacterium bifidum*. For instance, it was noticed that sub-lethal thermic stress increased *B. bifidum* viability and enhanced EPS production[93]. For *Sinorhizobium fredii* HH103, it seems that mannitol-induced non-ionic osmotic stress triggered the overproduction of EPSs through the positive regulation of key genes involved in EPS biosynthesis including, *exol*, *exoN* and *exoF*[94]. It is worthy to note that, in some cases, abiotic stresses do not prompt the overproduction of EPSs in some bacterial strains, such as *Pseudomonas tequilensis* J12 and *Pseudomonas aeruginosa* PM389[95], thereby suggesting that EPS production/overproduction is a strain-dependent phenomenon.

3. Extraction Methods

EPS extraction techniques can be divided into physical, chemical and physico-chemical methods[96,97]. The choice of an appropriate extraction method for EPS is typically influenced by factors such as the chemical composition of the EPS, extraction conditions, and the presence of interfering substances[98].

As presented in Table 1, the majority of studies used the following steps to extract EPS in various media, which include physical and chemical methods. EPS extraction involved incubation using the required growth media. The enzymes were then inactivated through cell suspension heating followed by centrifugation to remove insoluble compounds[20]. Trichloroacetic acid (TCA) was then added, followed by further centrifugation to remove proteins[99]. EPS was precipitated using an equal volume of ethanol and could be freeze-dried to obtain crude EPS[100]. In some studies, dialysis was used to remove residual molecules after co-precipitation with EPS[101].

Although there is no standardized extraction method recommended for EPS, many studies have compared the efficiency of different extraction protocols[97,102,103]. Three extraction techniques, including Cation Exchange Resin (CER), NaOH + formaldehyde, and ethylene diamine tetraacetic acid (EDTA), were compared[97]. The authors concluded that CER was the optimal extraction method of bound-EPS from Mediterranean soils as it provided a better compromise in terms of efficiency, handling time, and cost. In fact, although the CER method exhibited the lowest extraction efficiency, it also demonstrated the lowest level of EPS contamination[97].

In another study, Gangalla *et al.*[104] optimized the extraction of EPS produced by *Bacillus aerophilus* rk1 through the modification of several factors, including carbon and nitrogen sources, pH values varying from 6.0 to 8.0, time from 12 to 96 h, and incubation temperatures, from 20 to 45°C. The optimum conditions found were pH 7.0, temperature 30°C, and incubation time 72 h, yielding 3.73 g/L.

Hu *et al.*[99] investigated the effect of variation of three factors to optimize the extraction conditions: pH (7–9), sodium nitrate concentration (0.5–1.5%), and sodium citrate concentration (1.5–2.5%). The findings showed that the highest EPS yield (8.957 g/L) was reached at pH 8, 1% sodium nitrate concentration, and 2% sodium citrate concentration.

Zhao *et al.*[105] tested three factors, including precipitation temperature, precipitation time, and ethanol concentration in order to optimize EPS extraction. The maximum EPS yield

(53.43g/L) was obtained at 4°C precipitation temperature, 12 h precipitation time, and 80mL/dL ethanol concentration.

After the extraction, further purification may be required due to the possibility of residual proteins, DNA, and chemicals. This can be achieved by re-precipitation from an aqueous solution, chemical deproteinization, and membrane processes such as ultrafiltration[96]. The choice of the purification method depends on the specific characteristics and level of purity needed for the application of EPS. Some commonly used methods for the purification of exopolysaccharides include Sevag, TCA, ion exchange chromatography, and diethylaminoethyl (DEAE)-cellulose column chromatography methods[105–108]. Additional methods, such as gel filtration and size exclusion chromatography, can also be used during the purification process[109,110]. For example, Pei *et al*.[110] reported the application of gel filtration to purify levan produced by *Bacillus megaterium* PFY-147 through a Sephadex column, and EPS was eluted with Milli-Q water at a flow rate of 0.2 mL/min. Chen *et al*.[106] have been used anion exchange chromatography for the purification of EPS produced by *Lactobacillus reuteri* in order to remove the negative net charge, and the sample was eluted with NH_4HCO_3 at a flow rate of 2 mL/min. In addition, Patel *et al*.[111] investigated a study to compare the extraction of EPS obtained from *Porphyridium cruentum* by alcoholic precipitation, membrane separation, and dialysis techniques and found that diafiltration through a 300 kDa membrane proved to be the most efficient method. It should be noted that in order to select the suitable purification method, it is crucial to evaluate its effects on the properties, recovery, and purity of EPS, as certain purification methods may cause a decrease in product recovery or negatively impact EPS properties[96,112]. Furthermore, it was reported that it is not suitable to purify EPS from marine microalgae, since they are frequently contaminated by salts that co-precipitate with them[113].

Recently, efficient alternative tools have been developed to improve EPS production, including ultrasonication and microwave-assisted techniques. The application of ultrasonic treatment for six cycles of 5 minutes each resulted in an EPS yield of 218.78mg/g, which was 2.52-fold greater than the control group[114]. In addition, the application of microwave treatment also significantly increased the production of EPS by 2.3 folds compared to the untreated group[115]. Indeed, the combined effect of microwave and ultrasound showed a greater increase in EPS production compared to the combined impact of autoclave and ultrasound[116].

There are several factors that can be involved in the extraction of EPS. EPS producing microorganisms are found in several ecological sources. The source of EPS, such as the type

of bacteria strain, can affect the extraction process. Subsequently, the cultivation conditions and parameters, including the culture media composition and fermentation pattern, can adapted to optimize the growth of the chosen strain[117]. For instance, by using sucrose as carbon source, lactic acid bacteria strains from *Weissella confusa*/*cibaria* have been found to produce EPS with significant yields ranging from 3.2g/L to 47.1g/L, along with a high molecular weight dextran fraction[118].

The carbohydrate content of EPS is affected by several factors, including the strain, the type of carbon substrate used in the growth media, the availability of nutrients such as nitrogen (N) and phosphorus (P), and the extraction method employed for EPS extraction[119]. These factors can have an impact on the composition, quantity, and properties of the EPS. It was reported that *Tetragenococcus halophilus* produced two EPS fractions. EPS-1 had a significantly higher molecular weight (Mw) of 2613.4 kDa compared to EPS-2 with a MW of 93.4kDa, suggesting that a single strain has the ability to produce distinct exopolysaccharides with significantly different MWs[120]. *Lactobacillus plantarum* isolated from Egyptian cheese products produced the highest EPS yield of 11.86g/L compared with other *Lactobacillus* strains, indicating that EPS production varies among strains, even when obtained from the same origin[121]. Extraction conditions have a significant impact on yield, MW and composition[122]. The hydrolysis of probiotic EPS with trifluoroacetic acid (TFA) using various times showed that the highest concentration of monosaccharides was obtained at 4 hours of acid hydrolysis[106]. Chi *et al*.[123] found that acid extraction method provided the highest MW (41.1kDa) and the best yield (24.7%) of polysaccharide from *Enteromorpha prolifera* compared to the alkali and water extraction techniques. Furthermore, extraction of BPS-2 from *Bacillus thuringiensis* using multi-enzyme hydrolysis coupled with gel chromatography produced a heteropolysaccharide with a MW of 29.36kDa, and contained 63.1% amino sugars (D-Galactosamine and Glucosamine), showing its specific chemical composition[124]. The most commonly used technique to quantify the carbohydrate content is phenol-sulfuric acid method (Table 1). Anthrone-sulfuric acid method is another colorimetric technique that can be used for EPS quantification[124]. Quantification of EPS by nuclear magnetic resonance (NMR) and spectrophotometric methods was also used to validate the traditional techniques[125]. Indeed, NMR is the most efficient method, as acid hydrolysis may fail to release all monosaccharides or result in the degradation of certain carbohydrates during the process of acid hydrolysis[126]. In addition, the spectrophotometric method may also not be able to detect some complex carbohydrates in EPS samples[125,126].

There are several physicochemical properties that should be determined in order to evaluate the structural characteristics of EPS, including molecular weight, monosaccharide

composition, type of glycosidic linkage, etc. The different methods used for EPS characterization are shown in Table 1. To determine the average molecular weight (MW), high-performance size-exclusion chromatography (HPSEC) with refractive index (RI) and multi-angle laser light scattering (MALLS) were used[109]. Gel permeation chromatography (GPC) using RI and MALLS as detectors was also used to determine the MW of EPS[127]. The monosaccharide composition has been carried out by several methods, including high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), high-performance liquid chromatography (HPLC) system with pre-column derivatization, and gas chromatography coupled with mass spectrometry detector (GC–MS) after acid hydrolysis and derivatization[128–130]. The type of glycosidic bond was identified by methylation analysis using GC–MS following hydrolysis, reduction, and acetylation[109,127]. Furthermore, NMR is a powerful analytical technique that can be used to determine the specific glycosidic linkages between monosaccharide units, while Fourier-transform infrared (FT-IR) spectroscopy allows the identification of functional groups present in EPS[131,132]. Thermal properties of EPS, such as melting point and thermal stability, can be determined by thermogravimetric analysis (TGA), derived thermogravimetric (DTG) analysis, and differential scanning calorimeter (DSC) analysis[110,130,132]. In addition to these mentioned techniques, other potent analytical methods such as X-ray diffraction (XRD), scanning electron microscopy (SEM), and atomic force microscopy (AFM) have been employed to analyze the crystalline structure and morphological characteristics of EPS[124,133,134].

The ability of strains to produce EPS is widespread among different species, although the physiological function of these molecules is still not fully understood[101]. It was reported that certain monosaccharides, including uronic acids and aminosaccharides are commonly identified in the EPS produced by marine bacteria[135]. These findings are consistent with the data shown in Table 1. For instance, Alshawwa *et al*.[136] showed that EPSR5 isolated from *Kocuria* sp. marine bacteria contained sulfate, uronic, and hexose amine at proportions of 25.6%, 21.77%, and 13.55%, respectively. Selim *et al*.[137] reported that the major fraction of EPS-producing *Bacillus cereus* strain from marine sediment contained uronic acid at percentage of 28.7%. In addition, Awady *et al*.[138] found that EPSNC2 isolated from marine *Streptomyces hirsutus* was a highly acidic heteropolysaccharide which contained 72.73% uronic acid, and composed of glucuronic acid: galacturonic acid: mannose: glucose: arabinose with molar ratio 1.2: 0.6: 0.2: 0.1: 0.1, respectively. Indeed, EPSNC2 exhibited a strong antioxidant ability due to the high occurrence of hydroxyl groups in uronic acid[138]. On the other hand, it seems that the majority of the EPS extracted from lactic acid bacteria (LAB) are classified as heteropolysaccharides, which mainly consist of 3 to 8 units, with

glucose, galactose, mannose, and rhamnose as the major monosaccharides (Table 1)[117,130,139,140].

Table 1. EPS extraction methods, purification, and quantification.

Microbial strains	Source	EPS	Extraction and purification methods	Quantity	Quantification and Identification methods	Ref.
Lactobacillus kefiri	Chinese kefir grains	MSR101 EPS Carbohydrate (93.35%) Uronic acid (2.15%) Sulfated (2.7%) Glucose, rhamnose and arabinose in a molar ratio of 5:1:3, respectively	Boiled in a water bath, centrifugation, TCA treatment, ethanol precipitation, dissolved in sterile distilled water	0.75g/L	phenol-sulfuric method, meta-hydroxybiphenyl method, barium chloridegelatine method FTIR, NMR, TGA, XRD	[131]
Bacillus thuringiensis	Bean paste	Carbohydrate (91.29%) Monosaccharide composition (μmol/L) : D-Galactosamine 5.53 Arabinose 1.77 Glucosamine 4.74 Glucose 3.24 Mannose 1.00	Organic extraction, ethanol precipitation, dialyzing and freeze drying, centrifugation, purification by Superdex 200 column	16.19g/L	anthrone-sulfuric acid method, UV–visible, HPGFC, FT-IR, SEM, ion chromatography, NMR	[126]
Arthrospira Platensis	Microbial Culture Collection	ND	Centrifugation, purification by dialysis, and freeze drying	317.93 μg/mg	phenol-sulfuric acid method	[141]
Bacillus circulans	Slimy layer of coconut	Monosaccharide composition (Glucose, Mannose, and Galactose)	Centrifugation, incubation overnight in ethanol, purification by dialysis, lyophilization	0.065g/L	phenol-sulfuric acid method FTIR, NMR, HPLC, GC–MS	[142]
Lactococcus lactis subsp. cremoris FC	Fermented milk	Rhamnose, galactose, and glucose	Ethanol precipitation followed by TCA treatment, dialyzing against distilled water and lyophilisation	ND	HPLC, GC, GC-MS, NMR	[143]
Lactobacillus plantarum LRCC5310	Kimchi	ND	Centrifugation, ethanol precipitation, incubation for 30 min, drying at 4°C	0.64g/L	field-emission scanning electron microscopy	[144]

Microbial strains	**Source**	**EPS**	**Extraction and purification methods**	**Quantity**	**Quantification and Identification methods**	**Ref.**
Lactobacillus confusus TISTR 1498	Culture collection	Carbohydrate (81.9%) Homopolysaccharide (D-glucose)	Centrifugation, ethanol precipitation, TCA, precipitation using 3 volumes of ethanol, drying at room temperature	6.12g/L	phenol-sulphuric acid method, HPLC, GC-MS, NMR, HPSEC	[145]
Streptomyces hirsutus NRC2018	Marine sediments	Uronic acid (72.73%) Glucuronic:galacturonic: glucose:mannose:arabinose with molar ratio 1.2:0.6:0.1:0.2:0.1, respectively	TCA treatment, centrifugation, neutralization with NaOH, precipitation using 4 volumes of ethanol, centrifugation and dialyzing, purification by 4 volumes of absolute cold ethanol, dialyzing against distilled water	ND	Turbidity method, m-hydroxybiphenyl colorimetric method, HPLC, FTIR	[146]
Several bacterial strains	Fermented vegetables	Mannose, glucosamine, rhamnose, glucose, galactose, xylose	Precipitation by cold ethanol, centrifugation, purification using anion exchange chromatography	1.74–7.28g/L	Phenol-sulphuric acid method, HPCL	[106]
Lactobacillus	Tempoyak	ND	Precipitation using 2 volumes of ethanol, centrifugation, dialyzing against distilled water	0.10–0.85g/L	Phenol-sulphuric acid method	[147]
Rhodococcus erythropolis HX-2	Xinjiang Oil Field	Carbohydrate (79.24%) Glucose (27.29%), galactose (24.83%), fucose (4.79%), mannose (26.66%) and glucuronic acid (15.84%)	Heating at 90°C, centrifugation, TCA treatment to remove protein, precipitation using 3 volumes of ethanol, dialyzing and freeze drying, purification by gel filtration	3.736g/L	phenol-sulfuric acid method, anion exchange chromatography, high-performance size-exclusion chromatography, HPLC, UV-visible spectrophotometer, FT-IR, NMR, TGA, SEM, AFM	[99]
Lactobacillus acidophilus	Microbiological Resource Center	ND	Alcohol precipitation, drying at 60°C	0.35g/L	HPLC	[148]

Microbial strains	**Source**	**EPS**	**Extraction and purification methods**	**Quantity**	**Quantification and Identification methods**	**Ref.**
Bacillus thuringiensis	Marine ascidian	Fructose (43.8%), galatose (20%), xylose (17.8%), glucose (7.2%), rhamnose (7.1) and mannose (4.1%)	Deproteinization by Sevag method, purification through gel filtration chromatography	60.1%	phenol-sulfuric acid method, HPLC, FT-IR, ^{1}H NMR	[149]
Lactiplantibacillus plantarum DMDL 9010	Fermented pickles	Fucose (0.13%), arabinose (0.69%), galactose (8.32%), glucose (27.57%), mannose (62.07%), fructose (0.58%) and galacturonic acid (0.46%)	Sevag reagents precipitated ethanol and deproteinized to acquire crude EPS, lyophilization, purification using anion exchange chromatography, column purification, freeze-drying	42.40%	Phenol-sulfate method, HPGPC, UV, FT-IR, SEM, TG, NMR, HPLC, GC-MS	[150]
Lactiplantibacillus plantarum MM89	Human breast milk	Glucose and mannose	Precipitation by double volumes of ethanol, centrifugation, further purification by dialysis, purification by DEAE-52 chromatography, freeze-drying	95.63%	phenol–sulfuric acid method, FTIR, NMR, GC-MS, GPC	[107]
Pseudomonas Aeruginosa	Microbial Culture Collection	ND	Ethanol precipitation, incubation overnight, centrifugation, purification by dialysis against double distilled water and lyophilization	0.0924–0.1027 g/L	phenol-sulfuric acid method, FTIR	[151]
Leuconostoc mesenteroides	Semi-hard Italian Cheese	EPS_B3 Dextran	TCA treatment, centrifugation, precipitation using three volumes of cold absolute ethanol, dialyzing and freeze drying	1.06g/L	FT-IR, NMR, UV–Vis, HPLC, HPLC-SEC	[152]
Kocuria sp.	Red Sea	EPSR5 Sulfated (25.6%), uronic acid (21.77%), hexose amine (13.55%).	TCA treatment, centrifugation, precipitation by four volumes	6.84g/L	UV absorption spectrum, FTIR, Dodgson and Price technique, HPLC	[136]

Microbial strains	**Source**	**EPS**	**Extraction and purification methods**	**Quantity**	**Quantification and Identification methods**	**Ref.**
		Glucose, galacturonic acid, arabinose, and xylose in a molar ratio of 2.0:0.5:0.25:1.0	from cold ethanol, redissolving in deionized water and dialyzing			
Weissella cibaria	Korean kimchi	Glucose (38.95%) and galactose (61.04%)	Ethanol precipitation, centrifugation, deproteinization by the Savage solution, dialyzing and freeze drying, purification using a DEAE-sepharose (GE Healthcare, Sweden) fast flow column, second purification by sub-column and lyophilization	ND	Phenol-sulfuric acid assay, HPLC, FTIR, NMR	[153]
Streptococcus thermophilus and *Lactobacillus bulgaricus*	Labaneh (traditional yogurt-like product)	EPS-S: Glucose, mannose, and galactose, with molar ratios of 17.8:1.35:0.5 EPS-L: Glucose, mannose, and galactose, with molar ratios of 14.2:1.2:0.75	Precipitation by double volume of chilled absolute ethanol followed by TCA treatment, centrifugation, dialyzing and freeze drying	ND	GPC, GC-MS, NMR, FTIR	[154]
Lactobacillus curvatus SJTUF 62116	Gymnocypris przewalskii	Carbohydrate (96.03%) Glucosamine, glucose, mannose, and glucuronic acid (GluA) with a relative molar ratio of 0.36:1:2.49:0.39	TCA treatment, centrifugation, precipitation by chilled absolute ethanol, crude EPS was dissolved in distilled water and dialyzed against distilled water followed by lyophilizing	0.28g/L	Phenol-sulfuric acid method, HPSEC-MALLS-RI, HPAEC, UV–vis spectroscopy, FTIR, NMR, SEM, AFM, TGA	[140]
Lactiplantibacillus plantarum RO30	Romi cheese	Carbohydrate: 85.83% (w/w) Glucuronic acid, mannose, glucose, and arabinose with a molar	TCA precipitation, centrifugation, neutralization with NaOH, precipitation by three volumes of cold absolute ethanol,	4.23g/L	Phenol–sulfuric acid method, HPLC, HPLC/GPC, FT-IR, 1HNMR	[155]

Microbial strains	Source	EPS	Extraction and purification methods	Quantity	Quantification and Identification methods	Ref.
		ratio of 2.19:0.1:0.536:0.104	centrifugation, dissolved in ultrapure water and dialyzed for 2 days at 4 °C against the same solution, re-precipitation using three volumes of cold ethanol			
Pediococcus pentosaceus E8	Cereal vinegar	Mannose, glucose, and galactose at a molar ratio of 80.39: 18.12: 1.49	Precipitation by three volumes of pre-chilled ethanol, deproteinization using papain combined with Sevage reagent, dialyzing against distilled-deionized water followed by lyophilizing, purification by anion-exchange chromatographic column	97.9%	Phenol–sulfuric acid colorimetric method, MALLS, HPSEC, HPAEC-PAD, GC-MS, FTIR, NMR, TGA, DTG, XRD	[130]
Bacillus albus DM-15	Indian Ayurvedic traditional medicine Dasamoolarishta	Molar percentage: Glucose (71.32%), galactose (13.55%), xylose (9.38%), and rhamnose (5.75%)	Precipitation by two volumes of absolute ice-cold ethanol, centrifugation, dialyzing against sterile deionized water, purification using ion-exchange chromatographic column and gel-filtration chromatography followed by lyophilizing	0.29g/L	Phenol-sulfuric acid assay, size-exclusion chromatography, GC-MS, UV-VIS, FT-IR, NMR, XRD	[133]
Bacillus subtilis LR-1	Hunan fermented meat	Glucose (82.95%) and mannose (13.58%)	Centrifugation, deproteinization by the Sevage method, precipitation by three volumes of pre-cooled ethanol,	1.86g/L	Phenol-sulfuric acid method, gel permeation Chromatography, HPLC, HPGPC, FT-IR, NMR	[105]

Microbial strains	**Source**	**EPS**	**Extraction and purification methods**	**Quantity**	**Quantification and Identification methods**	**Ref.**
			dialyzing and freeze-drying, purification using anion-exchange column and gel column			
Lactiplantibacillus pentosus B8	Sichuan Pickle	LPB8-0: Carbohydrate (96.2%) Glucose (84.24%) and mannose (15.76%) LPB8-1: Carbohydrate (99.1%) Mannose (77.74%), glucose (21.08%), and galactose (1.18%) a	Ethanol precipitation, deproteinization by the Sevage method, dialyzing, lyophilizing, purification using anion-exchange chromatographic column, further EPS purification by size exclusion chromatographic columns followed by freeze-drying	1.40g/L	Phenol-sulfuric acid method, UV–vis spectrophotometer, HPSEC, GC–MS, FTIR, NMR, TGA, XRD, SEM, AFM	[109]
Bacillus cereus	Saudi Red Sea coast	Uronic acid (28.7%) Glucose, galacturonic acid, and arabinose at a molar ratio of 2.0: 0.8: 1.0	Treatment with TCA to remove protein, neutralization using NaOH, ethanol precipitation, centrifugation, dialyzing against deionized water	7.95g/L	FTIR, m-hydroxybiphenyl colorimetric technique, Aminex Carbohydrate Analysis Column, HPLC, GPC	[137]
Bacillus subtilis	Marine sediment	Sulfated polysaccharide (48%) Glucose, rhamnose, and arabinose at a molar ratio of 5:1:3	Treatment with TCA to remove protein, centrifugation, neutralization using NaOH, ethanol precipitation, dialyzing against deionized water	8.12g/L	FT-IR, UV-Vis, HPGPC, GPC, Aminex Carbohydrate Analysis Column, XRD	[156]
Bacillus haynesii CamB6	acidic (pH 5.82) Campanario hot spring	Mannose (66%), glucose (20%), and galactose (14%)	Treatment with TCA to precipitate the proteins, centrifugation, acetone precipitation, centrifugation, dialyzing followed by lyophilization	2.90g/L	AFM, SEM, HPLC, GPC, FTIR, NMR, TGA	[128]

Microbial strains	**Source**	**EPS**	**Extraction and purification methods**	**Quantity**	**Quantification and Identification methods**	**Ref.**
Weissella confusa KR780676	Idli batter (Indian traditional fermented food)	Galactan EPS	Treatment with TCA to eliminate the proteins, ethanol precipitation, centrifugation, dialyzing and freeze-drying	ND	SEM	[157]
Lacticaseibacillus paracasei Subsp. paracasei SS-01	Yogurt	Molecular weight: 49.68kDa	Ethanol precipitation, enzymatic hydrolysis, ethanol precipitation, freeze drying, dialyzing followed by lyophilization	ND	UV-Vis, FT-IR, GPC, SEM, AFM and TGA	[158]
Bacillus xiamenensis RT6	Sediments of the river source in Río Tinto (Huelva), Spain	EPS_t Glucose (60%), mannose (20%), and galactose (20%)	Precipitation by three volumes of cold ethanol, centrifugation, dialyzing, freeze-drying, purification using anion exchange column	0.75g/L	Phenol-sulfuric acid method, gel filtration chromatography, GC–MS, HPLC/MS-MS, ATR-FTIR, TGA, DSC	[129]
Saccharomyces cerevisiae Y3	Sweet glutinous rice (Chinese fermented food)	Y3 EPS Mannose (85.41%) and glucose (9.29%)	Treatment with TCA to remove the proteins, centrifugation, precipitation by three volumes of ethanol, centrifugation, dialyzing followed by lyophilization, purification by gel filtration chromatography	4.52g/L	HPLC, GPC, FT-IR, IR, GC–MS, NMR, XRD, UV–Vis, SEM, AFM, DSC, TGA, DTG	[127]
Leuconostoc mesenteroides SL and *Enterococcus viikkiensis* N5	Moroccan raw donkey milk	EPS-SL: Glucose (62.84%) and galactose (37.16%) EPS-N5: Glucose (79%), galactose (12.7%), and mannose (8.3%)	Treatment with TCA followed by centrifugation to remove the proteins, precipitation by two volumes of cold ethanol, centrifugation, dialyzing against deionized water, purification using gel filtration	0.67 g/L (EPS-SL) 0.90 g/L (EPS-N5)	Phenol-sulfuric acid method, GC–MS, FTIR, UV–Vis, NMR, SEM, TGA	[159]

Microbial strains	**Source**	**EPS**	**Extraction and purification methods**	**Quantity**	**Quantification and Identification methods**	**Ref.**
			chromatography, freeze-drying			
Rhizopus nigricans	ND	EPS2-1: Carbohydrate (98.56%) Man, Gal, Glc, Ara, and Fuc at a molar ratio of 0.519:0.204:0.065:0.031 and 0.029	Precipitation by four volumes of ethanol, deproteinization, decoloration, dialysis, lyophilization, purification by column chromatography	0.20g/L	Phenol–sulfuric acid method, HPGPC, HPIC, FT-IR, UV Spectroscopy, GC-MS, NMR, TEM, AFM	[160]
Lactobacillus kunkeei AK1	Bee pollen	EPS AK1 (dextran type EPS)	Precipitation by chilled ethanol, centrifugation, treatment with TCA to remove the proteins, neutralization, dialyzing, lyophilization	ND	HPLC, GPC, NMR, FTIR, TGA, DSC, SEM, AFM, XRD	[134]
Tetragenococcus halophilus	Soya sauce moromi	EPS-1: Carbohydrate (92.29%) Galactose, glucose, mannose and glucuronic acid at a molar ratio of (1.00:5.57:2.69:1.09) EPS-2: Carbohydrate (95.12%) Glucose and mannose at a molar ratio of (1.00:5.93)	Treatment with TCA to remove protein, precipitation by three volumes of pre-chilled absolute ethanol, dissolving in ultrapure water, dialyzing, lyophilizing, purification using both anion-exchange chromatography and gel filtration chromatography	0.45 g/L EPS-1 (25%) EPS-2 (27.5%)	Phenol–sulfuric acid method, UV–vis, HPAEC-PAD, GC–MS, NMR, SEM, AFM	[120]
Tetragenococcus halophilus SNTH-8	Soybean	Crude polysaccharide: Sugar contents (75.24%) Sulfated groups (0.25%) THPS-1: Sugar contents (92.16%) Uronic acid (0.35%) Sulfated groups (0.16%) Arabinose, xylose, fucose, galactose,	Treatment with TCA to remove protein, precipitation by thrice the volume of anhydrous ethanol, centrifugation, dialyzing, lyophilizing, purification using both ion-exchange chromatography and	1.00g/L	Phenol–sulfuric acid method, sulfuric acid carbazole method, barium chloride-gelatine colorimetry, HP-GPC, HPLC, UV, FT-IR, SEM, NMR, GC–MS	[108]

Microbial strains	Source	EPS	Extraction and purification methods	Quantity	Quantification and Identification methods	Ref.
		glucose, and glucuronic acid, at molar ratios of 1.66:38.95:2.11:26.12:29.73:1.43 THPS-2: Sugar contents (91.08%) Uronic acid (2.38%) Sulfated groups (0.19%) Arabinose, xylose, fucose, galactose, glucose, and glucuronic acid, at molar ratios of 0.46:40.3:0.54:30.8:1.36:25.54	gel filtration chromatography			
Bacillus enclensis AP-4	Deep-sea sediments	Mannose, glucosamine, glucose, galactose, and xylose in a molar ratio of 1.00: 0.09: 0.04: 0.09: 0.07	Treatment with TCA followed by centrifugation to remove protein, precipitation by three volumes of pre-cooling ethanol, centrifugation, dialyzing, freeze-drying, purification using column chromatography	4.23g/L	Phenol sulfuric acid method, UV-VIS, HPLC, FTIR, NMR, SEM, XPS	[161]
Aspergillus sp. DHE6	Soil	ND	Treatment with TCA to remove protein, neutralization, precipitation by 4 volumes of chilled absolute ethanol, centrifugation, dialyzing towards distilled water, drying at 60 ◦C	7.20g/L	Phenol-sulfuric colorimetric method, FT-IR	[162]
Lactobacillus plantarum JLAU103	Hurood (in Inner Mongolia of China)	EPS103: Carbohydrate (90.16%) Sulfuric ester (1.08%) Arabinose, rhamnose, fucose, xylose, mannose, fructose, galactose, and glucose in a molar ratio	Extraction and purification by ion-exchange chromatography, further purification using gel filtration chromatography	0.07g/L	Phenol-sulfuric acid method, Dische method, HPSEC, UV-vis, GC-MS, FT-IR, NMR, SEM, AFM	[163]

Microbial strains	**Source**	**EPS**	**Extraction and purification methods**	**Quantity**	**Quantification and Identification methods**	**Ref.**
		of 4.05: 6.04: 6.29: 5.22: 1.47: 5.21: 2.24: 1.83				
Lactobacillus pentosus 14FE, *Lactobacillus plantarum* 47FE, and *Lactobacillus pentosus* 68FE	Egyptian cheese products	EPSs: Carbohydrates (81.38–85.19%)	Treatment with TCA to remove protein, precipitation by four-volume of ethanol, dialyzing against distilled water	4.15–11.86g/L	Phenol sulfuric acid method, FT-IR, ^{1}H NMR, paper chromatography	[121]
Streptococcus thermophilus ZJUIDS-2-01	Traditional yak yogurt	EPS-3: Glucose, galactose, N-acetyl-D-galactosamine, and rhamnose in a ratio of 5.2:2.5:6.4:1.0	Treatment with TCA followed by centrifugation to remove protein, precipitation by four volumes of cold ethanol, centrifugation, dialyzing, lyophilizing, purification using anion-exchange chromatography and gel filtration chromatography	0.48g/L	Phenol-sulfuric acid assay, UV-Vis, HPGPC, HPAE-PAD, FT-IR, GC–MS, NMR, DSC	[164]
Lactobacillus plantarum C70	Camel milk	EPS-C70: Arabinose (13.3%), mannose (7.1%), glucose (74.6%) and galactose (5.0%) were the major monosaccharides constituents with molar ratio (2.7:1.4:15.1:1.0)	Extraction, purification, lyophilization, dialyzing, freeze-drying	ND	UV-Vis, GPC, GC-FID, FTIR, NMR, DSC, SEM	[165]
Pediococcus acidilactici MT41-11	Camel milk	Crude EPS: Carbohydrates (73.33%) EPS-1: Carbohydrates (94.61%) Mannose, rhamnose, glucuronic acid, glucose, galactose, xylose, arabinose, and fucose with a molar ratio of	Treatment with TCA followed by centrifugation to remove protein, ethanol precipitation, centrifugation, dialyzing followed by lyophilization, partial purification using anion-exchange	0.50g/L	Phenol-sulfuric acid method, GPC, PMP-HPLC, FT-IR, NMR, SEM	[139]

Microbial strains	**Source**	**EPS**	**Extraction and purification methods**	**Quantity**	**Quantification and Identification methods**	**Ref.**
		(52.82:0.15:1.02:1.26:0.29:0.03:0.95:1.83) EPS-2: Carbohydrates (84.82%) Mannose, rhamnose, glucuronic acid, glucose, galactose, xylose, arabinose, and fucose with a molar ratio of (2.58:1.17:1.27:53.52:1.49:0.30:0.15:9.39)	chromatography and gel filtration chromatography			
Leuconostoc mesenteroides SN-8	*Dajiang* (traditional fermented food in northeast China)	SN-8 EPS: Glucan and mannose	Centrifugation, cold ethanol precipitation, savage deproteinization, dialysis, and lyophilization	2.42g/L	HPLC, FT-IR, SEM, DSC, TGA	[166]
Lactobacillus plantarum S123	Traditional Chinese cheese	ND	Treatment with TCA followed by centrifugation to remove protein, precipitation by pre-chilled ethanol, centrifugation, dialysis, and lyophilization	0.75g/L	Phenol sulfuric acid method, FTIR, ^{1}H and ^{13}C NMR, XRD, SEM	[167]
Bacillus aerophilus rk1	Soil	ND	Precipitation by double volumes of chilled acetone, centrifugation, dialysis, freeze-drying, purification by anion exchange chromatography	3.73g/L	Phenol–sulfuric acid Method, UV–Vis, FT-IR, NMR, SEM, EDX	[104]
Leuconostoc mesenteroides	Sourdough	Levan type EPS	Ethanol precipitation, TCA precipitation, dialysis to remove the proteins, and lyophilization	13.20g/L	HPLC, NMR, FTIR, SEM, AFM, TGA, DTG, DSC	[132]
Pediococcus pentosaceus M41	Marine source (low water	EPS-M41: Arabinose (6.2%), mannose (9.5%), glucose	Precipitation by double volumes chilled absolute		UV–vis, GPC, GC-FID, FTIR, NMR, DSC, SEM	[168]

Microbial strains	Source	EPS	Extraction and purification methods	Quantity	Quantification and Identification methods	Ref.
	activity dried fish)	(79.0%) and galactose (5.2%) with a molar ratio of (1.2:1.8:15.1:1.0)	ethanol, centrifugation, treatment with TCA to remove protein, dialysis against distilled-deionized water, freeze-drying			
Rhodotorula mucilaginosa sp. GUMS16	Fallen leaf debris of Deylaman jungle	Carbohydrate (61.7%) Glucose (85%) and mannose (15%) with a molar ratio of 5.7:1	Ethanol precipitation, centrifugation, lyophilization	28.50g/L	Phenol sulfuric acid method, FT-IR, FE-SEM, SEC-HPLC, GC–MS, GC-EI-MS, NMR	[169]
Lactobacillus plantarum HY	Home-made Sichuan Pickle	HY EPS: Mannose, galactose, glucuronic acid and glucose in a mass percentage of 72.99%, 17.27%, 6.99% and 2.75%	Treatment with TCA followed by centrifugation to remove protein, precipitation by three volumes of pre-chilled ethanol, centrifugation, dialysis, lyophilization, purification by anion exchange chromatography, further purification by gel filtration	1.42g/L	Phenol sulfuric acid method, GPC, HPLC, FT-IR, NMR, SEM, AFM, TGA, DSC	[170]
Bacillus megaterium PFY-147	Hiqiher vineyard soil	Levan type EPS: Carbohydrate (90.37%), uronic acid (7.24%) and sulfated group (2.39%)	Protein precipitation by TCA and three volumes of pre-cooled ethanol, centrifugation, dialysis, lyophilization, purification by gel-filtration chromatography	4.82g/L	FT-IR, NMR, SEM, AFM, TGA, DSC	[110]

GPC : Gel Permeation Chromatography, FTIR : Fourier-Transform Infrared, NMR : Nuclear Magnetic Resonance, TGA : Thermogravimetric Analysis, DTG : Derived Thermogravimetric, XRD : X-Ray Diffraction, HPGFC : High Performance Gel Filtration Chromatography, SEM : Scanning Electron Microscopy, AFM : Atomic Force Microscopy, HPLC : High-Performance Liquid Chromatography, GC–MS : Gas Chromatography coupled with Mass Spectrometry Detector, HPSEC: High Pressure Size Exclusion Chromatography, HPAEC-PAD : High-Performance Anion-Exchange Chromatography With Pulsed Amperometric Detection, SEC-HPLC : Size Exclusion-High-Performance Liquid Chromatography, HPSEC-MALLS-RI : High-Performance Size Exclusion Chromatography-Multi-Angle Laser Light Scattering-Refractive Index Detector, DSC : Differential Scanning Calorimeter, HPIC : High Pressure Ion Chromatography, XPS : X-Ray Photoelectron Spectroscopy, HPGPC : High Performance Gel Permeation

Chromatography, GC-FID : Gas Chromatography-Flame Ionization Detection, PMP-HPLC : 1-Phenyl-3-Methyl-5-Pyrazolone High Performance Liquid Chromatography, FE-SEM : Field Emission Scanning Electron Microscopes, GC-EI-MS : Gas Chromatography-Electron Ionization-Mass Spectrometry.

4. Functional Properties

4.1. Anti-oxidant Activities

Oxidative stress can be defined as a phenomenon caused by an imbalance between the production of free radicals such as reactive oxygen species (ROS) and the ability of the antioxidant defences of a biological system to scavenge these reactive products[171]. ROS are normally generated during oxygen metabolism[172]. However, environmental stressors and xenobiotics lead to a significant increase in ROS production[171]. Harmful effects caused by oxidative stress on important biological molecules like nucleic acids, proteins, and lipids are linked to several disease, including diabetes, cancer, metabolic disorders, and cardiovascular diseases[173,174]. As synthetic antioxidants have been known to pose safety risks, considerable attention has been given to exploring the potential antioxidant ability of natural molecules[175]. Recently, EPS showed the capacity to inhibit the activity of free radicals, thus providing a notable antioxidant activity[176].

The antioxidant activities of bacterial EPS (Table 2) have mainly been investigated using *in vitro* techniques, including reducing power analysis, lipid peroxidation inhibition capacity, 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation, hydroxyl (•OH) radical, superoxide anion (O^{2-}) radical, and hydroxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays.

Table 2. Exopolysaccharides antioxidant activity.

Strain	Antioxidant assay	EPS concentration	Results	Ref.
Bacillus thuringiensis	DPPH radicals	5mg/ml	31.34%	[124]
	Hydroxyl radicals		32.43%	
	Superoxide anions		34.31%	
	ABTS radicals		48.53%	
Bacillus circulans	DPPH	1000μg/ml	97%	[142]
Bacillus subtilis F9	DPPH	800μg/ml	81.46%	[175]
	Hydroxyl radicals		66.34%	
	Superoxide radicals		78.03%	

Strain	Antioxidant assay	EPS concentration	Results	Ref.
Lactobacillus plantarum MTCC 9510	Reducing power	0.05–1mg/ml	3 (in terms of absorbance)	[177]
Streptomyces hirsutus NRC2018	DPPH	100–1500µg/ml	IC_{50} = 158.5 µg/ml	[146]
	Hydrogen peroxide	1500µg/ml	75.6% IC_{50} = 501.2 µg/ml	
Lactobacillus Strains	DPPH	ND	32.29% to 73.36%	[147]
Bacillus thuringiensis	DPPH	1mg/ml	79%	[149]
	Superoxide radical		75.12%	
Leuconostoc mesenteroides	ABTS	20mg/ml	24%	[152]
Kocuria sp.	DPPH	2000µg/ml	98%	[136]
Weissella cibaria	DPPH	1mg/ml	34%	[153]
	ABTS		90%	
Streptococcus thermophilus	DPPH	5mg/ml	31.36%	[154]
	ABTS		66.24%	
Lactobacillus bulgaricus	DPPH		36.69%	
	ABTS		82.52%	
Lactobacillus curvatus SJTUF 62116	DPPH		84.50%	[140]
	ABTS		92.53%	
Rhodotorula mucilaginosa sp. GUMS16	DPPH	1500µg/ml	up to 58%	[178]
Lactiplantibacillus plantarum RO30	DPPH	5mg/ml	43.60%	[155]
	Reducing power	10mg/ml	1.108 (in terms of absorbance)	
Pediococcus pentosaceus E8	DPPH		50.62%	[130]
	ABTS		52.17%	
	Hydroxyl radical		58.91%	

Strain	Antioxidant assay	EPS concentration	Results	Ref.
Bacillus albus DM-15	DPPH	3mg/ml	59.91%	[133]
	ABTS		64.24%	
	Nitric oxide radical		63.78%	
Bacillus subtilis LR-1	DPPH		80% and 56%	[105]
Lactiplantibacillus pentosus B8 (EPS: LPB8-0)	DPPH	10mg/ml	50.62%	[109]
	ABTS		47.17%	
	Hydroxyl radical		47.91%	
Lactiplantibacillus pentosus B8 (EPS: LPB8-1)	DPPH		62.82%	
	ABTS		58.36%	
	Hydroxyl radical		72.52%	
Lactiplantibacillus plantarum	DPPH	4mg/ml	33.60%	[179]
Bacillus cereus	DPPH	1500μg/ml	90.40% IC_{50} = 500 μg/ml	[137]
	Hydrogen peroxide radical		75.10% IC_{50} = 1500 μg/ml	
Bacillus subtilis	DPPH		97.60% IC_{50} = 300 μg/ml	[156]
	Hydrogen peroxide radical		64.80% IC_{50} = 1500 μg/ml	
Bacillus haynesii CamB6	DPPH	5mg/ml	72.30%	[128]
	Hydrogen peroxide radical		76.21%	
	ABTS		72.80%	
Weissella confusa KR780676	DPPH	500μg/ml	60% IC_{50} = 450 μg/ml	[157]
	Nitric oxide radical	200μg/ml	Almost 80%	

Strain	Antioxidant assay	EPS concentration	Results	Ref.
			IC_{50} = 138 μg/ml	
	Hydroxyl radicals	1 mg/ml	41.93%	
Lacticaseibacillus paracasei Subsp. *paracasei* SS-01	DPPH	10mg/ml	73.84% IC_{50} = 1.314 mg/ml	[158]
	Hydroxyl radicals		88.80% IC_{50} = 2.369 mg/ml	
	ABTS		90.46% IC_{50} = 0.449 mg/ml	
Bacillus xiamenensis RT6	DPPH	0.1–10mg/ml	Around 65% for all of the concentrations	[129]
	Hydroxyl radicals	2.5mg/ml	100%	
	Superoxide anions	10mg/ml	39.40%	
Saccharomyces cerevisiae Y3	DPPH	4mg/ml	35%	[127]
	Hydroxyl radicals		35.71%	
	Scavenging activity of Nitroso (NO^{2-})		17.85%	
Leuconostoc mesenteroides SL	DPPH	5mg/ml	52.70%	[159]
	ABTS		60.80%	
	Reducing power		1.08 (in terms of absorbance)	
Enterococcus viikkiensis N5	DPPH		64.90%	
	ABTS		75.20%	
	Reducing power		1.26 (in terms of absorbance)	
Rhizopus nigricans	DPPH		20.55%	[160]
	ABTS		12.09%	
	Hydroxyl radicals		13.76%	
	DPPH	4mg/ml	46.23%	[134]

Strain	Antioxidant assay	EPS concentration	Results	Ref.
Lactobacillus kunkeei AK1	ABTS	8mg/ml	91.60%	
Tetragenococcus halophilus (EPS-1)	DPPH	10mg/ml	22.61%	[120]
	Hydroxyl radicals		20.11%	
	ABTS		Around 20%	
Tetragenococcus halophilus (EPS-2)	DPPH		56.03%	
	Hydroxyl radicals		37.72%	
	ABTS		Around 40%	
Tetragenococcus halophilus SNTH-8 (THPS-1)	DPPH	12mg/ml	63.53%	[108]
	Hydroxyl radicals		50.19%	
	ABTS		44.19%	
	Reducing power		1.62 (in terms of absorbance)	
Tetragenococcus halophilus SNTH-8 (THPS-2)	DPPH		68.81%	
	Hydroxyl radicals		63.24%	
	ABTS		59.24%	
	Reducing power		1.86 (in terms of absorbance)	
Bacillus enclensis AP-4	DPPH	5mg/ml	83.1%	[161]
	ABTS		80.3%	
	Hydroxyl radicals		74.6%	
	Superoxide anion radical		87.5%	
Aspergillus sp. DHE6	DPPH	600μg/ml	52.3% IC_{50} = 573.6 μg/ml	[162]
Lactobacillus plantarum JLAU103	Hydroxyl radicals	10mg/ml	80.40%	[163]
	ABTS		65.50%	
	DPPH		60.50%	

Strain	Antioxidant assay	EPS concentration	Results	Ref.
Lactobacillus pentosus 14FE, *Lactobacillus plantarum* 47FE, and *Lactobacillus pentosus* 68FE	DPPH		54.50–68.90%	[121]
Streptococcus thermophilus ZJUIDS-2-01	DPPH		34.5%	[164]
Lactobacillus plantarum C70	DPPH	10mg/ml	75.91%	[165]
	ABTS		49.42%	
Enterococcus durans K48	DPPH	25mg/ml	53%	[180]
Enterococcus faecium R114	DPPH		58%	
Enterococcus faecium T52	DPPH		64%	
Lactococcus garvieae C47	DPPH	10mg/ml	67.52%	[181]
	ABTS		61.06%	
Pediococcus acidilactici MT41-11	DPPH	3mg/ml	Up to 71.65% IC_{50} = 0.53 mg/ml	[139]
Lactobacillus acidophilus LA5	DPPH	2mg/ml	59.30%	[182]
	Hydroxyl radicals		59.94%	
	Reducing power		1.047%	
Bifidobacterium animalis subsp. lactis BB12	DPPH		56.76%	
	Hydroxyl radicals		46.40%	
	Reducing power		1.270%	
Leuconostoc mesenteroides SN-8	Hydroxyl radicals	1mg/ml	17.76%	[166]
	DPPH	5mg/ml	40%	
	ABTS	10mg/ml	65%	

Strain	Antioxidant assay	EPS concentration	Results	Ref.
Lactobacillus plantarum S123	DPPH	100μg/ml	More than 65%	[167]
Bacillus aerophilus rk1	DPPH	4mg/ml	56.60%	[104]
	Hydrogen peroxide		67.50%	
Leuconostoc mesenteroides	Hydroxyl radicals	6mg/ml	100% EC_{50} = 1.7mg/ml	[132]
Pediococcus pentosaceus M41	DPPH	10mg/ml	76.50%	[168]
	ABTS		48.90%	
Rhodotorula mucilaginosa sp. GUMS16	DPPH	7.5mg/ml	28.70%	[169]
	Hydroxyl radicals		48.20%	
Bacillus sp. NRC5	DPPH	500μg/ml	100% IC_{50} = 31μg/ml	[183]
	ABTS		IC_{50} = 431.88μg/ml	
	Superoxide anion radicals		IC_{50} = 315.51μg/ml	
	Hydroxyl radicals		IC_{50} = 35.35μg/ml	
	Nitric oxide radical	1000μg/ml	98.11% IC_{50} = 14.65μg/ml	
	Lipid peroxidation	50–1000μg/ml	45.45% to 85.63% IC_{50} = 71.10μg/ml	
Lactobacillus plantarum HY	DPPH	10mg/ml	92.27% IC_{50} = 1.41mg/ml	[170]
Bacillus megaterium PFY-147	DPPH	5mg/ml	94.78%	[110]
	Superoxide anion radical		87.12%	
	Hydroxyl radical		79.29%	
	ABTS		96.12%	

EPS extracted from *Bacillus subtilis*, isolated from marine, was reported to have 97.60% DPPH scavenging activity at 1.5 mg/ml concentration, with an IC_{50} value of 300µg/ml[156]. This value was found to be higher compared to the EPS secreted by *B. subtilis*, which reached 80% toward DPPH at the concentration of 10mg/ml[105]. However, EPS isolated from *Bacillus* sp. showed a higher scavenging capacity toward DPPH (100%) at 300 µg/ml with an IC_{50} value of 31µg/ml[183].

Ramamoorthy *et al*.[149] evaluated the antioxidant activity of EPS from *Bacillus thuringiensis* RSK CAS4. The results regarding DPPH radical scavenging of the EPS showed a maximum activity of 79% with the concentration of 1 mg/ml of EPS. This value was higher than that observed for the EPS extracted from *B. thuringiensis*, which exhibited 31.34% DPPH antioxidant ability at a concentration of 5mg/ml[124].

At a concentration of 5mg/ml, the EPS from *Bacillus megaterium* had higher antioxidant activity for the DPPH compared to the EPS isolated from *Bacillus enclensis* with 94.78% and 83.1%, respectively. Similarly, the EPS extracted from *B. megaterium* exhibited a higher ABTS scavenging activity of 96.12% compared to the exopolysaccharide derived from *B. enclensis*, which had a scavenging activity of 80.3% against ABTS radicals. However, the EPS from *B. enclensis* had higher activity against hydroxyl radicals, although the difference is relatively small. Meanwhile, the superoxide anion radical scavenging activities of both exopolysaccharides were similar[110,161].

Exopolysaccharides isolated from *Lactobacillus plantarum* have antioxidant potential, but their activity can vary depending on the strain. At the same concentration of 10 mg/ml, Liu *et al*.[170] showed that EPS from *Lactobacillus plantarum* HY exhibited very high activity against DPPH radicals (92.27%). Min *et al*.[163] reported that EPS isolated from *Lactobacillus plantarum* JLAU103 had moderate antioxidant activity, with DPPH and ABTS values of 60.50% and 65.50%, respectively, and a relatively high hydroxyl radical scavenging activity value of 80.40%. Ayyash *et al*.[165] demonstrated that the EPS extracted from *Lactobacillus plantarum* C70 had a relatively high activity against DPPH radicals (75.91%), but its activity against ABTS was lower (49.42%). Furthermore, an investigation attempted to evaluate the antioxidant activity of EPSs from three strains *Lactobacillus pentosus* 68FE, *Lactobacillus plantarum*, and *Lactobacillus pentosus* 14FE. The results showed that EPS produced by *L. plantarum* displayed the highest antioxidant activity against DPPH radicals with a value of 68.90% at 10mg/ml[121].

Khalil *et al*.[147] evaluated the antioxidant ability of exopolysaccharide-producing *Lactobacillus* strains from Tempoyak using DPPH free radical assay. The isolated *Lactobacillus* strains showed high antioxidant activity, ranging from 32.29% to 73.36%.

On the other hand, the antioxidant activity of EPS can also be evaluated using *in vivo* studies. Glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) are important antioxidant enzymes in the human body that could protect the organs from oxidative stress by converting ROS produced in cells into non-toxic substances[184]. SOD functions by converting superoxide radicals to hydrogen peroxide (H_2O_2), effectively regulating superoxide concentrations at low levels[185]. CAT and GSH-Px are able to decompose H_2O_2 to form water, thereby preventing the generation of hydroxyl radicals[185,186]. Malondialdehyde (MDA) is a cytotoxic compound that is often used as a biomarker of cellular lipid peroxidation[187]. An increase in MDA levels serves as an indicator of tissue damage induced by oxidative stress[157,188]. Lactate dehydrogenase (LDH) is another indicator of cell damage that plays a significant role in the production of H_2O_2 and subsequent oxidative stress[189,190].

Previous research has shown that an excessive accumulation of ROS leads to an increase in MDA concentration and a decrease in the activity of enzymes like GSH-Px, CAT, and SOD[191]. Zhang *et al*.[187] showed that EPS (named LPC-1) from *Lactobacillus plantarum* C88 demonstrated notable defensive effects against oxidative damage in cells by inhibiting the production of MDA and enhancing the activities of SOD and total antioxidant capacities (T-AOC). These findings suggest that the EPS obtained from *L. plantarum* C88 could enhance both enzymatic and non-enzymatic antioxidant activities as well as mitigate lipid peroxidation. It was reported that acidic exopolysaccharide (EPS-LP2) isolated from *Lactiplantibacillus plantarum* DMDL 9010 improved the enzymatic antioxidant activity, alleviated the reduction of antioxidant substances, and enhanced the non-enzymatic antioxidant system, leading to a reduction of the cell damage[150]. In the presence of EPS-LP2, ROS, MDA, and LDH contents were reduced in a dose-dependent manner. EPS-LP2 could also protect cells against H_2O_2-induced oxidative stress by improving SOD, CAT, and GSH-Px activities[150].

Li *et al*.[192] showed that pre-treating PC12 cells with EPSs from *Lactobacillus plantarum* were able to protect against H_2O_2-induced oxidative damage by enhancing the activity of CAT, T-AOC, and SOD. In addition, the pre6incubating PC12 cells with EPS-3 resulted in an increase of each activity by 59.05%, 65.81%, and 41.34%, respectively, in comparison with H_2O_2 group[192]. In other studies, exopolysaccharides from *Lactococcus*

lactis and *Lactobacillus helveticus* were proven to protect hepatopancreas tissue and liver, respectively, against oxidative stress by increased SOD and GSH-Px activities and decreased MDA concentration[193,194]. Additionally, EPS from *Lacticaseibacillus rhamnosus* could reduce brain damage via increasing antioxidant capacity in a dose-dependent manner [24]. Recently, Yang *et al*.[195] reported that exopolysaccharide (LRSE1) produced by *Lacticaseibacillus rhamnosus* was able to mitigate the oxidative stress of the gastric mucosa of mice by reducing MDA levels. Furthermore, ROS content and the activities of GSH-Px and SOD in the gastric mucosal tissues significantly raised in the presence of LRSE1. Similarly, Chouchane *et al*.[196] also reported a considerable increase in GSH-Px, SOD, and CAT levels and a significant decrease in the content of MDA were observed after mice insoluble EPSs (iEPSs) administrations. Indeed, the SOD activities in the treatment group using EPS–1.5kGy (194UmL^{-1}) exceeded that of the acid ascorbic (positive control) group (192UmL^{-1})[196].

It should be noted that the structural properties of EPS, including its chemical composition, molecular weight, conformation, and types of glycosidic bonds, can affect its bioactivity. In addition, variations in source materials and extraction methods may also alter the physicochemical properties of EPS, leading to different levels of antioxidant activity[197].

4.2. Metal Chelating

The metals-binding capacities of EPS are thoroughly investigated and widely adopted in the bioremediation of heavy metals, providing an effective alternative to costly, inefficient and non-ecofriendly traditional techniques, such as solvent extraction, chemical precipitation, membrane separations, and activated carbon adsorption[198,199]. The chelating activity of exopolysaccharides (EPS) has gained increasing attention in recent years due to its potential applications in various fields, such as environmental remediation and wastewater treatment[200]. EPSs have been shown to exhibit chelating activity towards metal ions due to their carboxyl, hydroxyl, and amino functional groups, which can coordinate with metal ions and form stable complexes (Table 3)[201,202]. The chelating activity of EPS has been attributed to its net anionic composition, which allows it to bind with a wide range of positively charged metal ions[200]. Moreover, the chelating ability of EPS has been found to be influenced by factors such as pH, temperature, and the presence of other competing ligands[200]. Several studies have demonstrated the chelating activity of EPS. For example, Pie *et al*.[110] investigated the chelating activity of levan produced by *Bacillus megaterium*. The study found that levan exhibited strong chelating activity towards Cu^{2+} and Fe^{2+}, with metal adsorption capacities values of 99.20% and 97.12%, respectively, and had a higher binding

affinity compared to other tested metal ions (Pb^{2+} and Zn^{2+}). Similarly, dextran produced by *Weissella confusa* exhibited a strong binding affinity toward Cu^{2+} and Fe^{2+}, while its capacity to adsorb Pb^{2+} and Cd^{2+} was comparatively lower[203]. In another study, Huang-Lin *et al*.[129] revealed that EPS isolated from *Bacillus xiamenensis* exhibited a remarkable iron chelation capacity (100%) of over 5mg/mL. These results are higher than those obtained with the EPS extracted from *Lactobacillus plantarum*, which showed a ferrous ion chelating ability of 69.7% at 10mg/mL[163]. The crude EPS produced by *Pseudomonas stutzeri* AS22 showed an interesting metal-binding capacity with a maximum Pb uptake of 460mg/g[204]. EPS isolated from *Arthrobacter* ps-5 showed a high biosorption capacity, up to 216.09mg/g of Pb^{2+}, 169.15mg/g of Cu^{2+}, and 84.47mg/g of Cr^{6+}[205]. In addition, the authors evaluated the biosorption properties of EPS on the basis of various factors, including solution pH, ionic strength, and EPS concentration. Indeed, the maximum biosorption was achieved at pH 5.5 for Pb^{2+}, 5.0 for Cu^{2+}, and 6.0 for Cr^{6+}, respectively. Meanwhile, the biosorption becomes weak at low or high pH[205]. This can be explained by the competitive biosorption (high H_3O^+ content) in the first case[206] and metal hydroxide formation leading to metal precipitation in the second[207]. Furthermore, the metal biosorption capacity of EPS decreased with increasing ionic strength, which may be explained by competition between salt ions in solution and metal ions (Pb^{2+}, Cu^{2+}, and Cr^{6+})[205]. The functional group of O-H, C-O-C, C=O, and C=O-C have been implicated in metal biosorption, which contributed significantly to the binding of the metal ions by EPS[205,208]. These findings indicated that EPSs possess significant chelating activity towards various metal ions, making them potential candidates for the development of novel chelating agents. However, further research is needed to understand the relationship between the physicochemical properties of EPSs and their chelating activity, as well as their potential applications in various fields.

Table 3. Exopolysaccharides chelating activity.

Strain	EPS concentration	Chelating activity assay	Chelating activity	Ref.
Weissella confuse	10mg/ml	Heavy metal chelating activity	85.92% (Cu^{2+}) 94.71% (Fe^{2+}) 93.47% (Zn^{2+}) 71.84% (Pb^{2+}) 75.30% (Cd^{2+})	[203]
Pleurotus ostreatus	ND	Iron-chelating activity	IC_{50} = 5.23mg/ml	[209]

Strain	EPS concentration	Chelating activity assay	Chelating activity	Ref.
Lactobacillus plantarum YW11	3mg/ml	Ferrous ion chelating ability	41.09%	[210]
Lactic acid bacteria	10mg/ml		27%	[211]
Lactobacillus plantarum JLAU103			69.7%	[163]
Bacillus amyloliquefaciens GSBa-1	2mg/ml		30.5%	[44]
Lactobacillus acidophilus La	10mg/ml		75.80%	[212]
Bifidobacterium adolescentis Ba			80%	
Rhodobacter sp. BT18	100mg/l	Metal chelating activity	64% (As) 51% (Pb)	[213]
Lactobacillus bulgaricus	10mg/ml	Ferrous ion chelating ability	74.13%	[214]
Lactobacillus sp.Ca_6	7.5mg/ml		78.66%	[215]
Lactiplantibacillus plantarum RO30	5mg/ml		72.49%	[155]
	10mg/ml		89.78%	
Lactobacillus rhamnosus	4mg/ml		54–73%	[216]
Halomonas smyrnensis K2	10g/l		31.1%	[217]
Halolactibacillus miurensis	10mg/ml		49%	[218]
Streptomyces hirsutus NRC2018	400µg/ml		94.9%	[146]
	1000µg/ml		98.5%	
Lactobacillus helveticus MB2-1	4mg/ml		31.25–40.63%	[219]
Pleurotus fabellatus	5mg/ml		37.98% EC_{50} = 6.64mg/ml	[220]

Strain	**EPS concentration**	**Chelating activity assay**	**Chelating activity**	**Ref.**
		CUPRAC	EC_{50} = 3.48mg TE/g	
Bacillus xiamenensis RT6	7.5mg/ml	Iron chelating activity	100%	[129]
Lactobacillus helveticus MB2-1	4mg/ml	Ferrous chelating activities	63.33–73.33%	[221]
Streptomyces globisporus BU2018	400µg/ml	Ferrous ion chelating ability	85.2%	[197]
	1000µg/ml		90.2%	
Bacillus cereus KMS3-1	-	Maximum adsorption capacity	54.05mg/g for Cd(II) 71.42mg/g for Cu(II) 78.74mg/g for Pb(II)	[222]
Leuconostoc lactis KC117496	100–500µg/ml	Ferrous chelating activities	5.8–72.5%	[223]
Bacillus megaterium PFY-147	10mg/l	Metal adsorption capacity	99.20% for Cu^{2+} 97.12% for Fe^{2+} 92.93% for Zn^{2+} 91.40% for Pb^{2+}	[110]
Pseudomonas stutzeri AS22	0.75mg/ml	Ferrous ion chelating ability	88.5%	[224]
Streptomyces griseorubens GD5	1mg/ml		85.8%	[225]
Lacticaseibacillus plantarum 70810	4mg/ml		62.8%	[226]
Lactobacillus delbrueckii ssp. *Bulgaricus* SRFM-1	4mg/ml		62.33% EC_{50} = 1.25mg/ml	[227]
Weissella confusa AJ53	1mg/ml		90%	[228]
Leuconostoc citreum 1.2461	12mg/ml		42.4%	[229]

CUPRAC: Cupric ion Reducing Antioxidant Capacity.

4.3. Anticancer Activity

Cancer is a group of diseases characterized by the uncontrolled growth of abnormal cells. It is estimated that there are around 18.1 million new cancer cases and 9.6 million cancer deaths each year worldwide. Lung, colorectal, stomach, liver, and breast cancer are the most common types of cancer-related deaths worldwide[230]. Under the need for novel anticancer agents and because of their wide range of therapeutic properties, bacterial exopolysaccharides (EPS) are suggested as anticancer agents. Several studies have shown that EPS from different bacterial species can inhibit the growth and proliferation of different types of cancer cells *in vitro* (Table 4).

Table 4. *In vitro* studies on the anticancer properties of bacterial exopolysaccharides.

Bacteria	The test used	Results	Ref
Bacillus circulans	Effect of EPS on Hep 2 cell line	IC_{50} = 45µg/ml	[231]
	Effect of EPS on Hep G2 cell line	IC_{50} = 30µg/ml	
Lactobacillus kefiri MSR101	Effect of EPS on HT-29 cells	↘↘ HT-29 survival rate to 44.1% with 400µg/ml of EPS	[232]
		↗↗ Cyto-c gene expression	
		↗↗ BAX gene expression	
		↗↗ BAD gene expression	
		↗↗ caspase3 gene expression	
		↗↗ caspase8 gene expression	
		↗↗ caspase9 gene expression	
		↘↘ BCL-2 gene expression	
P. aeruginosa A	Effect of EPS on HT-29 cells	IC_{50} = 44.8µg/ml	[233]
P. aeruginosa B		IC_{50} = 12.7µg/ml	
Streptomyces sp. A5	Effect on 4T1 cells	↗↗ cytotoxicity to 96.2 ± 13.5%	[234]
	Effect on Caco-2 cells	↗↗ cytotoxicity to 73.9 ± 6.4%	

Bacteria	The test used	Results	Ref
	Effect on IEC-18 cells	↗↗ cytotoxicity to 29.9 ± 9.1%	
Streptomyces sp. M7	Effect on 4T1 cells	↗↗ cytotoxicity to 59.8 ± 1.5%	
	Effect on Caco-2 cells	↗↗ cytotoxicity to 73.3 ± 3.2%	
	Effect on IEC-18 cells	↗↗ cytotoxicity to 98.7 ± 5.7%	
Streptomyces sp. A14	Effect on 4T1 cells	↗↗ cytotoxicity to 84.5 ± 3.2%	
	Effect on Caco-2 cells	↗↗ cytotoxicity to 60.4 ± 1.9%	
	Effect on IEC-18 cells	↗↗ cytotoxicity to 84.5 ± 5.9%	
Streptomyces sp. MC1	Effect on 4T1 cells	↗↗ cytotoxicity to 76.1 ± 3.8%	
	Effect on Caco-2 cells	↗↗ cytotoxicity to 93.9 ± 1.9%	
	Effect on IEC-18 cells	↗↗ cytotoxicity to 96.9 ± 3.1%	
Streptomyces hirsutus NRC2018	Effect on Caco-2 cells	IC_{50} = 295.1µg/ml	[235]
Lactobacillus acidophilus (DSM20079)	Effect on Hep GII cells line	↗↗ apoptotic cells to 53.4%	[236]
	Effect on MCF-7 cells line	↗↗ apoptotic cells to 62.0%	
	Effect on Caco-2 cells line	↗↗ apoptotic cells to 54.3%	
Lactobacillus fermentum YL-11	Effect on HT-29 cells	↗↗ apoptotic cells to 34% with 600µg/mL of EPS	[237]
Rhodococcus erythropolis HX-2	Effect on A549 cell line	↘↘ A549 cell Viability to 21.86% at 800µg/ml	[238]
	Effect on SMMC-7721 cell line	↘↘ SMMC-7721 cell Viability to 31.24% at 800µg/ml	
	Effect on Hela cell line	↘↘ Hela cell Viability 37.65% at 800µg/ml	
Bacillus thuringiensis RSK CAS4	Effect on Hep-2 cell line	IC_{50} = 320µg/ml	[239]
	Effect on A549 cell line	IC_{50} = 115µg/ml	
Bacillus subtilis AG4	Effect on T-24 cell line	IC_{50} = 244µg/ml	[240]
	Effect on A-549 cell line	IC_{50} = 148µg/ml	

Bacteria	The test used	Results	Ref
	Effect on HepG-2 cell line	IC_{50} = 123µg/ml	
Lactococcus garvieae C47	Effect on Caco-2 cell line	Cytotoxicity of 59.35% at 10mg/ml of EPS	[241]
	Effect on MCF-7 cell line	Cytotoxicity of 42.82% at 10mg/ml of EPS	
Pediococcus pentosaceus M41	Effect on Caco-2 cell line	Cytotoxicity of 77.89 at 5mg/ml of EPS	[242]
	Effect on MCF-7 cell line	Cytotoxicity of 46.43 at 5mg/ml of EPS	
Bacillus velezensis SN-1	Effect on HepG-2 cell line	Cell inhibition ratio of 81.33% with 2mg/l of EPS	[243]
Bacillus cereus AG 3	Effect on T-24 cell line	IC_{50} = 121 ± 4.1µg/ml	[244]
	Effect on MCF-7 cell line	IC_{50} = 55.7 ± 2.3µg/ml	
	Effect on PC-3 cell line	IC_{50} = 61.4 ± 2.6µg/ml	
Lactobacillus plantarum S123	HT-29 cancer cells	the growth inhibition rates (52.4–12.1%) with (0–600µg/ ml) of EPS	[245]
Bacillus albus DM-15	lung cancer cell line (A549	IC50 value of 20 ± 0.97 −1µg mL	[246]

Exopolysaccharides isolated from four *Streptomyces* sp. had excellent anti-cancer activity against Caco-2 human colon cancer cells, IEC-18 intestinal cancer cells, and 4T1 breast cancer cells. EPS from *Streptomyces* sp. MC1 strain showed the highest cytotoxicity against Caco-2 cells (93.9 ± 1.9%) while EPS from *Streptomyces* sp. M7 strain displayed the highest cytotoxicity against ISC-18 cells (98.7 ± 5.7%) and the EPS from *Streptomyces* sp. A5 strain has the highest cytotoxicity against 4T1 cells (96.2 ± 13.5 %)[234]. Awady *et al*.[235] investigated the anti-cancer effect of EPS isolated from another species of *Streptomyces* genre, *Streptomyces hirsutus* NRC2018. The tested EPS also showed high cytotoxicity against Caco-2 cells with an IC_{50} of 295.1 µg/ml. EPS from *Lactobacillus kefiri* MSR101 exhibited high anti-cancer activity against HT-29 colon cancer cells by reducing cells' survival rate to 44.1% with a concentration of 400µg/ml of EPS. This effect is due to the modulation of apoptosis pathways by increasing the expression levels of the pro-apoptotic genes *Bax* and *Bad*, promoting Cyt-c gene expression which have a crucial role in the activation of intrinsic apoptotic pathways, notably Caspase-3 and Caspase-9. This isolated EPS is also involved in the activation of the extrinsic pathways by the up-regulation of caspase 8 gene expression, in addition to the down-regulation of BCL-2 gene expression,

which is an anti-apoptotic protein that prevents programmed cell death[232]. The EPS isolated from *P. aeruginosa* A and B strains significantly inhibited HT-29 colon cancer cells with IC_{50} of 44.8 and 12.7μg/ml, respectively.

The authors suggested that the anti-cancer activity of the isolated EPS is due to the presence of carbohydrates[233]. EPS from YL-11 can significantly suppress HT-29 colon cancer growth with an apoptotic cell rate of 34% with 600μg/ml of EPS[237]. The potential effect of EPS isolated from *Rhodococcus erythropolis* HX-2 on A549 lung cancer cells, SMMC-7721 liver cancer cells, and Hela col uterus cancer cells was also reported in the literature. For instance, the EPS produced by *Rhodococcus erythropolis* HX-2 significantly decreased the cancer cells' viability to 21.86%, 31.24%, and 37.65% with a concentration of 800μg/ml, respectively[238]. The EPS from *Bacillus thuringiensis* RSK CAS4 can significantly inhibit the proliferation of Hep-2 liver cancer cells and A549 lung cancer cells with IC_{50} of 320 and 115μg/ml, respectively[239]. In Abdel-Wahab *et al.*[240] study, they tested the anti-cancer effect of EPS isolated from *Bacillus subtilis* AG4 on three cancer cell lines, T-24 bladder carcinoma, A-549 lung cancer, and HepG-2 hepatocellular carcinoma. The EPS showed a high proliferation inhibitory activity with IC_{50} of 244, 148, and 123 μg/ml, respectively which is higher than the results showed by *Bacillus velezensis* SN-1 EPS against HepG-2 cell line (Cell inhibition ratio of 81.33% with 2 mg/l of EPS)[243]. The EPS isolated from *Bacillus cereus* showed high cytotoxicity against Caco-2 colon cancer cells, MCF-7 breast cancer cells, and PC-3 prostate cancer cells with IC_{50} of 121 ± 4.1, 55.7 ± 2.3, and 61.4 ± 2.6μg/ml, respectively[244]. In the study of [241], *Lactococcus garvieae* C47 EPS showed low antitumor activity against colon and breast (MCF-7) carcinoma cell lines compared with the cytotoxicity shown by the EPS isolated from *Pediococcus pentosaceus* M41, with cytotoxicity percentages of 59.35 and 42.82% with 10mg/ml of EPS *vs* 77.89 and 46.43% only with 5 mg/ml of EPS, respectively[241].

Emam *et al.*[236] investigated the potential effect of EPS isolated from *Lactobacillus acidophilus* (DSM20079) against the MCF-7 breast cell line, CaCo-2 colon cancer cell line, and HepG II liver cancer cell line. The tested EPS showed a dose-dependent effect with a high apoptotic rate of 53.4, 62.0, and 54.3%. This effect is related to the G1/S phase transition blockage. The *Lactobacillus acidophilus* EPS can moderate cancer proliferation underlying mechanisms by the up-regulation of the apoptotic agent's expression notably protein 53 (P53), the apoptosis-induced factor M1 (AIFM1), the retinoblastoma susceptibility gene (Rb1), Caspase-9, and Bax expressions. In addition, the isolated EPS can down-regulate the expression of anti-apoptotic genes like *Bcl2* expression and autophagic genes like the mammalian target of rapamycin (*mTOR*) and Microtubule-associated protein (LC3A).

Lactobacillus acidophilus EPS also acted by decreasing the proliferative gene expression notably the nuclear protein *KI-67* gene, proto-oncogenes *c-myc*, and *KRas*, and by the decrease of β-catenin expression in addition to the suppression of *miR-155* expression.

4.4. Anti-inflammatory Activity

Several studies have demonstrated the anti-inflammatory potential of exopolysaccharides. Table 5 summarized some studies underlying the anti-inflammatory potential of EPS. Gao *et al*.[247] tested the anti-inflammatory effect of EPS extracted from *B. thuringiensis* on Macrophages stimulated with LPS. As known, LPS stimulates the inflammatory response by inducing the activation of toll-like receptor 4 (TLR4), causing downstream signaling pathways activation and leading to the activation of several transcription factors, including nuclear factor-kappa. The activation of NF-kB induces the transcription of pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF-α)[248]. The treatment with EPS significantly inhibits the NO production and decreases the pro-inflammatory cytokine levels notably IL-1β, IL-6, and TNF-α. EPS isolated from *Lactobacillus reuteri* also showed a significant suppression of TNF-α overexpression induced by LPS in macrophages[249]. Following the same approach, Giri *et al*.[250] investigated the anti-inflammatory potential of *Bacillus subtilis* F9 EPS. The isolated EPS demonstrated high activity by decreasing the pro-inflammatory cytokines TNF-α and IL-1β concentrations and increasing the anti-inflammatory agents IL-10 and TGF-β.

Table 5. *In vitro* and *in vivo* studies on the anti-inflammatory properties of bacterial exopolysaccharides.

Bacteria	The test used	Key results	Ref.
B. thuringiensis	Effect on LPS Stimulated macrophages	↘↘ NO levels to 3.91 ± 1.03µmol/l vs 24.19 ± 1.65µmol/l	[247]
		↘↘ IL-1β levels to 273.80 ± 12.67pg/ml vs 341.92 ± 7.50pg/ml	
		↘↘ IL-6 levels to 57.21 ± 0.72pg/ml vs 87.38 ± 0.45pg/ml	
		↘↘ TNF-α levels to 159.58 ± 5.00pg/ml vs 232.08 ± 2.50pg/ml	
Lactobacillus reuteri	Effect on LPS Stimulated macrophages	↘↘ TNF-α levels to 209.20 ± 84.34pg/µg DNA with 1 ppm of EPS	[249]

Bacillus subtilis F9	Effect on LPS Stimulated macrophages	Suppression of the overexpression of TNF-α and IL-1β in a concentration-dependent manner	[247]
		↗↗ the expression level of IL-10 and TGF-β	
Bacillus cereus	EPS incubation with Lipoxygenase (LOX)	IC_{50} = 12.9 ± 1.3µg/ml	[244]
	EPS incubation with Cyclooxygenase (COX-2)	IC_{50} = 29.6 ± 0.89µg/ml	
	Membrane Stabilization inhibition	IC_{50} = 35.4 ± 0.67µg/ml	
Bacillus circulans	Effect on albumin denaturation	93% protection against albumin denaturation at 1000µg/ml.	[231]
	Effect on proteinase	71% inhibition of proteinase activity at 1000µg/ml	
	Effect on Red Blood Cell lysis	84% inhibition of heat-induced lysis of RBC at 1000µg/ml	
Lactococcus lactis subsp. cremoris	Effect on dermatitis mouse model induced by repeated exposure to TNCB	Suppression of skin thickening and mast cell infiltration in skin lesions.	[251]
		Suppression of the overexpression of IL-4, IFN-c, IL-6, and TNF-α levels by 0.5 mg/kg of EPS	
Lactobacillus sp. Ca6	Excision wound model in rats	Wound healing activity acceleration after treatment with EPS ointment at 15mg/ml	[252]

In addition to LPS, multiple *in vitro* assays are used, mainly the inhibition of cyclooxygenase and lipoxygenase enzymes, inhibition of hemolysis, inhibition of albumin denaturation, and proteinase inhibition. EPS from *Bacillus cereus* can significantly inhibit the lipoxygenase and cyclooxygenase enzymes with IC_{50} of 12.9 ± 1.3 and 29.6 ± 0.89µg /ml, respectively, in addition to the inhibition of hemolysis with an IC_{50} of 35.4 ± 0.67µg/ml[244]. EPS from *Bacillus circulans* demonstrated a high inhibition of albumin denaturation, proteinase, and hemolysis with 1000µg/ml[231].

To investigate the anti-inflammatory effect of EPS extracted from *Lactococcus lactis subsp. cremoris*, dermatitis was induced in BALB/c mice by repeated exposure to 2,4,6-trinitro-1-chlorobenzene (TNCB). The oral administration of EPS with a concentration of 0.05mg/kg or 0.5mg/kg decreased dermatitis severity in mice. The treatment showed a high suppression of skin thickening and mast cell infiltration in skin lesions. At the molecular axis, the EPS can significantly suppress the overexpression of IL-4, IFN-c, IL-6, and TNF-α levels [251]. Wound healing is the final phase of the inflammatory response characterized by damaged tissue reparation. These processes can be affected by various factors inducing multiple abnormalities. Trabelsi *et al.*[252] tested the therapeutic effect of *Lactobacillus sp.* Ca6 EPS on excision wound model in rats. The treatment with ointment formulated by EPS at a concentration of 15mg/ml optimized wound-healing process.

4.5. Immuno-modulatory Activity

In order to determine the possible future uses of non-dietary polysaccharides, their activities toward the immune system must be investigated in detail, as they could be potentially toxic, cause inflammation or magnify an ongoing inflammatory response. The expression of some biomarkers such as pro-inflammatory cytokines (IL-6, IL-1, and TNF-α) must be investigated after an EPS stimulation. Their expressions are major signs of proinflammatory proprieties of the EPS (Table 6). Other cytokines such as IL-4, IL-10 and IL-12 are in fact a sign of possible immunomodulatory proprieties of an EPS if expressed after stimulation by the latter. For example, Il-4 is required to drive the polarization of naïve T cells to type 2 phenotype that secretes IL-5, IL-10 and IL-13, each of them being of extreme importance to the maintaining of the immune homeostasis. IL-4 also promotes the regulation of the inflammatory response by decreasing the expression of IL-6 and TNF-α[253].

Table 6. *In vitro* and *in vivo* studies on the immuno-modulatory effects of bacterial exopolysaccharides.

Bacteria	The test used	Key results	Ref.
Leuconostoc mesenteroides S81	EPS incubation with HT-29 cell line	↗↗ IL-4 production levels	[254]
Lactobacillus confusus	Effect on LPS Stimulated macrophage- RAW264.7	↗↗ mRNA expression of NO, TNF-α, IL-1β, IL-6, and IL-10	[255]
		Facilitation of the degradation of Ik-B and the phosphorylation of c-Jun NH2-terminal kinase (JNK)	

Bacteria	The test used	Key results	Ref.
Leuconostoc citreum L3C1E7	Effect on LPS-stimulated HT-29 cells	↘↘ IL-8 secretion by 79% as compared to LPS stimulation	[256]
	Subcutaneous immunization of model animals with CFA	Normalization of Allergen-specific IgG1 plasma titer	
		↘↘ IgG2a plasma levels	
	Effect on OVA-induced asthma model of rats	↘↘ IgE plasma levels	
		↗↗ IgA plasma levels	
		↘↘ IgG1 plasma levels	
		↘↘ IgG2a plasma levels	
		↘↘ IgE plasma levels	
Lactobacillus plantarum MTCC 9510	Stimulation of human lymphocytes EPS	↗↗ lymphocyte proliferation by 20% with 0.1 mg/mL of EPS	[144]
Leuconostoc mesenteroides Strain NTM048	Stimulation of mouse Peyer′s patch cells with EPS	↗↗ IgA production with 1.5-fold and 1.7-fold, respectively with 25 and 250 μg/mL of EPS	[255]
	Stimulation of mouse Splenocytes cells with EPS	↗↗ IFN-γ gene expression with 20 μg/mL of EPS (14.5% vs 22.4%)	
		↘↘ IL-4 gene expression with 20 μg/mL of EPS (6.1% vs 2.7%)	
		↗↗ BCL6 gene expression	
		↗↗ RALDH1 gene expression	
		↗↗ TGF-β1 gene expression	
		↗↗ TGF-βR2 gene expression	
	BALB/cA Mice immune System stimulation with EPS	↗↗ CD4+T/ CD8+T cells ratio	
Enterobacter cloacae Z0206	Vaccination of Broilers with NDV “La Sota” vaccine	↗↗ antibody titers against NDV After 21 (7.75 ± 0.25 vs 6.67 ± 0.33)	[257]

Bacteria	The test used	Key results	Ref.
		↗↗ antibody titers against NDV After 35 (6.17 ± 0.31 vs 5.17 ± 0.40)	
		↗↗ antibody titers against NDV After 42 (4.80 ± 0.37 vs 3.80 ± 0.37)	
Lactiplantibacillus plantarum MM89	Incubation with RAW264.7 cells	↗↗ Phagocytosis index with 100 µg/ml of EPS	[258]
		↗↗ acid phosphatase activity with 50 µg/ml of EPS	
		↗↗ cytokine production in a dose-dependent manner	
	cyclophosphamide-induced immunosuppressed mouse model	↗↗ Spleen indice	
		↗↗ IgA levels	
		↗↗ splenic lymphocyte proliferation	
		↗↗ IL-2 and TNF-α levels with 100 mg/kg b.w	
Lactiplantibacillus plantarum DMDL 9010	LPS-stimulated RAW264.7 cells	Suppression of NO and IL-6 expression	[259]
		Inhibition of MAPK and NF-κB pathways activation	
Lactococcus lactis Z-2	Disease resistance against Aeromonas hydrophila in Cyprinus carpio L.	↗↗ TNF-α, IL-10, TGF-β, IL-1β and IL-6 expression levels with dose-dependent manner	[260]
Enterobacter cloacae	CP-induced immunosuppression in mice model	↗↗ splenic lymphocyte proliferation	[261]
		↗↗ TNF-α levels	

In the study of Taylan *et al*.[254], EPS from *leuconostoc mesenteroides* S81 showed a significant increase in IL-4 expression in HT-29 cell lines, without inducing the expression of TNF-α or IL-10, IL-12. Unlikely, in the study of Surayot *et al*.[255], the cleaved EPS from *lactobacillus confusus* showed important immunostimulatory activities. It proceeded to increase the expression of iNOS in RAW246.7 macrophages, resulting in an increase of NO

by the latter, moreover by activating degradation of Ik-B and increased the phosphorylation of JNK without affecting the phosphorylation of MAPK elements such as ERK and p38 but interestingly only hydrolyzed EPS showed these activities. Those EPS were either hydrolyzed by a dilute acidic solution in boiled water or by microwave oven with different boiling/microwaving times. The NO production for example, increased with the increase in the EPS dose, and the increase in the hydrolysis time. Those results are probably due to lower molecular weight saccharides having stronger conformational affinities with receptors expressed by the macrophages.

Mucosa is the entry of choice of pathogens. Indeed, those tissues are exposed to the environment on a daily basis. The intestinal mucosa, for instance, is in constant exposure to bacteria, viruses, parasites, and toxins. Immune activity is deeply important for limiting this exposure. Immunoglobulin A (IgA) is considered by many as the first line of defence against those infections, IgA directly binds to pathogenic antigens, stopping them from passing the intestinal barrier. They also directly block receptors, and inhibit bacterial virulence by inhibiting their secretory systems[262].

In Matsuzaki *et al*.[263] study, they extracted EPS from *leuconostoc mesenteroides* and studied their IgA stimulation proprieties in Peyer′s patch (PP) cells and splenocytes cells, their results showed an increase in baseline IgA production as well as an increase in specific IgA production when they treated the same cells with H1N1 antigen. After treatment with the EPS, the CD^{3+}, as well as the ratio of CD^{4+} to CD^{8+} significantly increased, showing distinct immunoregulatory propriety to the EPS. Their immunoregulatory proprieties also induce a rise in the CD^{4+} T cells releasing IFN while reducing IL-4-producing CD^{4++} T cells. *In vivo*, the oral gavage of the isolated EPS was found to increase the expression of TGF-A, RALDH, and BCL-6, probably due to a modulation of the T cells dependent pathway of IgA synthesis. Lu *et al*.[257] incubated *Enterobacter cloacae* with a medium rich in selenium, which resulted in a coupling of the EPS with selenium.

When fed to an avian broiler, this Se-EPS significantly decreased the levels of Malondialdehyde in the serum, while increased Superoxide dismutase, catalase, and glutathione in the serum. Moreover, after the treatment of the chicken with the NDV virus, the antibody production significantly increased in the broiler fed with Se-EPS.

4.6. Antiviral Activity

There is a growing number of studies investigating the potential of exopolysaccharides as antiviral agents[264–266]. Table 7 presents a comprehensive summary of these studies, including information about the origin of bacterial EPS and the viral strains

tested, in addition to the antiviral methods employed. The findings of these studies indicate that exopolysaccharides possess significant antiviral properties. Further research is needed to fully understand the potential of exopolysaccharides as antiviral agents, but these initial findings are promising.

The antiviral activity of *L. plantarum* LRCC5310 EPS was investigated *in vitro* using MA104 culture cells infected with Human rotavirus (HRV), and *in vivo* on infected BALB/c Neonatal Mice. The isolated EPS showed a high inhibition of virus replication *in vitro* by decreasing the viral RNA copy numbers to 7.46 log with a concentration of 1.95mg/ml. In mice, the treatment with 1 mg of EPS during 7 successive days, significantly decreased the number of newborns developing diarrhea and dehydration and enhanced the small Intestine state[144]. The potential anti-RVS activity of *Laminaria japonica* EPS was demonstrated *in vitro* using RVS-infected HEK293 cells. The treatment with EPS can up-regulate IRF3 signaling-mediated IFN-a production and induce viral replication inhibition[267].

The antiviral activity of EPS isolated from *Bacillus licheniformis* T14 was studied using three different virus Herpes Simplex virus type 1 (HSV-1), Hepatitis A virus (HAV), and Coxsackie B4 virus. The treatment with 125µg/ ml of EPS can significantly inhibit the enveloped virus HSV-1 with a percentage of 84.9%, and moderately HAV and Coxsackie B4 with 20.3% and 45.4%, respectively[235]. *Weissella paramesenteroides* MN2C2 EPS showed a complete reduction of Coxsackie B3 titer at a concentration of 3.0mg/ml[268]. EPS isolated from *Arthrospira platensis* showed significant suppression of the viral replication of koi herpesvirus with a concentration of less than 18µg/ml[269]. Biliavska *et al*.[270] investigated the antiviral potential of EPSs isolated from three different bacteria, *Lactobacillus* sp., *Leuconostoc* sp., and *Pediococcus* sp. against human adenovirus type 5 (HAdV-5). The EPS isolated from *Lactobacillus* sp. showed the highest viral inhibition compared to the other isolated EPS, 100% with a concentration of 20µg/ml, followed by the EPS extracted from *Leuconostoc* sp., 80% with a concentration of 20 µg/ml, and *Pediococcus* sp. EPS represented the lowest inhibition at the same concentration (42%). The EPS extracted from *Lactobacillus plantarum* exerted a high antiviral activity against Transmissible Gastroenteritis Virus (TGEV) by the inhibition of the virus proliferation and replication[269]. Overall, bacterial EPS can exert its antiviral activity through several mechanisms. EPS can inhibit viral replication and prevent the production of new virus particles, in addition to the stimulation of the antiviral pathways of infected cells notably IRF3 signaling-mediated IFN-alpha production.

4.7. Antibacterial Activity

Numerous studies have focused on the antibacterial properties of bacterial exopolysaccharides. Tables 7 and 8 provide a comprehensive summary of studies investigating these effects, including information about the bacterial exopolysaccharides' origin, and antibacterial tested, the results are expressed as inhibition zone diameter (Ø), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC).

The results of these investigations have demonstrated that exopolysaccharides possess significant antibacterial properties. The most sensitive bacteria to *Bacillus subtilis* F9 EPS were *Escherichia coli* MTCC 443 followed by *Staphylococcus aureus* MTCC 737, *Bacillus cereus* MTCC 6629, *Salmonella typhimurium* ATCC14028, and *Listeria monocytogenes* 1143 by IC_{50} of 3, 4.5, 6, 7, and 9.5 mg/ml, respectively[250]. The EPS extracted from *Lactobacillus sp.* showed significant inhibition of *Enterococcus faecium* DSMZ 2146, *Staphylococcus aureus* ATCC 6583, *Staphylococcus aureus* DSA_226, and *Listeria monocytogenes Scott* A with a concentration of 5mg/ml while *Salmonella enterica spp. arizonae* DSMZ 9386 and *Escherichia coli* DSA 8048 are less sensitive[271]. Khalil *et al*.[272] also investigated the antibacterial effect of *Lactobacillus* sp. EPS. The extracted EPS displayed a strong inhibitory action against *Salmonella Typhimurium*, *Escherichia coli*, *Pseudomons aeruginosa*, and *Listeria monocytogenes* with an inhibition zone diameter of 8–12mm, and against *Staphylococcus aureus* with an inhibition zone less than 12mm. *Lactobacillus curvatus* SJTUF 62116 EPS can completely inhibit the growth of *Salmonella enteritidis ATCC 1307, Escherichia coli ATCC 25922, and Staphylococcus aureus ATCC 13565 with a concentration of* 20mg/ml[273].

Table 7. *In vitro* and *in vivo* studies on the antiviral effects of bacterial exopolysaccharides.

Bacteria	The test used	Key results	Ref.
Lactobacillus plantarum LRCC5310	Human rotavirus (HRV)-infected MA104 cells	↘↘ The viral RNA copy numbers to 7.46 log (vs 8.13 log) at 1.95mg/ml	[274]
	Effect on Rotavirus EDIM (RV-EDIM)- infected BALB/c Neonatal Mice	↘↘ Number of newborn mice developing RV-EDIM-induced diarrhea	
		↘↘ Acute diarrhea and severe dehydration	
Laminaria japonica	Effect against RVS-infected HEK293 cells	Up-regulation of IRF3 signaling-mediated IFN-a production	[267]
Bacillus licheniformis T14	Effect against Herpes Simplex virus type 1 (HSV-1)	↗↗ Inhibition by 84.9% at 125µg/ml.	[235]

Bacteria	The test used	Key results	Ref.
	Effect against Hepatitis A virus (HAV)	↗↗ Inhibition by 20.3% at 125µg/ml.	
	Effect against Coxsackie B4 virus	↗↗ Inhibition by 45.4% at 125µg/ml.	
Weissella paramesenteroides MN2C2	Effect against Coxsackie virus (CVB3)	↗↗ Reduction of CVB3 titer by 99.99% at 3.0mg/ml	[268]
Arthrospira platensis	Effect against koi herpesvirus	Suppression of the viral replication at a concentration of >18µg/ml	[269]
Lactobacillus sp.	Effect against human adenovirus type 5 (HAdV-5)	↗↗ Viral inhibition to 100% at 20µg/ml of EPS	[270]
Leuconostoc sp.		↗↗ Viral inhibition to ≈ 80% at 20µg/ml of EPS	
Pediococcus sp.		↗↗ Viral inhibition to ≈ 42% at 20µg/ml of EPS	
Lactobacillus plantarum	Effect against Transmissible Gastroenteritis Virus (TGEV)	↗↗ Inhibition of TGEV proliferation (up to 78%)	[275]
		↗↗ Inhibition of TGEV RNA replication (71%)	

Table 8. *In vitro* studies on the bacterial effects of bacterial exopolysaccharides.

EPS origin	The tested bacteria	Key results	Ref.
Enterobacter sp. ACD2	*Escherichia coli*	Ø = 25.1 ± 0.2mm	[276]
	Staphylococcus aureus	Ø = 30 ± 0.2mm	
Bacillus subtilis F9	*Bacillus cereus* MTCC 6629	IC_{50} = 6mg/ml	[250]
	Listeria monocytogenes 1143	IC_{50} = 9.5mg/ml	
	Staphylococcus aureus MTCC 737	IC_{50} = 4.5mg/ml	
	Salmonella typhimurium ATCC14028	IC_{50} = 7mg/ml	
	Escherichia coli MTCC 443	IC_{50} = 3mg/ml	
Lactobacillus sp.	*Salmonella Typhimurium*	Ø = 8–12mm	[272]

EPS origin	The tested bacteria	Key results	Ref.
	Escherichia coli		
	Pseudomonas aeruginosa		
	Staphylococcus aureus	Ø = >12mm	
	Listeria monocytogenes	Ø = 8–12mm	
Leuconostoc mesenteroides (EPS_B3)	*Salmonella enterica spp. arizonae* DSMZ 9386	Significant inhibition at 10mg/ml	[271]
	Escherichia coli DSA_8048	Lag phase lengthening and reduced μmax and amplitude at 15mg/ml of EPS	
	Escherichia coli DSA_451	Total grow inhibition at 20mg/ml	
	Listeria monocytogenes Scott A	Significant inhibition at 5mg/ml	
	Staphylococcus aureus DSA_226		
	Staphylococcus aureus ATCC 6583		
	Enterococcus faecium DSMZ 2146		
Lactobacillus curvatus SJTUF 62116	*S. Enteritidis* ATCC 1307	Complete inhibition of bacterial growth at 20mg/ml	[273]
	Escherichia coli ATCC 25922		
	Staphylococcus aureus ATCC 13565		
Enterococcus sp.	*Staphylococcus aureus* (ATCC25923)	MIC = 5.25±0.3μg/ml	[277]
	Salmonella typhimurium (ATCC14,028)	MIC = 33.5±0.7μg/ml	
	Enterococcus faecalis (ATCC29,212)	MIC = 6.05±0.07μg/ml	
	Listeria monocytogenes (ATCC19,111)	MIC = 35.5±0.7μg/ml	
	Escherichia coli (ATCC25,922)	MIC = 10.5±0.7μg/ml	
	Bacillus cereus (ATCC11,778)	MIC = 11.5±0.7μg/ml	

EPS origin	The tested bacteria	Key results	Ref.
Lactobacillus plantarum S123	*Escherichia coli* ATCC25922	Ø = 11.5 mm at 3mg/ml	[245]
	Staphylococcus aureus ATCC29213	Ø = 7.2 mm at 3mg/ml	
Bacillus cereus	*Staphylococcus aureus*	MIC/MBC : 0.5/2mg/mL	[244]
Streptococcus thermophilus ZJUIDS-2-01	*Staphylococcus aureus* CMCC 26003	Ø = 11.4 ± 0.26 vs 15.1 ± 0.49mm (metronidazole)	[278]
	Listeria monocytogenes CMCC 54007	Ø = 11.9 ± 0.30 vs 13.5 ± 0.83mm (metronidazole)	
Leuconostoc mesenteroides SL and *Enterococcus viikkiensis* N5	*Staphylococcus aureus*	MIC = 1.75mg/ml	[279]
	Escherichia coli	MIC = 2.5mg/ml	
	Listeria monocytogenes	MIC = 5mg/ml	

In addition, the EPS extracted from the species *Lactobacillus plantarum* S123 exhibited a significant antibacterial effect against *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC29213 with an inhibition zone of 11.5 and 7.2mm at 3mg/ml, respectively Saleem *et al*.[245]. *Enterococcus* sp. EPS showed a high antibacterial effect against *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (ATCC29,212), *Escherichia coli* (ATCC25,922), *Bacillus cereus* (ATCC11,778), *Salmonella typhimurium* (ATCC14,028), and *Listeria monocytogenes* (ATCC19,111) with Minimal inhibitory concentration (MIC) of 5.25±0.3, 6.05±0.07, 10.5±0.7, 11.5±0.7, and 35.5±0.7µg/ml, respectively[277]. While the EPSs isolated from *Leuconostoc mesenteroides* SL and *Enterococcus viikkiensis* N5 exhibited relatively low activity against *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes* with MIC of 1.75, 2.5, and 5mg/ml [279]. *Bacillus cereus* EPS can significantly inhibit the growth of *Staphylococcus aureus* with MIC/MBC of 0.5/2mg/ml[244].

5. EPS Applications

Bacterial exopolysaccharides have numerous potential applications in medicine, agriculture, and environmental remediation. In medicine, bacterial EPS have been investigated for their immunomodulatory, antibacterial, and antiviral properties, as well as for their potential use as drug delivery systems and wound healing agents[280,281]. In agriculture, bacterial EPS have been explored as biofertilizers and soil conditioners[282]. Given their wide range of potential applications, bacterial EPS are an important area of research that continues to attract significant interest from scientists and industry alike.

5.1. Bacterial EPS in Medicine

Bacterial EPS have gained attention in recent years due to their potential applications in medicine. These EPS have been found to exhibit various biological activities, such as antibacterial and antiviral properties, wound healing, drug delivery, and immunomodulation[176,280,283,284]. Bacterial EPS are of interest as a potential source of new therapeutics due to their diverse chemical structures and biological activities (Table 9).

Table 9. Bacterial exopolysaccharides in medical applications.

EPS Type	Structure	Bacterial Source	Use in Medicine	Ref.
Dextran	α-1,6 and α-1,3 glucosidic linkages	*Leuconostoc mesenteroides*, *Streptococcus mutans*	Blood plasma expanders, wound healing	[285–287]
Xanthan	Glucose, mannose, and glucuronic acid	*Xanthomonas campestris*	Thickening agent, wound healing	[288–290]
Pullulan	α-1,4 and α-1,6 glucosidic linkages	*Aureobasidium pullulans*	Drug delivery, wound healing	[291,292]
Alginate	β-1,4 mannuronic and guluronic acids	*Pseudomonas aeruginosa*	Wound dressings, tissue engineering	[293–295]
Hyaluronic acid	β-1,3 and β-1,4 glucuronic and N-acetylglucosamine acids	*Streptococcus zooepidemicus*	Wound healing, anti-inflammatory	[296–298]
Gellan gum	Glucose, glucuronic acid, and rhamnose	*Sphingomonas elodea*	Drug delivery, tissue engineering	[299–301]
Curdlan	β-1,3 glucosidic linkages	*Agrobacterium* species	Drug delivery, immunomodulation	[302,303]
Fucoidan	Sulfated fucose, galactose, and mannose	Brown seaweed, marine bacteria	Anti-inflammatory, anticancer	[304–308]
Scleroglucan	β-1,3 and β-1,6 glucosidic linkages	*Sclerotium rolfsii*	Drug delivery, wound healing	[309,310]
Levan	β-2,6 fructosidic linkages	*Zymomonas mobilis*	Prebiotic, immunomodulation	[311–314]

5.1.1. Antibacterial and antiviral properties

Bacterial EPS are potential candidates for the development of new antimicrobial agents[315]. They can disrupt bacterial and viral cell membranes, preventing their growth and replication[316]. Some bacterial EPS have been found to exhibit activity against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and *Vibrio parahaemolyticus*, antibiotic-resistant pathogens that pose significant public health threats[317–322].

5.1.2. Wound healing and tissue engineering

EPS derived from bacteria have been discovered to facilitate the process of wound healing and regeneration of tissues[323]. These EPS have the capability to generate a protective layer on the wound site that thwarts infections and encourages cell proliferation and differentiation. Moreover, certain types of bacterial EPS have been shown to boost angiogenesis, which is vital for the restoration and regeneration of tissues via the creation of new blood vessels[324]. Additionally, bacterial EPS have been studied for their possible application in tissue engineering as they can serve as supportive structures to promote cell growth and differentiation[325,326].

5.1.3. Drug delivery systems

Bacterial EPS has been investigated as a promising approach for drug delivery systems. They have the capacity to encapsulate drugs and deliver them to specific cells or tissues. Some bacterial EPSs have been identified as selective towards cancer cells, which offers a potential application in targeted drug delivery for cancer treatment[307,327]. Furthermore, bacterial EPS can safeguard drugs against degradation and enhance their bioavailability, which can enhance their efficacy and decrease the need for frequent dosing[328].

5.1.4. Immunomodulatory effects

Bacterial EPS exhibits immunomodulatory effects by modulating the immune response, reducing inflammation, and stimulating the production of cytokines and chemokines. In addition, bacterial EPS can stimulate the production of antibodies, enhancing the immune response against pathogens[303,329–332].

5.2. Bacterial EPS in Environmental Sustainability

Bacterial EPS play an important role in environmental processes, particularly in bioremediation and biodegradation of pollutants. These molecules are able to interact with a wide range of environmental contaminants, such as heavy metals, pesticides, and hydrocarbons, and facilitate their removal from contaminated environments[333,334]. Bacterial EPS can also enhance soil quality and promote plant growth, making them useful in agriculture and environmental restoration[335,336].

5.2.1. Bioremediation and biodegradation of pollutants

Bacterial EPS has been shown to enhance the bioremediation of pollutants by facilitating the growth of microorganisms that can degrade contaminants[333,334]. For example, bacterial EPS can increase the bioavailability of nutrients and reduce the toxic effects of heavy metals, such as lead and cadmium, which can inhibit microbial activity. Moreover, bacterial EPS can sequester contaminants, making them more accessible to microorganisms that can break them down[337].

5.2.2. Soil improvement and plant growth promotion

Bacterial EPS can also play a role in improving soil quality and promoting plant growth. Bacterial EPS can help to enhance soil structure, water-holding capacity, and nutrient availability[338]. In addition, some bacterial EPS have been found to act as biofilm-forming agents, promoting the attachment of bacteria to plant roots and promoting plant growth[339,340]. The use of bacterial EPS in agriculture can help to reduce the need for chemical fertilizers and pesticides and can promote sustainable farming practices[282].

5.2.3. Biosurfactants and bioemulsifiers for industrial applications

Bacterial EPS are used as biosurfactants and bioemulsifiers for industrial applications[319,341]. These EPS can help to break down hydrophobic contaminants, such as oil, and can facilitate the removal of contaminants from industrial processes[342]. In addition, bacterial EPS can be used as natural surfactants in personal care and cleaning products, reducing the need for synthetic surfactants[343].

5.3. Bacterial EPS in Agriculture

Bacterial EPS have potential applications in soil improvement and plant growth promotion. They can enhance soil fertility, improve nutrient uptake by plants, and protect

plants from environmental stresses. Bacterial EPS can also improve the quality and yield of crops, making them useful in sustainable agriculture practices[338].

5.3.1. Soil improvement

EPS increase soil microbial activity, which is crucial for nutrient cycling and soil health. Some bacterial EPS have been found to increase the abundance of beneficial soil microorganisms, such as nitrogen-fixing bacteria, which can help to reduce the need for chemical fertilizers[344–346].

5.3.2. Plant growth promotion

Bacterial EPS can promote plant growth by increasing nutrient uptake and protecting plants from environmental stresses, such as drought and disease[347–349]. The EPS can enhance root growth and improve nutrient absorption by forming biofilms on plant roots [340]. In addition, some bacterial EPS have been found to induce systemic resistance in plants, making them more resistant to pathogens and pests[350]. Bacterial EPS can also enhance photosynthesis in plants, improving plant growth and yield[351].

5.3.3. Biofertilizers and biocontrol agents

Bacterial EPS have been explored as potential biofertilizers and biocontrol agents in agriculture. For example, some bacterial EPS can fix atmospheric nitrogen, which can be used by plants as a nutrient source[352]. In addition, bacterial EPS can promote plant growth by enhancing nutrient uptake and increasing root growth[340,348]. Bacterial EPS can also help to increase plant resistance to abiotic stress, such as drought and salinity, making them useful for sustainable agriculture practices[349]. *Streptococcus*, *Bacillus*, *Escherichia*[353], *Sphingomonas paucimobilis*[354] and *Sinorhizobium meliloti*[355,356] species are commonly utilized in this context.

6. Conclusion

Bacterial exopolysaccharides (EPS) have been found to have diverse applications in medicine, agriculture, and environmental remediation. EPS produced by different bacterial species have unique chemical structures and physical properties that make them suitable for various applications. In medicine, bacterial EPS have shown promise as immunomodulators, wound healing agents, and drug delivery systems. In agriculture, they have been explored as biofertilizers and soil conditioners, and in environmental remediation, they have been used to remove heavy metals and other pollutants from contaminated soil and water. While many

bacterial EPS have already found commercial applications, research in this field continues to explore new ways of harnessing their unique properties for a variety of uses. The potential for bacterial EPS to contribute to solutions for some of the world's most pressing challenges underscores the importance of continued research and innovation in this area.

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