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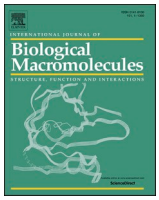

Review

Exopolysaccharides of *Paenibacillus polymyxa*: A review

Xuan-Ya Huang [a], Xin-Pei Ye [a], Yan-Yu Hu [a], Zhen-Xing Tang [b], Tian Zhang [a], Hai Zhou [a], Ting Zhou [a], Xue-Lian Bai [a], Er-Xu Pi [a], Bing-Hua Xie [a,*], Lu-E Shi [a,*]

[a] School of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, Zhejiang 311121, China
[b] School of Culinary Art, Tourism College of Zhejiang, Hangzhou, Zhejiang 311231, China

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ABSTRACT

Paenibacillus polymyxa (*P. polymyxa*) is a member of the genus *Paenibacillus*, which is a rod-shaped, spore-forming gram-positive bacterium. *P. polymyxa* is a source of many metabolically active substances, including polypeptides, volatile organic compounds, phytohormone, hydrolytic enzymes, exopolysaccharide (EPS), etc. Due to the wide range of compounds that it produces, *P. polymyxa* has been extensively studied as a plant growth promoting bacterium which provides a direct benefit to plants through the improvement of N fixation from the atmosphere and enhancement of the solubilization of phosphorus and the uptake of iron in the soil, and phytohormones production. Among the metabolites from *P. polymyxa*, EPS exhibits many activities, for example, antioxidant, immunomodulating, anti-tumor and many others. EPS has various applications in food, agriculture, environmental protection. Particularly, in the field of sustainable agriculture, *P. polymyxa* EPS can be served as a biofilm to colonize microbes, and also can act as a nutrient sink on the roots of plants in the rhizosphere. Therefore, this paper would provide a comprehensive review of the advancements of diverse aspects of EPS from *P. polymyxa,* including the production, extraction, structure, biosynthesis, bioactivity and applications, etc. It would provide a direction for future research on *P. polymyxa* EPS.

1. Introduction

The genus *Paenibacillus* contains over 200 species of facultative anaerobic species, which is an endospore-forming, neutrophil, peritrichous flagellate, heterotrophic, gram-positive bacilli [1,2] *P. polymyxa* (formerly called as *Bacillus polymyxa*) belonging to the genus *Paenibacillus*, has gained significant attention because of its great potential in a wide range of industrial processes and sustainable agriculture. It has a wide distribution in nature, showing a wide range of environmental adaptability, and can be isolated from soil, water, feed, insects, the rhizosphere in different plants such as wheat, rice, sugarcane, sunflower, barley, tomatoes, beans, sorghum, peppers, maize, etc.; forest trees, for example lodgepole pine, douglas fir, and marine sediments, etc. [1,3–9].

A variety of physiologically active substances, such as phytohormones, antibiotics, enzymes, volatile organic components (VOCs), and EPS, etc., are capable of being produced by *P. polymyxa* [10–14]. Due to the diverse range of active compounds produced, the wide range of applications of *P. polymyxa* in agricultural and food industries have been investigated [15–17]. Over the past decade, a great deal of research has also been carried out on using *P. polymyxa* to separate various minerals including pyrite, hematite and chalcopyrite through flotation and flocculation techniques [18,19]. In addition to mineral processing, *P. polymyxa* has a wide application in environmental remediation as a bio-flocculant for wastewater treatment [20,21].

Among these applications, the use of *P. polymyxa* in agricultural sector includes two main issues: the promotion of the growth of plants and the biocontrol for plant diseases. *P. polymyxa* uses several mechanisms to help the growth and adaptation of host plants, including fixing biological N, synthesizing phytohormone, solubilizing inorganic mineral phosphate, absorbing iron, and inducing systemic resistance [22–25]. In-depth paper of the plant growth-promoting properties of *P. polymyxa* including the fixation of N, phosphorus solubilization, iron uptake, the synthesis of phytohormones, was reviewed by Grady et al. [23]. Yegorenkova et al. [12] reported that the strains *P. polymyxa* CCM 1465 and *P. polymyxa* 92 were able to improve root cell mitotic index by 1.2 and 1.6 times, respectively, after inoculating wheat seedlings. The results showed that both strains and their EPS stimulated the growth and development of wheat, improving the length of root/shoot by around 22 % and dry weight of root/shoot by approximately 28 % over the control. A positive effect on the growth promotion of wheat and maize was also

* Corresponding author.
E-mail addresses: xbh840123@aliyun.com (B.-H. Xie), shilue@126.com (L.-E. Shi).

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confirmed in an investigation by Karnwal [26] with the strain *P. polymyxa* SbCT4. The length of root and dry weight exhibited a significant increase in contrast with the control group [26]. The growth parameters of canola and tomato were significantly improved after being treated by *P. polymyxa* P2b-2R. Tomato seedlings treated by *P. polymyxa* P2b-2R were able to assimilate almost 90 % more biomass, about 40 % longer compared to the control, and fixed around 17 % of atmospheric N [27].

As a sustainable agricultural solution, *P. polymyxa* shows the potential as a fertilizer and pesticide for the improvement of soil health and meeting the agricultural demands [5,28,29]. *P. polymyxa* has potential antagonistic activity through the production of a number of antibiotics, including polypeptins, gatavalin, jolipeptin, fusaricidin and polymyxin, which are effective for both bacteria and fungi [30,31]. In addition, they can also provide protection against a variety of insect herbivores and plant pathogens through the induction of a hypersensitive defense response in the host, which is referred to as induced systemic resistance [23]. *P. polymyxa* JY1–5 was obtained from turnip rhizosphere, showing excellent antagonistic activity against *Botrytis cinerea* which could cause gray mold on tomato [32]. Likewise, *P. polymyxa* NSY50 and *P. polymyxa* HX–140 have demonstrated the ability to control Fusarium wilt, a major destructive soilborne disease that affects cucumber [33,34]. *P. polymyxa* NSY50 was capable of effectively reducing the occurrence of Fusarium wilt (by 56.4 %) through the modification to the physiochemical characteristics of the soil including pH, C mic (microbial biomass carbon), R mic (basal respiration). After being treated by *P. polymyxa* NSY50, higher pH, C mic, R mic as well as the activities of all soil enzyme were observed [5,33]. In addition, *P. polymyxa* Nl4 was effective in the control of pear Valsa canker, which was induced by Valsa pyri, and *P. polymyxa* Y–1 successfully controlled bacterial disease of rice [35,36]. Chen et al. [4] found that *P. polymyxa* SF05 showed antagonism ability to pathogenic fungi of maize and was used in the field to control maize banded leaf sheath blight. It was suggested that the mechanism for controlling *P. polymyxa* SF05 may involve: (i) inhibiting pathogen growth by secreting secondary metabolites; (ii) inducing defense response of maize by producing VOCs. Wu et al. [37] assessed antifungal effects of VOCs derived from *P. polymyxa* CF05 against *Rhizopus stolonifer*, a significant pathogen responsible for postharvest fruit rot. The outcomes revealed that VOCs hindered the aerial mycelial growth and sporangia production of *Rhizopus stolonifer*. Additionally, VOCs were significantly effective in inhibiting on Rhizopus-based rot in cherry, mango and nectarine, with suppression ratios of 75.88 %, 60.64 % and 73.13 % of at 96 h after inoculation, respectively.

Currently, the research on the use of microbial EPS is growing rapidly as an important source of natural biopolymers [38]. EPS, one of the secondary metabolites, can be synthesized by various microorganisms. Many types of bacteria, such as *Bacillus subtilis*, *Pseudomonas*, *Lactobacillus*, etc., are able to synthesize EPS. EPS is a high-molecular-weight, water-soluble, long-chain polymer, and is present outside cells as capsular polysaccharide or mucopolysaccharide [39,40]. EPS called as Generally Regarded as Safe (GRAS) biomaterial, has a variety of functionalities including pseudoplastic rheology, emulsifying, and thickening properties [41,42]. It is now in use in food, biomedical, agricultural, environmental protection, and other industries because of its various beneficial effects [42–44]. In the last few years, many studies have reported that *P. polymyxa* is an excellent producer for both neutral and acidic EPS [16,45,46]. Though a majority of current studies on the metabolites from *P. polymyxa* are focused on various produced antibiotics, the growing interest is evidenced by large numbers of reports on the synthesis and utilization of *P. polymyxa* EPS [16,45–54]. EPS produced by *P. polymyxa* exhibits various biological activities such as antioxidative, anti-tumor, immune-regulatory, and many others [5,16,49,50,53]. Due to this, the synthesis of EPS by *P. polymyxa* can offer the potential to positively impact various applications within food, agricultural, and environmental industries [55]. Especially in the agricultural field, *P. polymyxa* EPS has been presented to play a crucial role in forming the associations between the plants and microbes. The EPS's surface localization gives on them the ability to act as the mediators in *P. polymyxa*'s interactions with other micro- and macroorganisms. Through the formation of a dense layer on the bacterial surface, EPS may potentially protect other cellular structures underlying it [12,16,56]. In view of the wide applications of *P. polymyxa* and its secondary metabolite EPS, in present paper, the aim of this review is to provide an overview of the main accomplishments in producing, isolating, and analyzing structure and functions of *P. polymyxa* EPS. Additionally, the synthesis mechanism of EPS is examined. The usage of *P. polymyxa* EPS in various fields is also highlighted.

2. The production of *P. polymyxa* EPS

Optimizing fermentation process is one of crucial means to enhance the growth and physiological metabolism of *P. polymyxa*, and subsequently elevate EPS yield. To enhance the productivity of *P. polymyxa* EPS fermentation, the researchers have examined the impacts of different factors on EPS synthesis. These factors include medium composition, temperature, pH, quantity of oxygen, and many others [22,45,47–54,57,58]. Table 1 presents a comprehensive list of *P. polymyxa*'s strains, culture conditions and EPS yields. The manufacture of EPS is not specific to any species, as every strain has the ability to produce unique EPS with varying biotechnological properties. The yield of EPS was presented significantly, ranging from 3.44 to 68 g/L (Table 1).

2.1. The optimization of fermentation medium

The medium composition greatly impacts the growth of microorganisms and the accumulation of the metabolites. The medium that supports *P. polymyxa* growth may not be optimal for metabolite accumulation. Various *P. polymyxa* strains have distinct nutrient requirements, including carbon and nitrogen sources, growth factors, and mineral ions. Therefore, optimizing the composition of the medium is necessary to increase the yield of EPS.

2.1.1. C sources

In general, the carbon source serves as the primary nutrient and energy source for cell growth. It has varying effects on microbial growth and EPS synthesis depending on the type of carbon source employed [64,65]. Numerous studies have confirmed that *P. polymyxa* can effectively utilize various carbon sources, including glucose, fructose and sucrose. However, glucose and sucrose are commonly employed as carbon sources in the medium (Table 1). Studies have indicated that sucrose is a more appropriate carbon source for producing *P. polymyxa* EPS than glucose [45,47–49,52,58,66]. Yang et al. [67] demonstrated that more EPS could be synthesized by *P. polymyxa* GA1 using sucrose compared to grape sugar, maltose, starch, glycerol and mannose as carbon sources. Additionally, C/N significantly impacted on the synthesis of EPS. Levansucrase, which exhibits potent hydrolyzing activity towards sucrose, is present in several strains of *P. polymyxa*, possibly contributing to the high EPS production using sucrose as a carbon source [68]. However, Rafigh and colleague [52] reported that the carbon source galactose produced the highest EPS synthesis, followed by fructose and starch. Increasing the amount of carbon source could simultaneously enhance EPS yield. The authors hypothesized that utilizing galactose as a carbon source would increase the metabolic flux of C source, thereby promoting greater EPS production. Similarly, the EPS production through the strain *P. polymyxa* SQR-21 was much lower with sucrose as a carbon source compared to galactose and fructose [53] Table 1. A higher concentration of galactose resulted in an increased in the EPS production. It is probable that an increased flux of metabolic C, with a limited availability for the growth of cells, could promote EPS synthesis [53]. Recently, Liyaskina et al. [60] achieved the maximum levan production of 68.0 g/L when using a molasses medium with a total

Table 1
EPS-producing strains in *P.polymyxa* and their fermentation conditions.

Strains	Fermentation conditions					Yield (g/L)	References
	Culture medium	Temperature (°C)	pH	Time (d)	Agitation speed (rpm)		
P. polymyxa SQR-21	galactose 48.5 g/L , Fe^{3+} 242 μM , Ca^{2+} 441 μM	30	6.5	4	170	3.44	[53]
P. polymyxa EJS-3	sucrose 188.2 g/L , Yeast extract 25.8 g/L , K_2HPO_4 5 g/L , $CaCl_2$ 0.34 g/L	24	8	2.5	180	35.26	[47]
P. polymyxa ATCC 21,830	glucose 100 g/L , Yeast extract 3 g/L	50	7	4	150	6.89	[52]
P. polymyxa JB115	MSM medium containing 10 % sucrose	30	/	3	180	10	[45]
P. polymyxa ZX-5	sucrose 200 g/L, $NaNO_3$ 3 g/L, $K_2HPO_4{\cdot}3H_2O$ 3 g/L, KH_2PO_4 1.0 g/L,Mg $SO_4{\cdot}7H_2O$ 0.50 g/L	20	6	0.875	150	34.55	[59]
P. polymyxa 2020	sucrose 200 g/L,yeast extract 7 g/L, K_2HPO_4 2.5 g/L, NH_4SO_4 1.6 g/L, $MgCl_2$ 0.4 g/L	30	7.2	4	250	68	[60]
P. polymyxa PYQ1	sucrose 50 g/L, tryptone 5 g/L, yeast powder 1 g/L, $Na_2HPO_4{\cdot}12H_2O$ 3 g/L	30	/	2	150	9.21	[61]
P. polymyxa 92	yeast extract 4 g/L, Na_2HPO_4 1.1 g/L, K_2HPO_4 2 0.5 g/L, Mg $SO_4{\cdot}7H_2O$ 0.2 g/L,$(NH_4)_2SO_4$ 0.1 g/L, $CaCO_3$ 0.2 g/L, sucrose, glucose, or fructose 30 g/L.	30	7.2	3–7	180	38.4	[1]
P.polymyxa CCM 1465	yeast extract 4 g/L, Na_2HPO_4 1.1 g/L, K_2HPO_4 2 0.5 g/L, Mg $SO_4{\cdot}7H_2O$ 0.2 g/L, $(NH_4)_2SO_4$ 0.1 g/L, $CaCO_3$ 0.2 g/L, sucrose, glucose, or fructose 30 g/L.	30	7.2–7.5	2	220	57 ± 2.8	[16]
P. polymyxa DSM 365	30 g/ L glucose, 0.05 g /L $CaCl_2 \times 2\ H_2O$, 5 g/ L tryptone, 1.33 g/ L MgSO4·$7H_2O$, 1.67 g/ L KH_2PO_4, 2 mL /L RPMI 1640 vitamins solution and 1 mL/ L trace elements solution (2.5 g/ L $FeSO_4$, 2.1 g/L $C_4H_4O_6Na_2 \cdot 2H_2O$, 1.8 g/ L $MnCl_2 \cdot 4H_2O$, 0.258 g /L H_3BO_3, 0.031 g/ L $CuSO_4 \cdot 5H_2O$, 0.023 g/ L $NaMoO_4{\cdot}2\ H_2O$, 0.075 g/ L $CoCl_2{\cdot}\ 7H_2O$, 0.021 g/ L $ZnCl_2$	30	6.8	1.2	160	/	[62]
P. polymyxa ATCC 824	sucrose 30 g/ L, yeast extract 30 g/ L, K_2HPO_4 5.72 g/ L, NH_2NO_4 5 g/ L, KH_2PO_4 1.9 g/ L and $MgSO_4$ 0.5 g/ L.	30	7.0	1.5	150	20.36	[22]
P. polymyxa HCT33–3	20 % molasses; KH_2PO_4 1.0 g/ L, $(NH_4)_2SO_4$ 1.0 g/L, $MgSO_4$ 0.5 g/ L.	37	7.0	48	/	35.8	[63]

sugar level of 200 g/L at 72 h. The maximum production of EPS was 53.78 g/L in the medium containing 150 g/L sucrose for 96 h. In contrast with sucrose medium, the highest EPS yield was achieved in the molasses medium within a shorter time frame (48–72 h).

Some studies have shown that high concentrations of sucrose can inhibit cell growth due to increased osmotic pressure within the culture medium [5,22,38]. To prevent the negative effect on the growth of cells induced by sucrose, Lakra [69] recommended maintaining a sucrose concentration between 20 and 30 g/L. Unfortunately, Daud et al. [22] optimized the production of EPS in *P. polymyxa* ATCC 824, and indicated that sucrose concentration at 30 g/L was effective in promoting the production of cell mass while also moderating EPS synthesis. Grinev et al. [1] found that the production of EPS increased up to 15 g/L with an increase in the initial sucrose concentration from 1.0 to 3.0 %. With 3.0 % sucrose, a gradual increased in fermentation time from 24 to 72 h, also improved the yield of EPS from approximately 7.0 to 15 g/L. A similar phenomenon on the synthesis of EPS in *P. polymyxa* EJS-3 was described by the group of Liu et al. [47]. However, compared with *P. polymyxa* EJS-3, the greater yields of EPS under the same substrate content in the medium, could be obtained by *P. polymyxa* 92 [1]. Using 10 % sucrose, *P. polymyxa* 92 generated the greatest amount of EPS (38.4 g/L), while lower yield of EPS (22.82 g/L) with 16 % sucrose was observed in *P. polymyxa* EJS-3 [47].

2.1.2. N sources

Nitrogen is an essential component of the cells, including amino acids, proteins, nucleic acids, and other nitrogen-containing metabolites. It serves as a critical nutrient for cell growth, and plays a vital role in EPS synthesis. Appropriate nitrogen sources and quantities contribute to the synthesis of EPS [22].

Compared to organic nitrogen sources, using inorganic nitrogen sources leads to lower yields of cell biomass and EPS. The phenomenon has been observed by some scholars [53,65,70,] Table 1. Liu et al. [50] initially identified the factors influencing EPS yield through single factor experimental method, and subsequently optimized the medium's carbon source, nitrogen source and inorganic salt components using response surface methodology (RSM). The authors found that the best medium combination was sucrose of 188.2 g/mL, yeast extract of 25.8 g/mL, and calcium chloride of 0.34 g/mL, producing a EPS yield of 35.26 g/L under the optimal medium conditions [50]. Kaziuniene et al. [71] optimized the medium components of *Paenibacillus* sp. MVY-024, finding that yeast extract of 10 g/L was the optimal nitrogen source. Raza et al. [53] examined the optimal nitrogen source for the growth of cells and the synthesis of EPS, by substituting separate nitrogen source at a content of 10 g/L using peptone and yeast extract in the basal medium. Yeast extract produced the greatest cell biomass production (1.89 g/L) and EPS (7.98 g/L). The findings of this study suggested that N source had an impact on the synthesis of EPS synthesized by *P. polymyxa* SQR-21, which was in agreement with Liu et al.'s Research [47]. The increase in EPS production by *P. polymyxa* strains with organic nitrogen source including yeast extract and peptone was because of their high content of protein, amino acids, and vitamins [22,46].

However, Lee et al. [72] work indicated that a higher of EPS production was achieved with nitrogen salts ($NaNO_3$, KNO_3, $NH4NO_3$, Ca $(NO_3)_2$, NH_2CONH_2) as the nitrogen source, while EPS remained at a relatively low concentration in the medium containing ammonium sulfate. With regard to this, it should be noted that in ammmonium sulfate medium, the pH of culture decreased from the initial value of 7.0 to 4.5 within 24 h, whereas it remained within the range of 6.5–7.0 in the nitrate salts medium. It is probable that the culture pH has a greater impact on EPS production than the nitrogen source [72]. Jian et al. [73] optimized the composition of the fermentation medium for *Bacillus polymyxides* PS04, and finally determined that inorganic nitrogen NH_4NO_3 was the optimal nitrogen source with a concentration of 1.4 g/ L.

2.1.3. Mineral sources

During cellular growth, the minerals impact the variety and output of EPS, proteins and enzymes that are released by microbial cells [74]. Appropriate minerals are beneficial to EPS synthesis. The presence of

phosphate salt in the culture medium affects EPS production by the cells. Phosphate, an inorganic agent also known as a salt of phosphoric acid, is typically employed in the medium to improve both cell growth and the production of metabolites [22,75]. It has been reported that incorporating phosphate into the medium improves cell growth and EPS production in the strain that produces levan [22,60]. Asghar et al. [76] found that the addition of phosphate to the medium produced advantageous results for EPS production. Raza et al. [53] examined the impact of several metal ions on EPS synthesis, and indicated that four metal ions of them (Ca^{2+}, Ni^{2+}, Cu^{2+}, Fe^{3+}) had a significant impact on the synthesis of EPS produced by *P. polymyxa* SQR-21. The addition of the minerals optimized the growth medium, resulting in a 12.5-fold increase compared to the normal medium. In contrast, Liu et al. [48,49] found only 1.55-fold increased using the same normal culture medium. Recently, Li et al. [77] also found that microbial cell EPS production increased after adding the mineral powder to the medium, and providing moderate stimulation.

2.2. The optimization of fermentation conditions

Additionally, besides the composition and concentration of the culture medium, fermentation conditions including fermentation time, fermentation temperature, initial pH, ventilation and inoculation amount, are also the principal factors that impact the production of EPS. EPS yield can be increased when the conditions are optimally controlled.

2.2.1. Temperature

Temperature affects not only microbial growth, but also enzyme activity and various biochemical reactions occurred in microorganisms. Within a specific range, optimal temperature stimulates the microbial growth and the production of metabolically active substances. As indicated in Table 1, a culture temperature of 30 °C was predominantly utilized in the majority of the research. This is because the ideal temperature for EPS production is typically lower than the optimal temperature for bacterial growth. Low temperature slows bacterial growth, allowing for increased precursor molecule use in the synthesis of EPS [59]. The investigation on the impact of the temperature on the production of EPS produced by *P. polymyxa* SQR-21 showed that the highest OD_{600} and EPS synthesis occurred at 30 °C, followed by a decline in both [53]. The results contradicted Liu et al.'s findings [47], which suggested that *P. polymyxa* EJS-3 required a temperature of 24 °C for optimal EPS synthesis. *P. polymyxa* EJS-3 exhibited a preference for a lower temperature when producing EPS compared to the other stains. Rafigh et al. [52] found that curdlan production demonstrated a rapid increase as the temperature fluctuated from 30 to 40 °C, followed by a slight increase during fermentation from 40 to 50 °C. The optimal temperature for curdlan production unexpectedly reached 50 °C. The findings suggested a temperature-dependent effect on curdlan product yield. The synthesis of curdlan was not suitable under a higher temperature (> 50 °C).

2.2.2. pH

The initial pH of a culture medium has a significant impact on cell membrane, morphology, structure, nutrient uptake, and EPS synthesis [65]. So far, numerous studies have been conducted on the optimal pHs for producing EPS in *P. polymyxa* EPS. The optimal pH range for producing EPS from *P. polymyxa* is between 6.0 and 8.0 [52–54,57] Table 1. Yang [78] found the ideal pH for the fermentation of *P. polymyxa* C-12 was 6.0. Similarly, Tian et al. [79] obtained *P. polymyxa* PS04 from soil samples. Fermentation under the conditions of pH 6.0, 37 °C for 48 h using Czapek's medium caused EPS yield of up to 13.3 g/L. Rafigh et al. [52] reported that curdlan gum and biomass yields increased by around 39.3 % and 4.8 %, respectively, upon increasing the initial fermentation pH varied from 5.5 to 7.0. Afterwards, EPS production decreased at higher values (pH 8.5). The EPS produced was consistent with a previous study which found that the optimal pH for the synthesis of EPS produced by *P. polymyxa* KCTC 8648P was 7.0 [53,72]. However, a limited number of reports have demonstrated the highest EPS production from *P. polymyxa* strains at a slightly alkaline pH [47–50]. Unlike *P. polymyxa* strains found in soil, *P. polymyxa* EJS-3 exhibited optimal EPS synthesis at a slightly alkaline pH. The authors indicated that the growth conditions of *P. polymyxa* EJS-3 may be influenced by its growth within the living tissues of plants [47–51]. Jian et al. [73] optimized the fermentation process for EPS from *P. polymyxa* PS04, and finally concluded that an initial pH of 8.22 could produce EPS up to 60.04 g/L.

2.2.3. O_2

The impact of aeration on the viability of *P. polymyxa* that produced EPS has been investigated [52,54]. Under anaerobic conditions, the cell population does not grow or produce EPS. Intensive aeration during fermentation results in a considerable rise in EPS yield. Kaziuniene et al. [71] reported that air flow significantly impacted on the yield of biomass and the formation of *Paenibacillus* sp. MVY-024 spore, with an optimal airflow rate of 0.4 vvm. Previous studies also suggested that agitation might benefit microbial cell growth and performance by enhancing mass transfer characteristics, including those relating to the substrates, products and oxygen [46]. The study conducted by Rafigh et al. [52] revealed that agitation speed improved from 120 to 150 rpm, resulting in significant enhancements in biomass and curdlan yields in *P. polymyxa* ATCC 21830. The highest curdlan gum and biomass yields were achieved at the agitation speed of 150 rpm. The yields of both curdlan gum and biomass were limited at the agitation of 120 rpm due to oxygen transfer restrictions. However, at the agitation of 180 rpm, the amounts of curdlan gum and biomass decreased, possibly because of bacterial fragmentation caused by various shearing mechanisms. Therefore, EPS production is believed to be favored in an oxygen-sufficient environment.

2.3. Effective strategies to improve EPS yield

Fermentation techniques can modulate EPS yield using methods to optimize cultivation conditions [22,48,55]. RSM is currently one of the primary means for optimizing the medium compositions to produce *P. polymyxa* EPS. Liu et al. [47] utilized RSM to enhance the cultivation parameters for producing *P. polymyxa* EPS EJS-3, and determined that sucrose and yeast extract served as appropriate carbon and nitrogen sources, respectively, for the synthesis of EPS. Rafigh et al. [52] developed a fermentation medium to cultivate high cell mass of *P. polymxya* ATCC 842 while simultaneously producing EPS. The primary components in the medium, namely sucrose, yeast extract, and K_2HPO_4, were chosen as parameters and optimized using the Box-Behnken design. Batch fermentation culture was optimized using a Box-Behnken experimental design. Optimal culture conditions were at a temperature of 50 °C, pH of 7.0, fermentation time of 96 h, glucose concentration of 100 g/L, yeast extract of 3.0 g/L, and agitation speed of 150 rpm. The curdlan production amount was 6.89 g/L under optimized conditions. Model cultural conditions for curdlan production was accomplished using both artificial neural network (ANN) and RSM. ANN and RSM models predicted the maximum curdlan yield to be 6.85 and 6.68 g/L, respectively. The ANN model demonstrated higher accuracy in prediction compared to RSM model, as evidenced by the results.

Since culture media accounts for roughly 30 % of the overall fermentation cost, it is crucial to seek out more cost-effective carbon sources, for example, agricultural or industrial byproducts, to reduce production expenses [46,80]. Wang et al. [54] first produced and identified EPS derived from squid pen powder (SPP) culture supernatant of *P. polymxya* TKU023. For the medium with a 1.5 % content, *P. polymxya* TKU023 produced EPS yields of 4.55 g/L. However, according to other sources, EPS yields could go up to 35.26 g/L when the medium contained high concentrations of sucrose [47,50]. This motivated the researchers to enhance EPS yield by increasing SPP concentration. The findings indicated that *P. polymxya* TKU023 produced 41.25 g/L of EPS with 10 % SPP in the medium. Ağçeli & Yücel [63]

carried out levan production using *P. polymxya* HCT33–3 in the molasses medium. The maximum levan yield (35.8 g/L) was obtained when the ratio of molasses in the medium was 20 % (*v*/v). However, levan yield decreased when the molasses content in the medium exceeded 20 %. The results suggested that the use of waste molasses as a carbon source would be highly advantageous for levan production. Cheng et al. [81] established *Paenibacillus* sp. FP01 as a new strain for cost-effective levan production. *Paenibacillus* sp. FP01 was cultivated using a relatively inexpensive inorganic nitrogen source ($NaNO_3$) instead of more expensive employed organic nitrogen sources (yeast extract, peptone, etc.). Maximum levan production (89.5 g/L) was achieved in the optimal medium following 62-h incubation at 30 °C with 180 g/L sucrose. This indicated that *Paenibacillus* sp. FP01 could be a viable option for large-scale production of levan in industry.

Li et al. [30] used low-intensity ultrasound to improve the fermentation process, and found a 24 % increase in EPS yield with the optimal ultrasonic treatment (90 W, 45 s in 9 h). The authors revealed that low-intensity ultrasound increased the production of membrane transporters responsible for transporting sucrose and polysaccharides. As a result of these modifications, carbon source consumption was expedited, and EPS transport was facilitated to the exterior of bacterial cells, resulting in an improvement of EPS production.

3. The extraction and purification of *P. polymyxa* EPS

Currently, EPS isolation and extraction follows a sequential process consisting of centrifugation, organic solvent precipitation, protein removal, dialysis, freeze-drying and additional steps as shown in Fig. 1. The crude EPS obtained with low purity commonly comprises impurities, including pigments, proteins, and various small molecules. The presence of the impurities not only lowers EPS purity, but also has an impact on its structural and biological activity analysis. Therefore, obtaining EPS samples with high purity through column chromatography technology is highly necessary [46].

3.1. Extraction

The removal of cells from fermentation broth of *P. polymyxa* is necessary in order to separate EPS. The most frequently used method for cell removal is centrifugation. However, the high viscosity of fermentation broth of *P. polymyxa* requires heat treatment before cooling and diluting the broth by centrifugation for the optimal EPS extraction [82]. Ethanol precipitation is the most frequently employed technique for extracting EPS [64,83]. Huang et al. [84] utilized gradient ethanol precipitation to isolate EPS fractions from fermentation broth. The findings indicated that the technique is both simple and effective for the initial separation of EPS. It is observed that the extraction rate of EPS from fermentation broth is significantly impacted by the concentration of ethanol used. Jia et al. [85] isolated EPS from *P. polymyxa* HY96–2 using one-volume ethanol precipitation. Jian et al. [74] used 95 % ethanol at 3 times the volume to precipitate EPS of *P. polymyxa* PS04. The use of organic solvents can enhance the precipitation amount and promote the precipitation rate at lower temperatures. Therefore, some researchers prefer to pretreat fermentation broth with organic solvents before low-temperature EPS precipitation. Xu et al. [86] found that the highest extraction rate of EPS occurred at 4 °C.

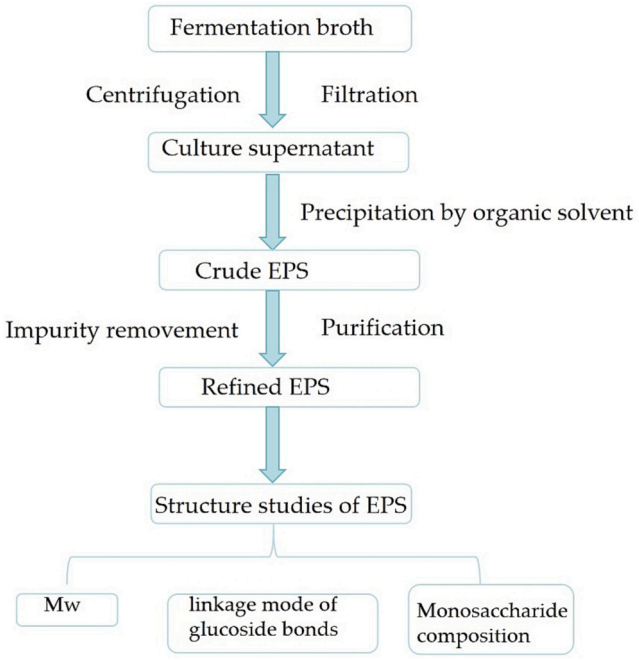

Fig. 1. The isolation and purification procedures of EPS from *P. polymyxa* fermentation broth.

3.2. Removing the proteins

Diverse impurities, particularly proteins and pigments, would co-precipitate with EPS during ethanol precipitation, which complicates the subsequent purification process. At present, there are three techniques to remove protein impurities [2,87,88]: (1) Strong acid treatment, such as TCA, can induce a positive charge on proteins, leading to combinatory reaction with negatively charged ions to create insoluble salts [88]; (2) Sevage method, the denaturation of proteins is achieved by using organic solvents for separation; (3) Enzymatic hydrolysis, strong acid hydrolysis may yield higher protein removal efficiency compared to enzymatic hydrolysis, which can lead to the decomposition of EPS and ultimately decrease EPS extraction rate. Enzymatic hydrolysis can take place under mild conditions, resulting in a minimal loss rate of EPS. However, it can not fully eliminate proteins from EPS samples. Sevage method, despite requiring repeated operations and being less efficient, has little impact on the structure of EPS and is considered more gentle and reliable protein removal method. Therefore, it is commonly used to remove proteins from the crude *P. polymyxa* EPS.

The methods mentioned above have been utilized to remove the proteins from crude EPS extracts. However, it is likely that these methods cause partial hydrolysis of EPS which leads to varying bioactivities [48,89]. Therefore, it is imperative to develop innovative techniques for the removal of proteins from crude EPS samples. Liu et al. [48] created a simple method to concurrently deproteinize crude levan samples obtained from *P. polymyxa* EJS-3. The authors demonstrated that macroporous resin S-8 could effectively remove the proteins from crude EPS samples without compromising the integrity and functionalities of EPS.

3.3. Purification

The deproteinized EPS extracts undergo sequential purification via column chromatography, which includes ion-exchange chromatography, gel filtration chromatography and affinity chromatography [16,45–47,53,54]. In numerous instances, EPS produced by *P. polymyxa* is either acidic or neutral. The primary purification methods utilized are ion-exchange chromatography and gel filtration chromatography. Elution is performed with a suitable buffer, followed by sample collecting, concentrating, dialyzing, and freeze-drying (Fig. 1). The samples are then analyzed employing the phenol-sulfuric acid method, with glucose as the standard [46]. Zhang et al. [90] purified the crude EPS using DEAE-cellulose column, yielding components B and D. HPLC was used to detect their purity, and GC analysis was conducted on component B's monosaccharide composition. Asker et al. [83] identified three EPS constitutes of the crude EPS of *P. polymyxa* through DEAE-cellulose and Sephadex G-150 column. The results showed that three types of EPS

were acquired. Further studies indicated that the variations in monosaccharide proportions primarily accounted for the differences among these EPS. Liu et al. [47,51] found that EPS1, characterized as an acidic polysaccharide, was further purified EPS1 using DEAE-52 and Sephadex G-100. Similarly, the fine EPS from *P. polymyxa* PYQ1 was also obtained through DEAE-52 and Sephadex G-100 [61].

3.4. Structure analysis

The culture conditions can enhance EPS production instead of cell proliferation. In harsh environments such as enhanced osmotic pressure of the medium, water stress, the cells produce significant yields of EPS to create a physicochemical barrier for protecting against environmental changes. The analysis of EPS structure primarily involves determining the monosaccharide composition, M_W and glycosidic bonds linkage mode (Fig. 1). EPS possesses a complex structure, and many researchers often employ diverse methods such as electrophoresis, acid hydrolysis, methylation analysis, Smith degradation, infrared spectroscopy (IR), ultraviolet and visible spectrophotometry (UV), gas chromatography (GC), high performance liquid chromatography (HPLC), mass spectrometry (MS), high performance gel permeation chromatography (HPGPC), and nuclear magnetic resonance (NMR), to analyze it [45–47,53]. The composition and typical structures of EPS are outlined in Table 2 and Fig. 2, respectively.

3.4.1. M_W distribution of EPS

Various techniques including HPLC, viscometry, and HPGPC, have been utilized to calculate the average M_W of the polymer. Among them, HPGPC is commonly used to determine M_W of EPS. The average M_W of EPS from *P. polymyxa* varies greatly, ranging from several hundreds to thousands of kDa, which depends on the factors such as carbon source concentration, the strain, fermentation pH, C/N ratio, fermentation temperature, and fermentation techniques [46] Table 2. Sucrose-containing medium could stimulate the output of high M_W EPS of *P. polymyxa* [47]. *P. polymyxa* EJS-3 generated high M_W EPS on a medium containing sucrose. The estimated high M_Ws of EPS-1 and EPS-2, extracted from *P. polymyxa* EJS-3, were 1220 and 869 kDa, respectively [50]. In addition, Yegorenkova et al. [93] reported that EPS 1465 comprised both neutral and acidic fractions. It was a diverse polysaccharide, consisting of a complex of macromolecules with an M_w between 70 and 2000 kDa. When cells are cultured with glucose, the acidic constituent dominates, and is corrected with elevated viscosity in EPS solutions [93]. Similarly, EPS 88A comprised an acidic high-viscosity EPS (with an M_w of 1–10 mDa) and a neutral low-viscosity EPS (with an M_w of 100–300 kDa). However, the average M_w of curdlan synthesized employing glucose as a carbon source by *P. polymyxa* ATCC 21830, was 170 kDa, which was lower than that of other EPS synthesized by *P. polymyxa* JB115 (with Mw of 576 kDa) and *P. polymyxa* SQR-21 (with Mw of 896 kDa) [45,52,53] Table 2. Besides, *P. polymyxa* 92 also produced a significant quantity of EPS, with the main fraction having a M_W distribution ranging from 110 to 229 kDa.

3.4.2. Monosaccharide composition

The analysis of monosaccharide composition typically requires the cleavage of glycosidic linkages via acid hydrolysis, followed by derivatization, and detection and quantification through GC. Many diverse EPSs have been extracted from *P. polymyxa* (Table 2, Fig. 2). The composition of monosaccharides typically consists of glucose, mannose, galactose and glucuronic acid in varying mole ratios (Table 2, Fig. 2). A diverse array of EPS is produced by *P. polymyxa* (Fig. 2). The EPS production is dependent on the specific strain of *P. polymyxa*, cultural conditions and the composition of the medium [53]. EPSs from *P. polymyxa* EJS-3 and its fractions were investigated by Liu's group. The compositions of EPS derived from *P. polymyxa* EJS-3 were mannose, fructose and glucose [47]. As a comparable EPS production study on *P. polymyxa* EJS-3, the primary components of EPS1 from *P. polymyxa* EJS-3 were mannose (20.40 %) and glucose (66.20 %). The IR spectrum of EPS1 revealed the existence of characteristic absorption peaks for EPS, which contained β-type pyranose [50]. EPS derived from *P. polymyxa* SQR-21 consisted of mannose, glucose, fructose and glucuronic acid [53]. Jian et al. [94] conducted filtration chromatography on *P. polymyxa* PS04's EPS using S-200 HR. They found that EPS was made up of fructose and glucose in a ratio of 7:1. According to NMR analysis, it revealed the presence of furan nucleus and the chain was connected by β-2,6 and β-1,2 linkage. Rütering [95] introduced "Paenan", a heteropolysaccharide synthesized by *P. polymyxa*, which was produced through fermentation (Fig. 2). Paenan was composited of glucose, galactose, mannose and glucuronic acid with a ratio of 3.5:2:1:0.1. Recently, Wang et al. [61] found *P. polymyxa* PYQ1 could produce a homogeneous glucomannan-type EPS. The structural analysis revealed that the monosaccharides within the EPS were pyranoses linked by β-glycosidic bonds. The EPS contained mannose, ribose, glucuronic acid, glucose, galactose and xylose, with a molar ratio of 20.40: 1.58: 2.67: 66.20: 6.10: 3.05. Notably, mannose and glucose were the main type of monosaccharides [61].

Generally, the structure of EPS generated by *P. polymyxa* varies greatly and depends on the employed strain of *P. polymyxa* and the medium's composition [50,53]. Numerous studies have indicated that EPS monosaccharide composition ratio is impacted by carbon source. Sathishkumar et al. [96] found that sucrose, when used as a carbon source, primarily produced fructan type EPS. However, Jung et al. [45] obtained a specific type of EPS derived from *P. polymyxa* JB115, which consisted of β-(1 → 3) and β-(1 → 6)-linked glucan, using a sucrose-containing medium. EPS derived from *P. polymyxa* EJS-3 in a culture medium composed of sucrose and yeast extract, was identified as levan-type EPS (Fig. 2). The findings showed the similarities to EPS produced by *P. polymyxa* NRRL B-18475, despite differences in M_W and branching degrees [97]. Modifying fermentation conditions can enhance the length and M_W of EPS in some studies, however, the monomer composition of EPS remains unaffected [98]. Rath et al. [99] demonstrated that cultivating Acetobacter glucose in a low peptone medium resulted in the production of EPS with a significant ribose content. Furthermore, the structure and composition of EPS can be influenced by precursor

Table 2
Structural Characterization of EPS.

Strains	Molecular weight(Da)	Monosaccharide composition	Sugar chain	References
P.polymyxa SQR-21	8.96×10^5	Mannose: glucose: fructose: glucuronic acid =2.74: 2.54: 2.23: 0.45	/	[53]
P.polymyxa EJS-3	EPS1: 1.22×10^6, EPS2: 8.69×10^5	Mannose: fructose: glucose EPS1: 2.59: 29.83: 1 EPS2: 4.23: 36.59: 1	The main chain was composed of fructose groups linked by β-(2 → 6).	[49]
P.polymyxa ATCC21830	1.7×10^5	Glucose	β-(1 → 3) glucoside bond constituted linear glucan.	[52]
P.polymyxa JB115	$>1.0 \times 10^5$	Glucose	Dextran-linked by β-(1 → 3)- and β-(1 → 6)	[45]
P.polymyxa HY96–2	1.22×10^6	Rhamnose: xylose: mannose: galactose: glucose =2.52: 0.25: 1: 1.61: 2.24	/	[91]
P.polymyxa PS04	4.0×10^6	Fructose: glucose =7: 1	Fructose residues existed in a furan ring configuration, and were connected by β-(2 → 6) and β-(1 → 2) bonds.	[92]

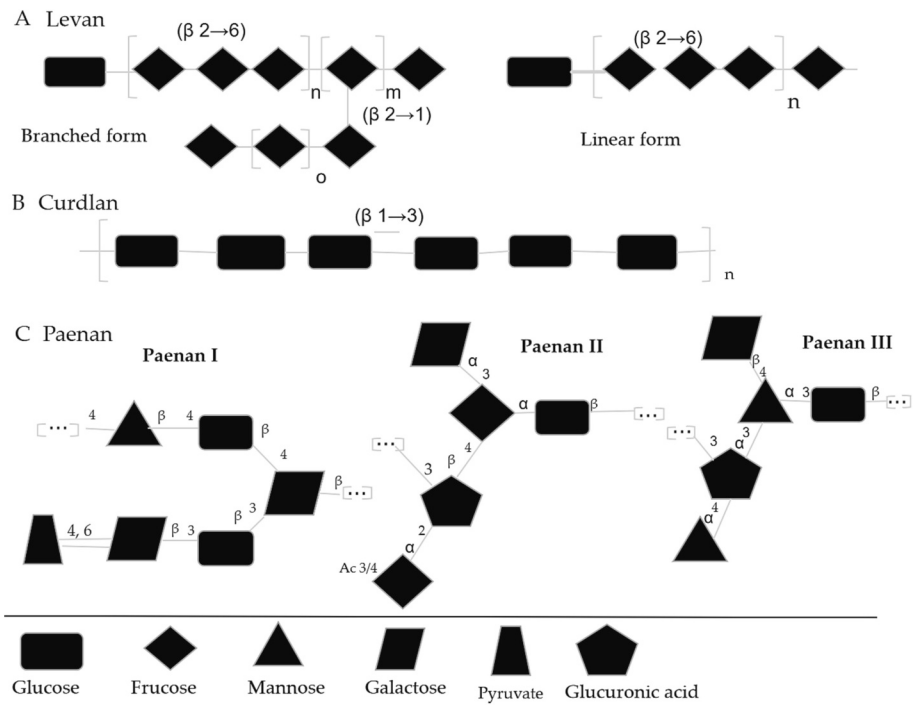

Fig. 2. Some typical structures of EPS from *P.polymyxa.*

molecules and mineral salts [100].

4. The bioactivity of EPS of *P. polymyxa*

Recent studies have demonstrated that EPS serve both structural and energy substances for microbial cells, while also functioning as multi-functional bioactive substances. In addition, functional EPS possesses anti-tumor, anti-inflammatory, and antioxidant properties, rendering them useful in various industries (Table 3).

4.1. Biological activity

4.1.1. Immune regulation

EPS exhibits significant biological activity, enhancing lymphocyte and macrophage numbers, boosting cytokine release, regulating the body's immune system, and strengthening overall immunity [64]. Hwang et al. [106] found that supplementing piglet feed with EPS extracted from a strain of *P. polymyxa* resulted in elevated IL-10 levels and decreased IFN-γ and TNF-α levels. These findings suggested that EPS might have immunomodulatory effects in piglets. Liu et al. [49] demonstrated that administering EPS to D-galactose-induced aging mice could boost their spleen and liver index, thereby improving their immune function. *P. polymyxa* 1465 EPS boosted the phagocytic activity of macrophages, increased metabolism in human and animal leukocytes, and had a minor impact on the production of proinflammatory cytokines (IL-1, TNF-α) in human mononuclear cells [16]. Recently, Chen et al. [107] reported that EPS substantially improved the immunity in three aspects: cellular immunity, humoral immunity, and innate immunity.

NO plays a crucial role in various biological functions, such as transmitting neurons, regulating blood vessel toxicity, modulating immunity, and exerting cytotoxicity against tumors. Nonetheless, excessive NO levels in the cells show dual effects. It may cause DNA damage and trigger proinflammatory responses [16]. In an investigation on the activation of EPS in murine splenocytes carried out by Yegorenkova et al. [16], the glucan concentration-dependently induced NO synthesis, which was crucial for the macroorganism's non-specific defense against

Table 3
The activity and potential applications of EPS from *P. polymyxa.*

Strains	Origin	Activity	Applications	References
P.polymyxa B_1 and B_2	Wheat rhizosphere	Biofilm formation	Biocontrol agent	[101]
P.polymyxa B_5 and B_6	Soil around peanut roots	Biofilm formation	Biocontrol agent	[102]
P.polymyxa A26	Barley rhizosphere	Biofilm formation	Biocontrol agent	[56]
P.polymyxa WLY78		Biofilm formation	Biocontrol agent	[55]
P.polymyxa HY 96–2		Biofilm formation	Biocontrol agent	[103]
P.polymyxa 1465	Soil	Biofilm formation	Biocontrol agent	[93]
P.polymyxa CCM 1465		Immunomodulatory	Food and medical industries	[16]
		Biofilm formation	Biocontrol agent	[12]
P.polymyxa 92	Wheat roots	Biofilm formation	Biocontrol agent	[104]
		Production of levan	bioflocculant	[1]
P.polymyxa JB115	Soil	Production of β-glucan	Animal feed additive	[45]
P.polymyxa EJS-3	Root tissue of *Stemona japonia* Miquel	Production of levan, antioxidant, anti-tumor	Antioxidant in foods, anti-tumor agent for animals	[47,51]
P.polymyxa SQR-21		Antioxidant	Food antioxidant, bioflocculant	[53]
P.polymyxa ZCY-79	Activated sludge	The removal of arsenic	Bioflocculant	[21]
P.polymyxa 96–2		Antioxidant	Pesticide adjuvant	[85]
P.polymyxa PYQ1		Cytoprotective activity	Skin-care agent in cosmetic industry	[61]
		Anti-inflammatory activity	Functional food for gastrointestinal protection	[105]

intracellular infections. Additionally, the glucan stimulated the synthesis of interleukin-6 (IL-6) and inducible NO synthase (iNOS), an enzyme that could potentially contribute to NO production [108].

4.1.2. Anti-oxidant activity

Reactive oxygen species (ROS), which are generated during normal cell metabolism, serve a dual function. Low levels ROS play a crucial role in both growth factor response and immune response. However, excessive levels of ROS can cause damage to the DNA, lipids and proteins inside the cells, subsequently increasing the risk of carcinogenesis. According to the existing research, several species of *P. polymyxa* EPS demonstrate strong antioxidant activity [53,109] Table 3. Raza et al. [53] reported that *P. polymyxa* SQR-21 was able to produce a specific type of EPS with yeast extract and galactose serving as nitrogen source and carbon source, respectively. The EPS produced demonstrated effective ROS scavenging capabilities, as well as moderate lipid peroxidation inhibition and reducing activities. The activities of the β-glucan derived from *P. polymyxa* JB115, could boost antioxidant properties by increasing NO production in macrophages via the signaling pathways of MAPK and NFkB [109]. As further research conducted by Hong & Jung [110], *P. polymyxa* JB115 generated β-glucans with high M_W (> 100 kDa), which exhibited hydroxyl radical- and ROS scavenging properties. EPS from *P. polymyxa* TKU023 also exhibited antioxidant activity. They observed that EPS yield reached a maximum of 4.55 g/L on the fifth day of incubation with the highest total phenolic concentrations and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Furthermore, the crude EPS produced by *P. polymyxa* HY96–2 showed a UV protection for its cells, possibly attributed to the antioxidant properties of EPS [111].

Many levan-type EPSs produced by the strains of *P. polymyxa* show strong anti-oxidant activity (Table 3). In particular, the levan-type EPS produced by *P. polymyxa* EJS-3 demonstrated effective scavenging ROS and hydroxyl radicals [47]. In vitro antioxidant assays showed that both the raw EPS and its purified fractions, EPS-1 and EPS-2, exhibited moderate scavenging activity against DPPH radicals, hydrogen peroxide, as well as the inhibition of lipid peroxidation. Additionally, they exhibited potent ferrous ion chelating activity [16,47]. A levan-type EPS produced by *P. polymyxa* ATCC 21830 underwent acetylation, phosphorylation, and benzylation, resulting in the production of acetylated, phosphorylated, and benzylated levan derivatives. These compounds showed greater ability to reduce, as well as effectively scavenge ROS and hydroxyl radicals [5,51].

4.1.3. Anti-tumor activity

Tumor is a prevalent disease, particularly the malignant type, which poses a significant threat to human health. Tumor cells can trigger lymphocyte apoptosis, resulting in organ atrophy and reduced host immunity. Consequently, many researchers are now focused on discovering substances with anti-tumor activity to combat this threat. EPS produced by *P. polymyxa* can significantly hinder the advancement of tumor cells, whilst its glucan can also terminate tumor cells by enhancing the immune response of macrophages (Table 3). EPS-1, one levan-type polysaccharide derived from *P. polymyxa*, underwent successful modifications through the methods of acetylation, phosphorylation and benzoylation techniques [51]. The results demonstrated that the anti-tumor activity of EPS-1 was enhanced because of chemical modifications to EPS-1 molecules. The authors suggested EPS-1 derivatives could be explored as potential anti-tumor agents. Chen et al. [107] also found that EPS could regulate blood sugar concentration, and inhibit the proliferation of tumor cells.

5. Biosynthesis of EPS of *P. polymyxa*

Generally, EPS production in bacterial strains involves four mechanisms. These include Wzx/Wzy-dependent pathway, ABC transporter-dependent pathway, synthase-dependent pathway, and extracellular biosynthesis by sucrase protein [112,113]. It is important to note that EPS biosynthesis pathway is intricate, and these four mechanisms play a crucial role. Homopolysaccharides are typically synthesized through the synthase-dependent pathway and extracellular biosynthesis pathways, whereas Wzx/Wzy-dependent and ABC transporter-dependent pathways are responsible for the synthesis of heteropolysaccharides [112]. At present, the studies on EPS biosynthesis in *P. polymyxa* remain limited, with a focus on Wzx/Wzy-dependent pathway.

In Wzx/Wzy-dependent pathway, a glycosyl-phosphotransferase initiates the transfer of the initial sugar residue to an undecaprenyl-phosphate lipid anchor located on the inner cellular membrane. Afterwards, highly specific glycosyltransferases lengthen the newly formed oligomer via activated sugar nucleotides's binding energy. The complete repeating unit is then moved to the periplasmic space using a Wzx flippase, which belongs to the Wzy protein family, and then polymerized using a Wzy protein before being released into the cellular environment [62,114]. The length of biopolymer chain is regulated by the Wzz chain length regulator, which interacts with the Wzy polymerase, ultimately resulting in a unique M_w [62,115,116].

Despite numerous attempts over the past five decades, the structural elucidation of EPS in *P. polymyxa* has ultimately proven unsuccessful attributed to the exceedingly complex structure [46,70]. A wide range of structurally related EPS have been synthesized by diverse *P. polymyxa* strains. The gene cluster accountable for the synthesis of its heteroexopolysaccharide in *P. polymyxa* DSM 365 was identified and preliminarily annotated using on the reported genome [70,117,118]. The gene cluster underlying the production of EPS comprised 28 coding sequences that encode eleven glycosyltransferases, two polymerases and flippases, and additional genes responsible for regulating chain length, export, and synthesizing nucleotide precursor. This intricate system reflects the complexity of the produced EPS [70,117]. Usually, Wzx flippases and glycosyltransferases typically show strong preference for their natural substrate, accepting only slight variations in the repeating units's composition [70,119]. As determined by previous bioinformatic analysis, *P. polymyxa* DSM 365 had the capability of producing various heteroexopolysaccharides through Wzx/Wzy pathway [117]. The authors confirmed that a 32.8 kb pep cluster, consisting of 28 genes, was responsible for the synthesis of paenan, a glucose-derived EPS from *P. polymyxa*. The 32.8 kb pep cluster contained 28 genes that facilitate the synthesis of paenan. There is a limited understanding of EPS clusters' role in biofilm formation for *P. polymyxa*. *P. polymyxa* WLY78, a bacterium that fixes N, has the ability to form biofilm. He et al. [55] sequenced the genome of *P. polymyxa* WLY78 and identified as a sizable genetic cluster responsible for EPS production that closely resembles that found in *P. polymyxa* DSM 365 [120]. The authors also identified two putative gene clusters that were accountable for the synthesis of EPS, referred to as the pep-1 cluster and pep-2 cluster. Specifically, the pep-1 cluster comprised of 12 potential genes, labeled as pepO-lytR, while the pep-2 cluster contained 17 potential genes, labeled as pepA-pepN. 17 potential genes in the pep-2 cluster were arranged as an operon using gene organization and RT-PCR. Specifically, only the pep-2 cluster was accountable for both EPS synthesis and biofilm formation in *P. polymyxa* WLY78, where biofilm provided a microaerobic habitat for nitrogen fixation.

Genome editing technology is one of popular technologies for studying the biosynthesis and structure of *P. polymyxa* EPS. Using a Cas9-based system, the biosynthetic gene clusters responsible for paenan production, are purposefully eliminated. Deleting the pep cluster resulted in the absence of paenan biosynthesis, leading the mutants to exhibit slimy mutants when cultivated on EPS-inducing plates utilizing glucose as a carbon source [121]. Schilling et al. [122] also utilized CRISPR-Cas9 genome editing technology to improve the production 2,3-butanediol in *P. polymyxa* DSM 365. The authors eliminated the formation of EPS through co-knocking out of the *clu1* gene cluster accountable for the production of heteroexopolysaccharide and the levansucrase SacB. It would result in none of EPS formation and a

direct impact on cell growth. The gene that encodes levansucrase, is the primary enzyme responsible for EPS biosynthesis in *P. polymyxa*. Okonkwo et al. [123] found that the deactivation of the levansucrase gene in *P. polymyxa* DSM 365 resulted in reduced EPS accumulation. The levansucrase null mutant showed 8.7- and 2.6-fold decreases in EPS formation on sucrose and glucose, respectively, compared to the wild type. In order to clarify the structure of EPS, Schilling et al. [70] applied a CRISPR-Case9 knock-out approach to delete each individual glycosyltransferases and Wzy polymerases within the associated gene cluster. The results indicated that the absence of paenan II was linked to the knockout of the glycosyltransferases PepQ, PepT, PepU and PepV among with the Wzy polymerase PepG, highlighting the significance of these enzymes in the formation of the repeating unit.

6. The applications of EPS of *P. polymyxa*

6.1. Ecological agriculture

6.1.1. Biological control

Numerous studies have demonstrated that *P. polymyxa* can effectively prevent crop diseases, including cucumber, tobacco and rape [11,34]. The biofilm formation of biocontrol agents is believed to function in resisting pathogen invasion of plant roots, restricting their ability to colonize and access nutrients in the rhizosphere of plants. This limits pathogen population and thus controls disease [4,124] Table 3. The formation of biofilm by *P. polymyxa* on plant roots provides protection to plants against pathogen infections [103,125]. Yi et al. [124] demonstrated that *luxS* facilitated biofilm formation in *P. polymyxa* and improved its biocontrol capacity against *Ralstonia solanacearum*. The biocontrol efficacy of the *l*uxS-deficient strain against *Ralstonia solanacearum* was the weakest with 50.70 ± 1.39 %. In contrast, the *luxS* over-expression strain exhibited the highest biocontrol efficacy at 75.66 ± 1.94 %. Zhao et al. [126] found that a transcription factor, MsmR1, in *P. polymyxa* showed a high correction with carbohydrate metabolism pathways. These findings lay a theoretical foundation for employing *P. polymyxa* SC2 in controlling different pepper pathogens. Many studies have been carried out by the group of Timmusk (Table 3). They found that the strains of *P. polymyxa* B_1 and B_2, which were plant growth promoting rhizobacteria, exhibited excellent biofilm formation capabilities and effectively combat *hytophthora palmivora* and *Pythium aphanidermatum* in *Arabidopsis thaliana* [101,127]. Two isolates of *P. polymyxa* isolates B_5 and B_6, prevented root colonization and *Aspergillus niger* infection, which caused crown rot disease in peanuts, by forming biofilms [128]. The authors reported that strain B_5 was significantly more effective in root colonization and its inhibitory activity against *A. niger* due to its higher production of polysaccharidic polymers. Recently, Timmusk et al. [56] found that EPS in *P. polymyxa* A26 biofilms had the ability to act against *Fusarium graminearum*, and that the uronate content of EPS was vital for this antagonistic effect.

6.1.2. Promote plant growth

Research have showed that *P. polymyxa* can boost the growth of diverse crops, including tomato, wheat, pepper, lily, cucumber, sesame, and others, through EPS, which effectively promotes plant growth and stimulates root meristem cell mitotic activity [12,28,63,102,129,130]. At the same time, EPS can enhance plant nutrient absorption through biofilm formation, degrade soil phosphorus and potassium, increase availability of these nutrients for plants, facilitate nitrogen fixation and phosphorus dissolution in the roots, and ultimately enhance the growth and yield of plants [29,120,131].

EPS produced by *P. polymyxa* is required for bacterial adhesion and the formation of biofilm on different non-living surfaces [1,132]. These biofilms play a crucial role in colonizing plant roots, enabling host plants to adapt and survive in severe conditions [56,101]. The EPS also promotes the distortion of root hairs in wheat seedlings, plays a role in cell attachment to plant roots, and boosts plant resistance against living and non-living threats [1,56,104] Table 3. Treating seedlings with EPS derived from *P. polymyxa* CCM 1465 and *P. polymyxa* 92 exhibited 1.9 and 2.8 times increase in the rate of cell division at the root tips. Measurements of the seedling morphometric variables indicated that EPS derived from *P. polymyxa* CCM 1465 and *P. polymyxa* 92 enhanced wheat growth, resulting in a rise of up to 22 % in the length of root/shoot and up to 28 % in dry weight of root/shoot, in comparison to the control group [12]. Earlier, Yegorenkova et al. [133] demonstrated that treating wheat seeds with EPS derived from *P. polymyxa* 1465 resulting in the promotion of plant growth and defense responses. This suggested that *P. polymyxa* EPS, acting as active metabolites, are responsible for the microbial cell interactions with wheat roots, and therefore involved in inducing plant responses to these interactions.

6.2. Environmental remediation

Water pollution caused by heavy metal ions has become an increasingly serious issue worldwide due to the rapid development of industries and the heightened competition for freshwater resources. Heavy metal ions in water have long been a major concern due to their harmful effects on aquatic life, plants, animals, human health and the environment. Several processes have been developed to remove wastewater pollutants, including physical and chemical methods. Among these methods, flocculation is a popular choice due to its affordability and efficacy. Bioflocculants, which are natural organic polymers created by microbes with flocculating abilities, are a safer, more biodegradable, and cost-effective alternative to chemical flocculants. In the context of removing metals with bioflocculants, EPS plays a vital role. These bioflocculants contain various functional groups such as carboxyl, amino, and hydroxyl, which have negative charges that can bind with metal ions. The presence of the functional groups offers abundant binding sites for metal ions, making it easier for the metal ions and flocculants to bridge effectively [134,135].

It has been found that EPS of *P. polymyxa* demonstrates exceptional flocculation activity (Table 3). Specifically, EPS synthesized by *P. polymyxa* SQR-21 proved highly effective in the flocculation of activated carbon within water. Meanwhile, the crude extract of *P. polymyxa* GA1 successfully flocculated high-concentration particulate matter wastewater, including kaolin solution, soil suspension, coal washing wastewater and landfill leachate via adsorption bridging [111,136]. *P. polymyxa* EPS is capable of adsorbing heavy metal ions, including cadmium and copper found in sewage, thereby contributing to water purification [58,137]. *P. polymyxa* P13 was identified as an EPS producer, had the ability to adsorb large amounts of Cu^{2+} generated by various industries [138]. Mokaddem et al. [58] found that EPS produced by *P. polymyxa* CHL0102 could adsorb Cd^{2+} in sewage with a maximum Cd^{2+} content of 520.09 mg/g. The most significant factors that affect the adsorption of Cd^{2+} were pH and initial Cd^{2+} concentration, followed by the used amount of EPS. The findings suggested that this strain of *P. polymyxa* could be implemented for eliminating heavy metal ions in wastewater, leading to sewage purification. Colak et al. [139] investigated the adsorption abilities of *P. polymyxa* to heavy metals, and found that it had the ability to adsorb copper of 49.8 mg/g and nickel of 35.02 mg/g. In addition, lead binding capability was also linked to the production of EPS as found by Hassiba et al. [140]. The researchers noted that immobilized *P. polymyxa* EPS was able to remove lead at 111.11 mg/g in accordance with the Langmuir model. This method could be useful for bioremediating lead in contaminated soil for agricultural purposes. Recently, Zhao et al. [21] developed a new bioflocculant (MBF-79) utilizing formaldehyde wastewater as a carbon source. The monosaccharides found in MBF-79 included glucose, galactose, xylose, and galacturonic acid. Maintaining a proper uronic acid content in MBF-79 could potentially supply sufficient carboxyl groups. The carboxyl groups on the molecular chain offer better attachment sites for particles, allowing numerous particles to be adsorbed onto the elongated molecular chain [141,142]. Arsenite and arsenate had maximum removal

rates of 84.6 % and 98.9 %, respectively, at 1.0 mg/L arsenic concentration.

6.3. Mining industry

In the mining sector, *P. polymyxa* has the ability to adhere to mineral surfaces, form biofilms, and releases active metabolic substances that modify their composition. Numerous studies have focused on utilizing *P. polymyxa* in biological mineral processing. The primary mechanism responsible for this phenomenon involves the alteration of mineral surfaces through EPS produced by *P. polymyxa* [143–145]. EPS can interconnect mineral particles via polymer bridging, selectively flocculating mineral fines. Deo & Natarjan [146] found that *P. polymyxa* displayed greater adhesion to hematite and corundum than to quartz. These findings indicated that the selective flocculation of hematite and corundum was due to their increased cell and EPS affinity, allowing for rapid settling in water. The interaction between *P. polymyxa* and iron ore minerals including hematite, corundum, calcite, quartz and kaolinite, induced notable surface chemical alterations in all the minerals. The surface chemical changes were attributed to the predominance of EPS on the interacted hematite, corundum and calcite, as well as the abundance of the proteins on quartz and kaolinite. *P. polymyxa* cultivated in the presence of quartz yielded a greater amount of surface proteins, whereas the same bacterium cultivated in the presence of calcite caused higher production of surface EPS. Natarajan and co-workers have recently investigated the beneficiation of bauxite and iron ore utilizing *P. polymyxa*. Natarajan & Neo [147] attempted to selectively remove galena from a mixture of galena and pyrite using *P. polymyxa* and its extracellular biopolymers. Furthermore, Patra & Natarjan [148,149] conducted a detailed study of the electrokinetic analysis and flocculation adsorption experiments to explain the selective separation mechanism of pyrite and galena by *P. polymyxa.*

6.4. Other applications

EPS produced by *P. polymyxa* also has been found to have diverse roles in multiple fields, including feed, food, cosmetics, fine chemicals, materials and many others [63,111]. *P. polymyxa* JB115 could produce β-glucan, which was then utilized to develop animal feed additives. These additives displayed potential as natural immunomodulators, biological response modifiers, with potential anti-tumor properties, that proved beneficial for livestock overall [45,150]. Li et al. [151] conducted a study on β-glucan production through *P. polymyxa*, establishing a basis for developing β-glucan in the feed industry. A new β − 1,3/1,6-glucan was obtained from *P. polymyxa* JB115 [109]. The β-glucan from *P. polymyxa* JB115 stimulated macrophages through the MAPKs and NF-κB signaling pathways, and showed potential applications as an immunostimulant or an adjuvant in certain animal vaccines [108,109].

Emulsification is a characteristic shared by numerous biological macromolecules like EPS and proteins. *P. polymyxa* NCIB 11429 produced heteropolysaccharide Biopolymer PS 87, which could act as a salad emulsifier to create a more effective emulsifying system between water and vegetable oil [46]. EPS 88 A is utilized in baking to enhance the flour's baking properties [113]. Rheology-controlling agents are crucial for various industries. According to the study by Rütering et al. [152], paenan showed strong shear-thinning flow behavior at the concentrations >0.1 % in 0.5 % NaCl solution. Furthermore, in the mixtures of paenan with surfactants such as sodium lauryl ether sulfate, cetrimonium chloride, cocamidopropyl betaine, or lauryl glucoside, paenan exhibited exceptional compatibility with all surfactant classes, surpassing partially incompatible xanthan and gellan. Based on these results, paenan/surfactant systems exhibited a weak-gel characteristic, which highlighted the high potential of paenan for diverse applications. Cheng et al. [81] obtained the levan, which was produced by *Paenibacillus* sp. FP01. The linear high M_W levan possessed favorable rheological characteristics. The levan solutions remained stable and displayed relatively a low intrinsic viscosity and a specific non-Newtonian flow behavior, making them suitable for food manufacturing industry. The authors indicated that levan demonstrated potential as a thickener and prebiotic supplement.

EPS of bacterial origin is vital in the cosmetic and food industries [70,105]. *P. polymyxa* PYQ1's EPS has been showed to provide protection against short-wavelength UV radiation. This type of radiation is known to cause cytotoxicity in HaCaT cells. Specifically, the EPS scavenges excess ROS, mitigates mitochondrial membrane potential decline, enhances catalase activity, and maintains membrane integrity for cell protection [30,61]. With these biological activities, EPS may serve as a potential skin care agent. *P. polymyxa* PYQ1's EPS also exhibited anti-inflammation activity in the gastrointestinal tract by modulating lipid metabolism. It made it a promising candidate for the development as a functional food for gastrointestinal protection [105].

Tregubova et al. [113] utilized the viscous and superviscous EPS produced by the strains *P. polymyxa* CCM 1465 and 88 A to synthesize silver nanoparticles, and followed by an investigation of the characteristics of the resulting nanoparticles. The nanoparticles prepared were spherical, oval, and triangular in shape and demonstrated stability, the size between 2 and 40 nm, depending on the experimental conditions and particular strain used. The authors highlighted that the role of EPS obtained from the strains *P. polymyxa* CCM 1465 and 88 A in reducing silver ions and stabilizing nanoparticle. In addition, Taran et al. [153] produced the nanocomposites using magnetic iron nanoparticles and levan from *P.polymyxa* PTCC1020. The authors proposed that levan and Fe_3O_4 could be utilized to synthesize antibacterial nanocomposites with significant potential for applications in medical, food, and pharmaceutical industries.

7. Conclusions

The current paper reviewed the yield, influencing factors, structure properties, synthesis pathway, biological functions, and promising applications of *P. polymyxa* EPS. We can modify the yield and structure of EPS by adjusting fermentation conditions, leading to improved characteristics and biological activities for wider adaptability and application range. Currently, *P. polymyxa* EPS has been utilized in ecological agriculture, environmental remediation, food, mining industry and other areas. However, further applications are still being developed. Extensive research is required to comprehend the synthesis mechanism and structure-activity relationship of *P. polymyxa* EPS. The EPS yield of well-known *P. polymyxa* strains is currently too low for industrial production. Therefore, it is necessary to produce *P. polymyxa* EPS in an economical, environmentally friendly and high quantity for various applications. By optimization of cultivation conditions, fermentation techniques, and genetic engineering, it is feasible to increase the amount of *P. polymyxa* EPS. Because of the costly production of EPS, numerous recent publications have proposed strategies to overcome the issue, mainly the utilization of diverse agricultural and industrial wastes. Currently, the entire genomes in certain *P. polymyxa* strains have been sequenced. All these studies build the foundation for regulating the metabolism of EPS production and developing genetically engineered strains, which have yet not to been developed for industrial use. Biocatalytic technologies and cell-free synthesis are other promising avenues for obtaining EPS more effectively. In a word, it is highly necessary to establish a highly efficient production methodology for EPS in the future.

CRediT authorship contribution statement

Xuan-Ya Huang: Writing – original draft. **Xin-Pei Ye:** Writing – original draft. **Yan-Yu Hu:** Writing – original draft. **Zhen-Xing Tang:** Conceptualization, Funding acquisition. **Tian Zhang:** Writing – original draft. **Hai Zhou:** Writing – original draft. **Ting Zhou:** Writing – review & editing, Supervision. **Xue-Lian Bai:** Writing – review & editing, Supervision. **Er-Xu Pi:** Writing – review & editing, Supervision. **Bing-Hua**

Xie: Supervision, Writing – review & editing. **Lu-E Shi:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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