Wang *et al. Chem. Biol. Technol. Agric.* (2023) 10:137
https://doi.org/10.1186/s40538-023-00515-3

Chemical and Biological Technologies in Agriculture

REVIEW

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Structural characteristics of microbial exopolysaccharides in association with their biological activities: a review

Wei Wang[1], Yuhao Ju[3], Nan Liu[1], Shengbo Shi[1] and Lujiang Hao[1,2*]

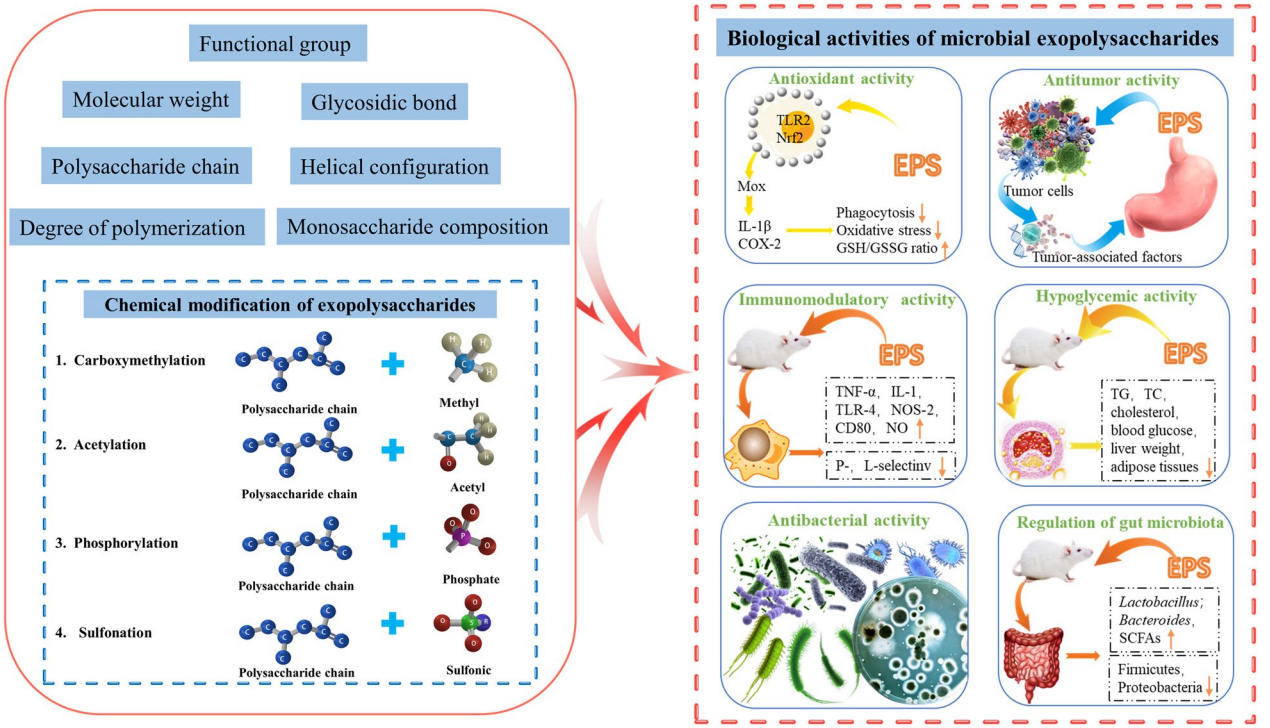
Abstract

Many microbial exopolysaccharides (EPS) have been reported in the last decade, and their fermentation processes, functional properties and applications, structural characterization, and biological activities have been extensively studied. Despite the great diversity of biological activities already described for EPS, only a few have been exploited industrially. The main reason for this is that the structure–activity relationship of EPS has not been clearly defined. In this review, we collected EPS-related publications from two databases, the Web of Science and China National Knowledge Infrastructure, and reviewed the correlation between the structural characteristics of EPS and observed biological activity, as reported in studies over the last decade. This review focused on the antioxidant, antitumor, immunomodulatory, hypoglycemic, antibacterial, and gut microbial-modulating activities of EPS. This review aimed to lay a foundation for researching the structure–activity relationship of EPS and provide a theoretical basis for important scientific studies and applications of EPS.

Keywords Exopolysaccharides, Structure–activity relationship, Structure, Biological activity, Modification

*Correspondence:
Lujiang Hao
lujiang_hao@qlu.edu.cn
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Microorganisms are the most abundant source of biological diversity on Earth. They exhibit novel functions and extensive biological characteristics, and are special sources of various metabolites [1, 2]. Exopolysaccharides (EPS) are extracellular carbohydrate polymers produced by microorganisms, including bacteria, fungi, and microalgae [3–5]. In the natural environment, EPS usually participate in protecting microorganisms; they resist adverse conditions (e.g., desiccation, cold, and hypertonic conditions), enhance resistance, and promote nutrient uptake [6–8].

EPS have anticancer [9], antitumor [10], anti-inflammatory [11, 12], antidiabetic [13], antiviral [14], antioxidant [15], cholesterol-lowering [16], hypoglycemic/hypolipidemic [17, 18], immunomodulatory [19], and probiotic activities [20, 21]. Owing to their novel physiological functions and extensive biological activities [22], their formability is advantageous in terms of chemical composition and structure. EPS have been widely used in the fields of food, chemicals, and cosmetics [23, 24], and have also shown great potential for medical applications [25, 26]. Currently, the applications of microbial EPS in medicine include drug targeting [27], delivery [28, 29], vaccine preparation [30], tissue engineering [31], wound healing [32], anti-proliferation [33], cell carriers, and diagnostic tool manufacturing [3]. A number of carbohydrate-based drugs are also clinically used, including carragelose [34, 35] (Fig. 1A), cethromycin [36] (Fig. 1B), sodium oligomannate [37] (Fig. 1C), and lactitol [38] (Fig. 1D).

The biological activities of EPS are closely related to their structure [39], including monosaccharide composition, molecular weight, glycosidic linkage type and position, and chain conformation [40]. Therefore, in this review, we summarized the structural characteristics and biological activities of microbial EPS and explored their structure–activity relationship provide a reference and theoretical basis for the research and application of microbial EPS.

Research status of EPS

With the mining of EPS bioactivities and their wide range of applications in numerous research areas [41, 42], EPS research is gradually becoming an internationally cutting-edge topic with a large number of literature reports [3, 43, 44]. The EPS-related publications from 2012 to 2022 were statistically analyzed using the Web of Science (WOS) and China National Knowledge

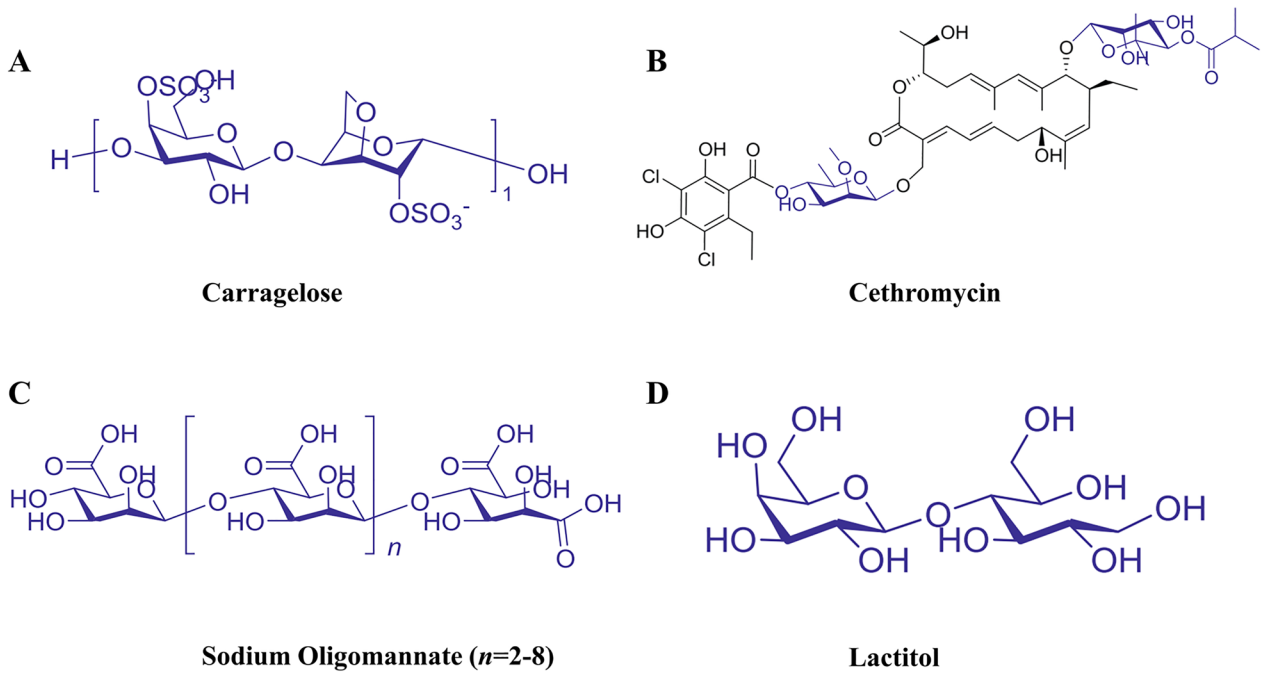


Fig. 1 Chemical structures of four carbohydrate-based drugs

Infrastructure (CNKI) series databases with "exopolysaccharides" as the subject term (Fig. 2). The plot in Fig. 2 is similar to the global reasoning approach proposed by Chen et al. [45]. We projected the data of the two databases from the coordinate space to the nodes in an interaction space graph, which allowed us to directly analyze the information of the two databases from a global perspective.

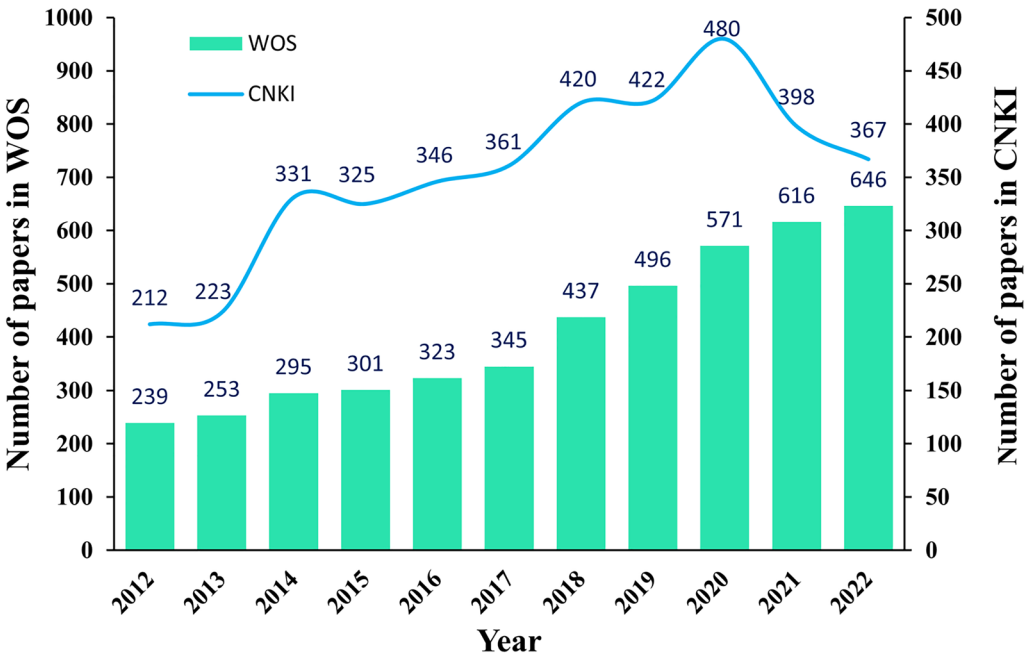


Fig. 2 Statistics on the number of publications on EPS in WOS/CNKI per year from 2012 to 2022. *EPS* Exopolysaccharides, *WOS* Web of Science, *CNKI* China National Knowledge Infrastructure

The number of EPS-related publications on the WOS has been increasing annually since the past decade; the number of EPS-related publications was 239 in 2012, increasing to 646 in 2022—an increase of 170%. Over the past five years, the number of publications on EPS has increased, with an average annual increase of approximately 70 articles. As of March 9, 2023, the total number of EPS research publications reached 4,522 in the last decade. An annual analysis of the CNKI database shows that the number of EPS-related publications has increased annually over the past decade. As of March 9, 2023, the total number of EPS research papers published in the last decade was 3,885. An increase in the number of EPS-related studies in recent years has shown that EPS have gradually become a focus of attention and a research hotspot.

Currently, research on EPS focuses on four aspects: preparation process, functional properties and applications, structural characterization, and biological activities. The classification results of the studies on EPS in the two databases over the past decade are shown in Fig. 3, which shows that the focus of EPS research on CNKI differs significantly from that on the WOS.

In recent years, many studies on the EPS preparation processes for CNKI have been reported and are increasing annually. Research on the biological activity of EPS has been steadily increasing annually, with a slower increase than that of the preparation process studies. However, the number of studies on the structural characterization of EPS is relatively small, with approximately 20 papers published each year (Fig. 3A). In contrast, the number of research publications on the structural characteristics of EPS in WOS remained above 60, but the overall number accounted for a small percentage of the publications (Fig. 3B). In summary, basic research on the structural characteristics of EPS is still very limited; therefore, conducting relevant research on these aspects is a key direction for researchers focusing on EPS.

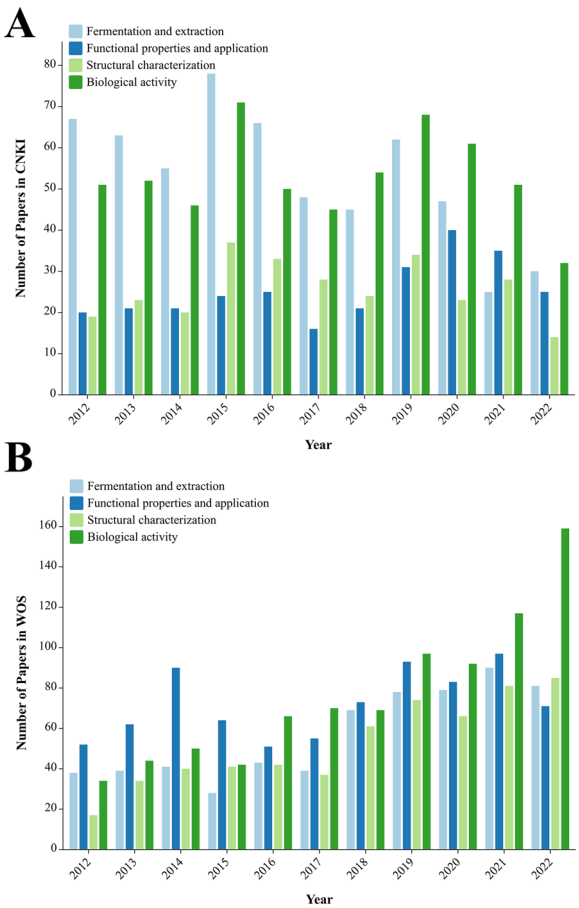


Fig. 3 Comparative statistical analysis of CNKI/WOS published literature from 2012 to 2022. Comparative general data of the number of CNKI/WOS published papers using different keywords/phrases to search: **1** fermentation and extraction; **2** functional properties and applications; **3** structural characterization; and **4** biological activity. *WOS* Web of Science, *CNKI* China National Knowledge Infrastructure

Structural features of EPS

EPS have two different extracellular secretion states: capsular polysaccharides that adhere to the microbial cell wall to form a capsule, or slime polysaccharides that are loosely attached or even completely released into the surrounding environment to form slime [46, 47]. EPS can be homopolysaccharides composed of the same monosaccharide, such as curdlan, or heteropolysaccharides composed of different monosaccharides[48] (Fig. 4A). Heteropolysaccharides consist of different monosaccharides, including not only commonly observed sugars (such as glucose, galactose, and fructose), but also some rare monosaccharides (such as rhamnose, xylose, fucose, and mannose), uronic acids and amino sugars [20, 49] (such as xanthan) [50] (Fig. 4B). EPS with straight chains of monosaccharides, such as pullulan, are called linear polysaccharides [8, 51] (Fig. 4C). EPS with arms and bends, such as EPS-W1 extracted from *Lactobacillus plantarum* W1, are called branched polysaccharides [52–54] (Fig. 4D).

The structural description of EPS usually includes monosaccharide composition and conformation, molecular weight range, glycosidic bond conformation, repeating units, linkage sites, and spatial structures [55]. Table 1 summarizes the various EPS obtained from different microorganisms from 2018 to 2022. According to Table 1, we can speculate that EPS with a porous structure is more likely to have antioxidant activity, and that EPS with immunomodulatory activity are all triple-helical structures. Analyses of the monosaccharide composition, molecular weight, conformation, and biological activity

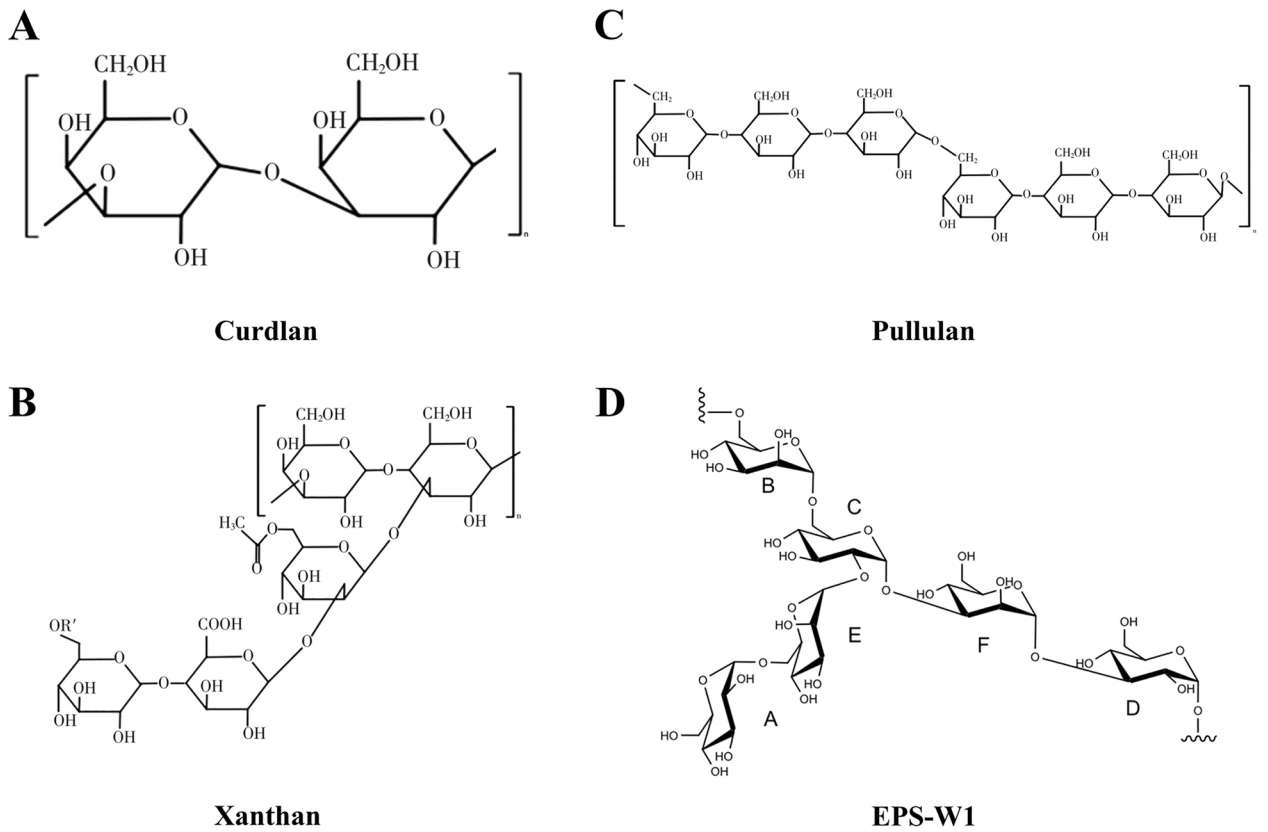


Fig. 4 Structural formulae of EPS, including homopolysaccharides [e.g., curdlan (**A**)]; heteropolysaccharides [e.g., xanthan (**B**)]; linear polysaccharides [e.g., pullulan (**C**)]; and branched polysaccharides [e.g., EPS-W1 (**D**)]. *EPS* Exopolysaccharides

of these EPS can provide useful information about their structure–activity relationships.

Structure–activity relationship of EPS

The composition and structure of EPS determine their microstructure and surface morphology, which affect their biological activity [77]. In this section, we focus on the antioxidant, antitumor, immunomodulatory, hypoglycemic, antibacterial, and gut microbial-modulating activities of microbial EPS (Fig. 5).

Antioxidant activity

Studies have shown that EPS have significant antioxidant activity. Similar to the mechanism of other sources of polysaccharides, the hydrogen-donating capacity of bacteria-derived EPS is considered the main property of its antioxidant function, but the underlying mechanism is not clear [80]. The antioxidant activity of EPS is affected by several factors, including monosaccharide composition, glycosidic bond type, and branching patterns.

The monosaccharide composition and composition ratio of EPS significantly a effected the antioxidant activity of EPS. As an example, EPS consisting of glucose-repeating units exhibit strong superoxide and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) scavenging activities and low hydroxyl radical scavenging activity, similar to that of the ascorbic acid standard [81]. The EPS obtained from *Weissella cibaria* SJ14, purified with a mannose content of 75.9%, exhibited excellent hydroxyl radical scavenging activity. This may be due to the higher mannose content [82]. In addition, high galactose-containing heteropolysaccharides obtained from *W. confusa* show strong scavenging ability and effective reduction of DPPH and hydrogen peroxide radicals [83]. Similarly, the two EPS components produced by *Lactobacillus delbrueckii* ssp. *bulgaricus* SRFM-1, r-EPS1 and r-EPS2, had higher proportions of galactose and exhibited stronger antioxidant activities [84]. Moreover, EPS, which consist of galactose, glucose, and rhamnose as the main monosaccharides exhibit antioxidant activity. This has been verified in EPS studies on *Porphyridium cruentum* and *Bacillus* sp. S-1, and *Enterobacter ludwigeii* [85, 86].

These results suggest that the glycosidic bond type and branching pattern can affect the antioxidant activity of EPS [84]. It has been suggested that α-1,2 and 1,6 glycosidic bonds are more flexible than β-1,3 and 1,4 glycosidic

Table 1 Monosaccharide compositions and conformational of selected microbial EPS and their related biological activities

EPS Producing Microorganisms	Monosaccharide composition	Molecular weight	conformation	Biological activities	Refs.
Aureobasidium pullulans CGMCC 23063	Beta-glucan	2.949×10^5 Da	Composed spheres joined by triple-helix conformation into chains and circles	Immunomodulatory	[56]
Panteoa alhagi NX-11	Glucose Galactose Mannose	1.326×10^6 Da	Flaky structure	Antioxidant	[57]
Morchella esculenta	Glucose Mannose	1.392×10^6 Da	Fragmental and rod-like structure	Immunomodulatory	[58]
Lysinibacillus fusiformis KMNTT-10	Xylose Rhamnose Arabinose Galactose Glucose	—	A highly porous web-like structure with an irregular surface	Flocculation Emulsification Antioxidant	[59]
Alteromonas infernus	Glucose Rhamnose Galactose	1.0×10^3 Da	Random-coiled conformation	Anti-metastatic	[60, 61]
Potential Probiotic Leuconostoc mesenteroides LM187	Arabinose Galactose Rhamnose	7.757×10^7 Da	A large number of scattered, fluffy, porous cellular network flake structures	Antioxidant	[62]
Weissella confusa XG-3	Glucose	3.19×10^6 Da	A smooth, porous, and branched structure; round lumps and chains on irregular surfaces	Antioxidant	[63]
Cordyceps militaris	Galactose Mannose	1.56×10^6 Da	Helix structure when dissolved in weakly alkaline solution; branched and intertwined form on the surface	Hypoglycemic	[17]
Weissella cibaria MED17	Glucose	–	Compact sheet-like morphology	Antioxidant	[64]
Pleurotus citrinopileatus	Galactose Glucose	1.062×10^6 Da	Triple-helix conformation	Hypoglycemic	[65]
Antrodia cinnamomea	Galactose	1.18×10^5 Da	Spherical and flexible chain morphologies	Food /drug delivery	[66]
Lactobacillus plantarum HY	Mannose Galactose Glucose	9.549×10^4 Da	A highly porous structure, the presence of spherical lumps	Antioxidant Alpha-amylase inhibitory activity	[67]
Abortiporus biennis	Glucose Mannose Galactose	2.207×10^4 Da	A random coil conformation	Antioxidant Antitumor	[68]
Lactobacillus plantarum JLAU103	Arabinose Rhamnose Fucose Xylose Mannose Fructose Galactose Glucose	1.24×10^4KDa	A smooth and glittering cube structure, and the presence of many homogeneous rod-shaped lumps	Antioxidant	[69]
Lactococcus lactis F-mou	Galactose Glucose	–	A porous structure characterized by a flake-like basic configuration with an extremely dense assembly	Antioxidant Anticoagulant	[70]
Alteromonas sp. PRIM-28	Glucose Mannose	7.80×10^5 Da	Exists as a triple helical structure in an aqueous solution	Promote wound healing	[71]
Lactobacillus fermentum S1	Mannose Rhamnose Glucose Galactose	7.19×10^5 Da	A hollow porous structure	Antioxidant	[72]

Table 1 (continued)

EPS Producing Microorganisms	Monosaccharide composition	Molecular weight	conformation	Biological activities	Refs.
Lactobacillus plantarum KX041	Arabinose Mannose Glucose Galactose	2.638 ~ 7.073 × 10^4 Da	A triple helical structure; flake shapes piling up into compact structures with a rough surface	Immunomodulatory Antioxidant Anti-inflammatory	[73]
Rigidoporus microporus (*Agaricomycetes*)	Beta- glucopyranose; Mannose	34.1 × 10^4 Da	A flexible, linear random coil chain structure	Antioxidant	[74]
Bacillus amyloliquefaciens GSBa-1	Glucose	5.4 × 10^4 Da	Appeared as ellipsoid or globose with a smooth surface	Antioxidant	[75]
Streptococcus thermophilus AR333	Galactose Glucose	3.137 × 10^5 Da	As compact and semi-stiff or stiff chains in an aqueous solution	Emulsification	[76]

EPS Exopolysaccharides

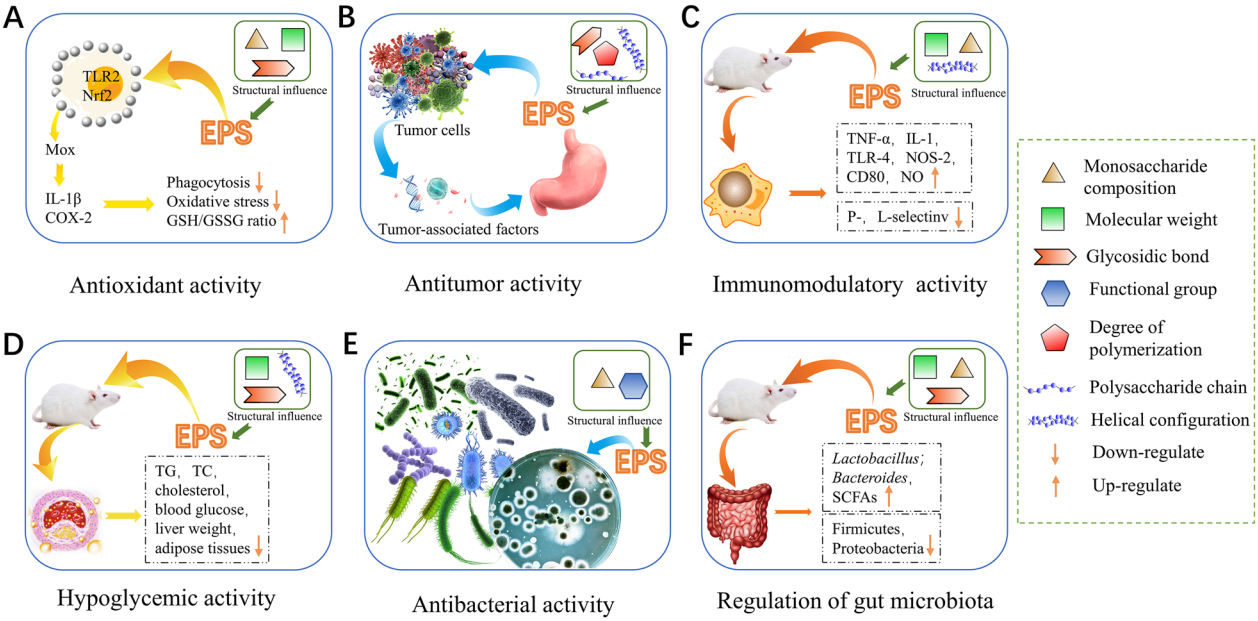


Fig. 5 Main biological activities of microbial EPS, including antioxidant (**A**), antitumor (**B**), immunomodulatory (**C**), hypolipidemic (**D**), antibacterial (**E**), and regulation of gut microbiota (**F**). Figure modified according to [78, 79]. *EPS* Exopolysaccharides, *TG* Triglycerides, *TC* Total cholesterol

bonds [87–89]. The EPS produced by *B. amyloliquefaciens* is an α-glucan composed of glucose with two α-(1 → 3) and one α-(1 → 6) glycosidic bonds, which has a superoxide anion-scavenging ability [75]. However, the role of glycosidic bonds in antioxidant activity remains unclear and requires further investigation. It has been found that EPS with a high degree of branching also has good antioxidant activity. Yang et al. isolated and purified two fractions, THPS-1 and THPS-2, from the *Tetragenococcus halophilus* SNTH-8, both of which were highly branched polysaccharides with high antioxidant and emulsifying abilities [90].

Furthermore, changes in bacterial fermentation conditions (e.g., pH) can alter the structure of EPS, thereby affecting its antioxidant activity. For example, the EPS produced by *Alteromonas australica* QD under different pH conditions. The results revealed that acidic pH EPS (AC-EPS) and alkaline pH EPS (AL-EPS) contained similar types of monosaccharides with different proportions of Man, Gal, and GlcA. AL-EPS has been found to have high antioxidant activity [91]. Similar results have been reported by Ju [92].

Antitumor activity

According to a study on EPS antitumor activity regarding its structure, the high-order structure of EPS is more important than the primary structure for EPS antitumor activity [93, 94]. It includes the main chain composition, flexibility, molecular chain conformation, degree of branching, helical conformation, and spatial structure.

Antitumor EPS structural studies have shown that β-1,3 glycosidic bonds on glucose chains and β-1,6 glycosidic bonds on branched chains are required for their activities [95]. For instance, a variety of polysaccharide were isolated from *Porphyra mushroom*, whose antitumor active fraction is β-(1,3)-D-glucan with (1 → 6) branched chains [96]; the antitumor polysaccharide extracted from *Auricularia auricula-judae* was also composed of β-1,3-bound straight-chain glucan [97].

The flexibility of the polysaccharide backbone determines the antitumor activity of EPS to a certain extent [98]. Flexibility consists of a combination of hydrogen bonding and electrostatic repulsion of substituents within the polysaccharide molecule. High flexibility facilitates the interaction between the polysaccharide and the immune system, thus enhancing the antitumor activity of EPS [41, 99]. It has also been reported that polysaccharide branches can weaken intramolecular interactions and disrupt intermolecular binding, thus affecting antitumor activity [100, 101]. Bohn suggested that EPS with branching degrees of 0.2–0.33 have higher antitumor activity [102].

Morphological characteristics and chain conformation may also influence EPS antitumor activity [103]. Polysaccharides with a triple-helical conformation exhibit antitumor activity [104]. For instance, Misaki et al. found that lentinan and *Auricularia auricula-judae* polysaccharides with antitumor activity have β-triple helix conformation [97]. It has been found that chain conformation facilitates the interaction of polysaccharides with the immune system and enhances the antitumor activity of EPS [105]. The in vivo antitumor activity of different chain conformations of lentinan showed that the triple-helix conformation plays an important role in the antitumor activity of lentinan. Once the helical chain is disrupted, the antitumor activity decreases significantly or even disappears [106]. *Poria* polysaccharide is similar to lentinan; both have β-1,6 side chain glucan and no tumor activity, but through the oxidation of periodate and by Smith degradation after the removal of β-1,6 chain, the antitumor activity of polysaccharides was observed. X-ray diffraction analysis revealed that the polysaccharides formed a triple helical configuration [107]. Similarly, several other polysaccharides with antitumor activity extracted from mushrooms exhibit a triple-helical conformation in solution [108, 109]. Furthermore, characteristic viscosity is a key factor. An appropriate characteristic viscosity is conducive to the adhesion of polysaccharides to tumor cells [110].

The antitumor activity of sulfated polysaccharides was higher than that of non-sulfated polysaccharides. EPS from *Lactobacillus plantarum* 70810 (e.g., r-EPS1 and r-EPS2) inhibited tumor cell growth at a higher rate than the inhibition associated with r-EPS1; the authors hypothesized that the significant antitumor activity of r-EPS2 may be closely related to the composition of the sulfate group and β-glycosidic bond in r-EPS2 [111]. Sulfated galactans isolated from *Halomonas aquamarina* EG27S8QL also exhibit antitumor activity [112, 113]. However, EPSR3 from *Bacillus cereus* is a sulfate-free EPS, and its main component is uronic acid (28.7%). The results of this study showed that EPSR3 exhibited antitumor activity. The authors suggested that the antitumor activity of EPSR3 may be due to its uronic acid content [114]. Therefore, the relationship between acidic polysaccharides and their antitumor activities requires further investigation.

Immunomodulatory activity

Many studies have reported that EPS with certain compositions and molecular weights may be involved in immune responses [39, 115]. Results based on the structural features and immunomodulatory activity revealed that the presence of galactose is closely related to the immunomodulatory activity of EPS [115]. Reactions between polysaccharide antigens and antibodies produced in rabbits for galactose were reported as early as 1988 [116]. EPS from probiotic *Enterococcus hirae* WEHI01 are composed only of galactose, with a molecular weight of 2.59×10^3 Da, and it effectively improves macrophage-mediated immune responses [117].

Structure–activity relationship analysis showed that the molecular weight was significantly correlated with the immunomodulatory activity of EPS. EPS with higher molecular weights may inhibit immune responses [118]. In general, the degradation of higher-to lower-molecular-weight EPS significantly improves their biological activity [119]. Surayot et al. investigated the effect of EPS produced by *Lactobacillus confusus* TISTR 1498 on immunomodulatory activity, which consisted only of glucose with a high molecular weight of 65 000–506 000 kDa and was unable to stimulate the production of the pro-inflammatory factors nitric oxide and cytokines by RAW264.7 cells. After partial acid hydrolysis, its molecular weight was less than 70 kDa, and it was able to significantly stimulate macrophages and induce the production of nitric oxide as well as cytokines such as TNF-α, IL-1β, IL-6, and IL-10 [120]. The mechanism may be that lower-molecular-weight EPS and cell receptors bind more

strongly and are more conducive to stimulating the production of pro-inflammatory factors in RAW264.7 cells. However, another study purified two homogeneous EPS, EPS53 (high molecular weight) and EPS53d (low molecular weight), from skimmed milk fermented by *S. thermophilus* XJ53; EPS53 showed stronger immune activity by promoting phagocytic ability and NO release from macrophages [121]. Therefore, the relationship between the molecular weight and immune activity of EPS needs to be further studied.

Acidic heteropolysaccharides are better at inducing immune responses [118]. For example, high-molecular-weight sulfated heteropolysaccharides from *Lactobacillus paracasei* VL8 are mainly composed of glucose and galactose, which have strong immunomodulatory activities [122]. Nishimura-Uemura studied a heteropolysaccharide produced by *Lactobacillus delbrueckii* subsp. *Bulgaricus* OLL1073R-1, consisting of both neutral and acidic polysaccharides in a 3:2 ratio of glucose to galactose. The acidic polysaccharides contained a small amount (0.1%) of phosphate, which was able to strongly induce the proliferation of various types of macrophages, whereas the neutral polysaccharides were unable to function, and dephosphorylation of this heteropolysaccharide caused a significant reduction in the stimulatory effect [123].

Furthermore, EPS with a triple-helical conformation may have immunomodulatory activity. For instance, EPS have been isolated from *Aureobasidium pullulans* CGMCC 23063, which has a triple-helical conformation linked to chains and round spheres. In an in vitro cellular assay, EPS showed immunoreactivity in RAW264.7 cells [56]. A novel crude EPS with a triple helical structure produced by *Lactobacillus plantarum* KX041 possesses prominent immune activity, promoting the proliferation and phagocytosis of Raw264.7 [73].

Hypoglycemic activity

Current studies on the hypoglycemic mechanism of EPS mainly focus on the regulation of related enzyme activities [124] and the improvement of insulin sensitivity [125]. EPS can reduce blood sugar by inhibiting digestive enzymes [126]. The hypoglycemic activity of EPS is closely related to its molecular weight, branched structure, and high-order structure [127, 128].

The hypoglycemic activity of EPS is closely related to its molecular weight [129–131]. The optimal activity of EPS can only be achieved at the appropriate molecular weight. Generally, EPS with low molecular weights exhibit better hypoglycemic activity [128]. A novel *Codyceps* polysaccharide with low molecular weight of 28 kDa was obtained by acid hydrolysis, and its inhibition rate on α-d-glucosidase was calculated as 40.01% [132]. Wang et al. used birch mushroom polysaccharides to simulate digestion in the intestine; the digested polysaccharide (UIOPS-1I) had a reduced molecular mass and significantly higher inhibitory activity against glucosidase [127].

Glycosidic bonds play an important role in the hypoglycemic activity of EPS [133]. It was found that most of the EPS with hypoglycemic activity have 1 → 3, 1 → 4, and 1 → 6 glycosidic bonds [128]. For example, the EPS backbone of *Cordyceps militaris* is dominated by a galactose 1 → 4 linkage, which effectively inhibits-glucosidase activity and restores glucose tolerance in mice [134].

It has also been suggested that EPS with a helical structure are more likely to have hypoglycemic activity, which has been verified in studies on *Cordyceps militaris* and *Pleurotus citrinopileatus* [17, 65]. Furthermore, sulfated EPS exhibited better hypoglycemic ability than that of natural EPS. For example, the EPS isolated from the fermentation broth of *Lachnum* sp. YM240 is sulfated, and sulfated EPS have a higher ability to inhibit glucosidase and amylase activities than that of unsulfated EPS [135].

Antibacterial activity

EPS contain various functional groups, such as carbonyl, phosphate, and hydroxyl groups. To some extent, these functional groups interact with bacterial cell membranes to exert antimicrobial activity [136, 137]. Novel *Aspergillus* spp. DHE6 produces EPS with the main functional groups -OH,–CH,–C = C, and C–O–C, which exhibit strong antibacterial activity against harmful human pathogens (*Staphylococcus aureus*, *Bacillus subtilis, Bacillus pertussis*, and *Pseudomonas aeruginosa*) [138].

EPS composed of glucose and rhamnose are more likely to exhibit antimicrobial activity, as verified in studies on EPS obtained from *Lactobacillus gasseri* FR4, *Streptococcus thermophilus* GST-6, and *Lactococcus garvieae* C47 [139–141]. Similarly, EPS are produced by *Pediococcus pentosaceus* SSC-12, with monosaccharide fractions of mainly glucose and rhamnose; they exhibit a strong antibacterial capacity [142]. However, an EPS with a molecular weight of 53,887 Da is produced by *Lactobacillus crispatus*, consisting mainly of mannose and glucose. It also has excellent antibacterial activity, which can effectively limit bacterial translocation and increase the abundance of *Lactobacillus* and *Bifidobacterium* [143]. *Enterobacter* sp. ACD2 EPS, with monosaccharide fractions of glucose, galactose, and fucose, containing 10% uronic acid and a small amount of fructose, showed high antibacterial activity against *S. aureus* and *Eschericia coli* [144]. In another study, dextran formed by *Lactobacillus* inhibited biofilm formation by *C. albicans* [145]. Moreover, negatively-charged EPS can interact better with

pathogens through their sulfate groups, exhibiting antifungal activity [70].

Regulation of gut microbiota

EPS can also regulate the composition and function of the gut microbiota [20, 146]. *Cordyceps sinensis* polysaccharides (CSPs) are composed mainly of glucose, galactose, mannose, galacturonic acid, arabinose, trace proteins, and phenolic compounds. The backbone of CSPs consist of 1,4-glucose and 1,4-galactose, with a molecular weight of approximately 28 kDa [147]. CSPs increase the abundance of probiotics (*Lactobacillus*, *Bifidobacterium*, *Bacteroides*) and decrease that of pathogenic bacteria (*Clostridium* and *Flexispira*) [148]. *E. coli* EPS (EPS-m2) are composed of glucuronic acid, glucose, fucose, galactose/N-acetyl glucosamine, arabinose, xylose, and ribose in a molar ratio of approximately 77:44:29:28:2:1:1. EPS-m2 increases the abundance of *Alistipes*, *Acinetobacter*, *Alloprevotella*, *Howardella*, and *Oxalobacter*, and GC detection illustrates that EPS-m2 enhances the production of SCFAs [149]. The dextran (LM742) produced by *Leuconostoc mesenteroides* SPCL742, with a molecular weight of 1.3×10^6 Da, contains α-1,6 and α-1,3 glycosidic bonds in a ratio of 26.11:1. The LM742 glucan is resistant to digestive enzymes in the human gastrointestinal conditions [150]. Additionally, EPS from *Paecilomyces cicadae* TJJ1213 regulates the gut microbiota and metabolism and increases the abundance of probiotics [151].

However, few studies have reported on the role of EPS in the regulation of gut microbiota, and current studies have some shortcomings; thus, the mechanism of how EPS regulate gut microbiota is yet to be further elucidated. Therefore, researchers should strengthen the study of EPS structure and its relationship with the regulation of gut microbiota in the future, and reveal the intrinsic mechanism of EPS regulation of the gut microbiota.

Other biological activities

In addition to the aforementioned biological activities, EPS exhibit other biological activities. These include emulsifying, anti-inflammatory, and antimetastatic properties. EPS containing galactose generally have emulsifying properties, as demonstrated by the EPS generated by *Lysinibacillus fusiformis* and *Streptococcus thermophilus* [59, 76]. Sulfated heteropolysaccharides with branched and multichain structures may exhibit anti-inflammatory activities [152, 153]. Similarly, EPS isolated from *Lactobacillus crispatus* with a molecular weight of 53,887 Da, consisting mainly of mannose and glucose, possesses excellent anti-inflammatory activity [143]. Acidic EPS can degrade cholesterol more effectively than neutral polysaccharides can [154]. Moreover, EPS with irregularly curled conformations may have antimetastatic properties [61].

Chemical modification of EPS

The modification of EPS by group substitution to alter the structure of polysaccharides and enhance targeted biological activity has been reported as an emerging trend [138, 155, 156]. Current polysaccharide modification methods include carboxymethylation, acetylation, phosphorylation, and sulfonation. Lasiodiplodan, an exocellular fungal (1→6)-β-D-glucan, was used to illustrate the linkage of functional group to the glucan chain [157, 158] (Fig. 6).

Carboxymethylation modifications

Carboxymethylation involves the introduction of carboxymethyl groups to polysaccharide chains via etherification reactions of polysaccharides with acids or carboxylic acid derivatives to achieve changes in the spatial structure and water solubility of the polysaccharides; thus, affecting their biological activities [13, 159]. This modification has been shown to have a significant role in enhancing EPS bioactivity [160, 161]. For example, polysaccharides obtained from *Lachnum* YM240 fermentation broth were carboxymethyl-modified, and the results showed that diabetic mice fed carboxymethylated *Lachnum* polysaccharides had significantly lower fasting glucose and serum triglyceride levels and significantly higher insulin sensitivity [162]. Similarly, the EPS extracted from *Lachnum* YM281 were modified by carboxymethylation and exhibited enhanced biological activity [163].

Acetylation modifications

Acetylation alters the spatial structure of polysaccharides, thereby affecting their biological activities [159, 164]. EPS derived from *Paenibacillus polymyxa* EJS-3 have a higher reducing power than that of native EPS after various chemical modifications, including acetylation and phosphorylation [165]. Similarly, the EPS produced by *Lactobacillus plantarum* 70810 exhibited antioxidant activity after the introduction of a new acetylation moiety [166].

Phosphorylation modifications

Phosphorylation is a reliable method for enhancing the bioactivity of EPS [167]. EPS obtained from *Lactococcus lactis* subsp. *lactis* were phosphorylated and showed antioxidant effects in vivo and in vitro [168]. Similarly, EPS produced by *Lachnum* YM405 were subjected to sulfonation and phosphorylation treatments. The antioxidant activity of the modified derivatives was significantly enhanced [169].

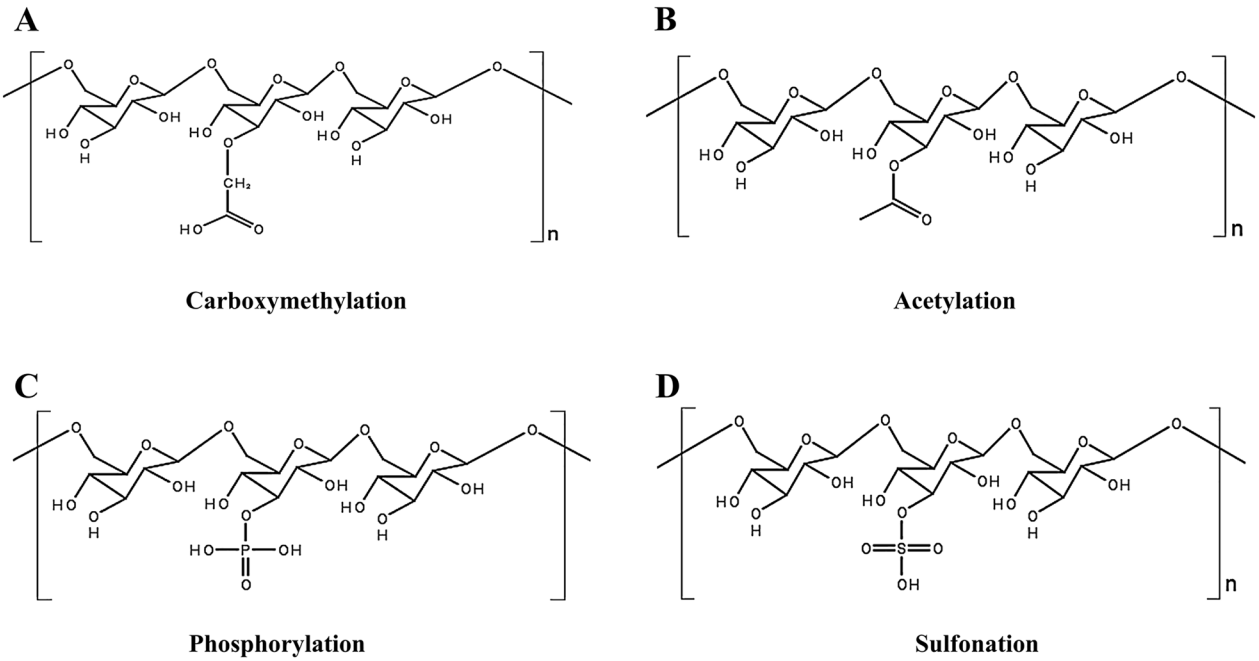


Fig. 6 Structural representation of D-glucan modification, including carboxymethylation (**A**); acetylation (**B**); phosphorylation (**C**); and sulfonation (**D**), where a group is added to the hydroxyl group on the monosaccharide

Sulfonation modifications

EPS are sulfonated to achieve the desired chain length and water solubility of the polysaccharide, which affects its biological activity. The sulfonation EPS extracted from *Enterobacter cloacae* Z0206 protected RAW264.7 mouse macrophages from H_2O_2-induced oxidative damage and inhibited DNA breakage. These results suggest that sulfonation enhances antioxidant activity by modulating water solubility and chain length and protects cells by exchanging more hydrogen atoms [170]. Similarly, sulfonated EPS produced by *Streptococcus thermophilus* GST-6 and *S. thermophilus* ASCC1275 showed stronger antimicrobial efficacy against various Gram-positive and harmful pathogens than the efficacy of non-sulfonated EPS [141, 171].

Conclusion and future perspectives

EPS produced by microorganisms have attracted attention worldwide owing to their safety, diverse potent biological activities, and favorable advantages over other natural agents for industrial and therapeutic applications. However, the structure of EPS is complex and difficult to analyze, resulting in difficulties in investigating their structure–activity relationship, and its specific mechanism of action has not yet been revealed. In this review, we suggest that the structural characteristics of EPS, such as molecular weight, monosaccharide composition, glycosidic bond type, branching pattern, spatial structure, and chemical modifications may affect their biological activity. We believe that more advanced technology should to be used to analyze the structure of EPS. On this basis, the mechanism of action of the structure–activity relationship should be revealed from a new perspective to lay the foundation for the targeted synthesis and design of glycans. Moreover, many studies have shown that incubation conditions (e.g., time, temperature, and pH) can also affect the structure and biological activity of EPS. Therefore, changing the composition and structure of EPS through the influence of external factors can broaden its applications to some extent.

Acknowledgements

We would like to thank Editage (www.editage.cn) for English language editing.

Author contributions

WW writing-review & editing. LH supervision, writing review & editing. YJ and SS writing-review & editing. YJ writing-review & editing, visualization. NL project administration, funding acquisition. All authors read and approved the final manuscript.

Funding

This research was supported by the Natural Science Foundation of Shandong Province (ZR2022MD097, ZR2012CM019), Education and Industry Integration Innovation Pilot Project of Qilu University of Technology (Shandong Academy of Sciences) (2022JBZ01-06), the Foundation of State Key Laboratory of Biobased Material and Green Papermaking (No. ZZ20190302), the Foundation of Shandong Provincial Key Laboratory of Biosensors (SWCG 2018–01), and the Foundation (No. 202002) of Qilu University of Technology of Cultivating Subject for Biology and Biochemistry, Science, Education and Industry Integration

Innovation Pilot Project of Qilu University of Technology (Shandong Academy of Sciences) (2020KJC-ZD08).

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

Author details
[1]School of Bioengineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250353, China. [2]State Key Laboratory of Biobased Material and Green Papermaking, Qilu University of Technology (Shandong Academy of Sciences), Jinan, China. [3]State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, and Chemical Biology Center, Peking University, Beijing, China.

Received: 24 October 2023 Accepted: 22 November 2023
Published online: 28 November 2023

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