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Characterization and valorization of thermophilic exopolysaccharides as natural carriers for antibiotic encapsulation from Algerian strains: *Brevibacillus borstelensis* Gp-1 and *Bacillus licheniformis* AS28

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ABSTRACT

Exopolysaccharides (EPS) produced by thermophilic bacteria offer promising potential as bioactive compounds in medical and pharmaceutical applications. This study focuses on the extraction and characterization of EPS from *Brevibacillus borstelensis* strain Gp-1 and *Bacillus licheniformis* strain AS28, with a comparative analysis of their structural and functional properties. The findings reveal that *B. licheniformis* displayed a higher EPS yield than *B. borstelensis*, indicating potential metabolic and significant environmental adaptations. Molecular mass analysis identified low-molecular-weight EPS (<5 kDa), which were associated with enhanced bioactivity. Structural characterization using FTIR and DRX confirmed that the EPS are predominantly curdlan-type β-(1,3)-glucans with prominent thermal stability. Furthermore, the encapsulation of antibiotics using these EPS demonstrated significant synergistic effects against both sensitive and resistant bacterial strains (*E. coli* and *Staphylococcus aureus*), suggesting potential applications in controlled-release drug formulations. The exceptional structural and functional properties of these thermophilic bacterial EPS position them as viable candidates for drug delivery systems and regenerative medicine. Further investigation into their biocompatibility and therapeutic efficacy could unveil innovative strategies for combating antibiotic resistance and advancing targeted drug delivery approaches.

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Introduction

Exopolysaccharides (EPS) have received significant interest in microbial physiology and biopolymer research due to their biological and technical efficacy Extracellular carbohydrates are produced by diverse microorganisms, including bacteria, archaea, and fungus, to address environmental challenges, such as biofilm formation under hostile environments to metal sequestration in polluted ecosystems (Prateeksha et al. 2021, Zhang et al. 2022). The growing interest in EPS is due to their biological functions and structural adaptability (Decho & Gutierrez 2017). Every monosaccharide unit, glycosidic bond, and functional substituent plays a role in a wide

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range of biological functions that are just starting to be comprehended (Asgher et al. 2020).

Exopolysaccharides (EPS) are polymers consisting of repetitive sugar units interconnected by diverse glycosidic bonds (β and α) which provides significant structural flexibility. Multiple studies have demonstrated that minor alterations in monosaccharide composition can affect tertiary structures (Zhao et al. 2021, Jiang et al. 2021). The β-(1→3)-glucan configuration of curdlan produces strong triple helices, while the α-(1→6) linkages in dextran yield extensively branched and soluble structures. This structural diversity is directly linked to functional multiplicity, emphasizing the chemical uniqueness of nature (Moradali et al. 2022).

Recent advancements in analytical techniques have provide insight into the complex behaviour of extracellular polymeric substances (EPS). Nuclear magnetic resonance (NMR) spectroscopy has demonstrated that even minor substitutions, such as acetyl or pyruvate groups, can significantly modify molecule conformation and interaction capabilities (Yang et al. 2024). Investigations employing small-angle X-ray scattering (SAXS) have revealed that specific low-molecular-weight EPS can self-assemble into stable nanostructures, thereby questioning the conventional understanding of polymer length requirements for functional efficacy (Rinaudi et al., González 2009, Palanisamy et al. 2016). Extracellular polymeric substances (EPS) are essential for the survival and functionality of microorganisms. Microbial communities utilize these polymers as molecular instruments to establish and maintain ecological niches (Whitfield et al. 2020, Solmaz et al. 2018). Pathogenic bacteria often utilize EPS to evade the host immune response (Whitfield et al. 2022). Recent metagenomic analysis have revealed that fewer than 10% of EPS structures and their functions have been characterized (Al-Nabulsi et al. 2022).

The correlation between polymer length and bioactivity remains ambiguous. Although high-molecular-weight EPS (>100 kDa) is prevalent in industrial applications, recent research indicates that shorter chains(<5kDa) may provide distinct benefits in drug delivery and precision biotechnology (Stenzel 2022). This research addresses this substantial gap by thoroughly detailing a novel low-molecular-weight EPS (<5 kDa) synthesized by two thermophilic bacteria, *Brevibacillus borstelensis* strain Gp-1and *Bacillus licheniformis* strain AS28, thereby challenging conventional beliefs by exhibiting significant bioactivity despite its short chain length.

The methodology utilized integrates advanced analytical techniques characterize bacterial EPS. Fourier-transform infrared (FTIR) spectroscopy provides comprehensive insight into functional groups and substitutions, while X-ray diffraction (XRD) analysis reveals unexpected crystallinity patterns in this small molecule polymer. Thermal degradation provides insights on the structural integrity of EPS under many environmental conditions. This study aims to influence various discipline. In biomedicine, the small size of EPS and its terminal carboxyl groups suggests its potential as a targeted drug carrier capable of crossing biological barriers. Environmental researchers have recognized its metal chelation abilities, particularly due to its stability across a wide pH spectrum.

This study presents novel opportunities for investigating nature's molecular insights by integrating microbial ecology, polymer physics, and applied biotechnology. By examining the vast diversity of microbial EPS, we approach the realisation of their full potential addressing global challenges in health, industry, and environmental sustainability.

Materials and Methods

Bacterial strains and chemicals

The study involved the isolation and identification of the thermophilic bacteria *Brevibacillus borstelensis* strain Gp-1 16S ribosomal RNA gene and *Bacillus licheniformis* strain AS28 16S ribosomal RNA gene from the hammam bouhanifia hot water spring in Mascara and hammam serguinn in Tiaret, (Northwestern Algeria). Pathogenic microorganisms (*Escherichia coli, Staphylococcus aureus*) were acquired from the laboratory of Ibn Khaldoun University for antibacterial assays. Chloramphenicol was purchased from Acros Organics (Belgium), Tween 80 from Sigma-Aldrich (USA), and Dichloromethane (DCM) with a purity greater than98% from Fluka (Germany).

Production and extraction of EPS

Strains are incubated in a semi-synthetic agar medium (2g/l NaCl, 4g/L yeast extract, 8g/L peptone, 20g/L glucose; pH=4) at 55°C for 5 days to produce exopolysaccharides. Bacterial cells are isolated using centrifugation (5000rpm for 10 minutes) following a 15 minutes boiling at 80°C (Han et al. 2022, Nouha et al. 2017). Precipitation of EPS: Ultrafiltration of the supernatant (conducted thrice at 4°C); incorporation of three volumes of ethanol at -20°C; centrifugation at 10,000 g for 20 minutes at 4°C; re-dissolution of the pellet in three volumes of ethanol followed by centrifugation under the similar conditions; re-dissolution of the resultant pellet in physiological water; lyophilization. (Yadav et al. 2024, Ruas-Madiedo & Reyes-Gavilán 2005, Bergmaier et al. 2001).

Dosage and quantification of EPS

The same methodology as Dubois et al. (1956) was utilized for quantifying carbohydrate concentrations in bacterial samples. Carbohydrates are dehydrated using sulphuric acid under heat, resulting in furfural derivatives that react with phenol to generate a pinkish-salmon color. Specifically, glucose yields hydroxy furfural. The permanent color is measured with a spectrophotometer at a wavelength of 490 nm, and the technique is sufficiently sensitive to identify 1 µg of carbohydrate (Zeng et al. 2021)

Thermal degradation of exopolysaccharides

The continuous heating of exopolysaccharide samples in a muffle furnace, accompanied by periodic weighing, offers a practical and reproducible method for assessing thermal stability, especially in the absence of analytical instruments (Kozlowski & Wladyka-Przybylak 2008, Schnabel 1981).Most exopolysaccharides demonstrate a distinct multi-stage degradation mechanism, beginning with moisture loss below 150 °C, then polymer backbone decomposition between 250 and 400 °C, and leading to carbonisation beyond 400 °C (Poletto et al. 2014).

Molecular masses

The molecular weight of the synthesized polymers was determined utilizing the Ubbelhode viscosimeter. This experiment involved measuring the flow time of various polymeric solutions to construct the viscosimetric line and determine the molecular mass utilizing the equation below:

$$[\eta] = K M_v^a$$

The “K” and “a” parameters are attributes of “polymer/solvent” system, derived from Masuelli’s research (2014).

The pectin value derived from the Mark-Houwink equation closely resembles those of our polysaccharides. The selected parameters are K= 0.0242 cm^3/g and a=0,82 for acetone and K= 0.0213 cm^3/g and a=0,81 for toluene.

FTIR spectroscopy

Infrared spectroscopy was employed to characterise the samples utilizing an FTIR-8300 Shimadzu spectrophotometer. KBr disks were utilized to prepare the samples.

Valorisation of exopolysaccharides

Encapsulation of antibiotics

Antibiotics were encapsulated utilizing the isolated bacterial exopolysaccharide (EPS) to assess its potential as a natural carrier matrix.

Microsphere preparation using EPS as Matrix

The conventional water/oil emulsion-solvent evaporation technique, outlined in prior publication (Abdelmalek et al. 2014), was employed to prepare the Chloramphenicol-loaded microspheres. A dispersion solution was prepared using a four-blade turbine impeller stirrer, consisting of 1g of extracted polymer (EPS68 or EPS79), 0.5g of chloramphenicol and 100 mL of DCM. The mixture was subsequently emulsified with 100mL of continuous solution (Tween80 at 1%) for two hours. Upon solvent evaporation, the solidified microspheres were filtered, rinsed with deionised water, and vacuum-dried at 40°C.

Description of Microsphere Characterization

Encapsulation Efficiency EE Determination

The equations from the literature (1 and 2) were employed to determine the drug-loaded (DL) and encapsulation efficiency (EE) (Mouffok et. al. 2016). By dissolving 0.1g of crushed microspheres in 100mL of methanol while stirring for 24 hours, UV-VIS spectroscopy was employed to experimentally ascertain these parameters for analysis of the final solution.

% DL = (Drug mass in microspheres/mass of microspheres) x 100 **(1)**

% EE = (Actual drug loading / Theoretical drug loading) x100 **(2)**

Particle Size

The mean particle size and size dispersion (δ) of a minimum 500 analysed microspheres were determined using Optical Microscopy (OPTIKA 4083. B1) and several formulas (Kaczmarski & Bellot 2003).

Scanning Electron Microscopy (SEM)

The Scanning Electron Microscopy (SEM Quanta 200 FEI) at the CRAPC-Laghouat Center in Algiers was utilized to analyse the dimensions and surface morphology of the produced microspheres.

X-ray powder diffractometry

The D8 Advance BRUKER diffractometer at the synthetic and catalysis laboratory of IBN Khaldoun University (Tiaret, Algeria) was utilized for X-ray diffraction analysis at a diffraction angle of 2θ ranging from 5° and 70°.

Inhibition of pathogen growth

The antibacterial activity of EPS was assessed using agar well diffusion as method reported by Hossain (2024). *E. coli* sensitive and resistant, and *S. aureus* resistant were utilized as indicator microorganisms and cultured overnight at 37°C in LB broth and suspension, subsequently adjusted to 10^7 to 10^8 CFU/ml. Prior to the experiment, EPS samples containing chloramphenicol (varying concentrations (6%, 13%, 25%,50%, and 100%) were dissolved in deionised water and subsequently sterilised using a 0.45 µM Millipore filter. After that, 100µl of bacterial suspension was evenly spread on Mullar Hinton agar plates, and wells with diameter of 4mm were created in the agar. Then, 60µl of the sample (5mg/ml) was introduced into each well. The inoculated plates were incubated at 37°C for 48 hours. The antimicrobial efficacy was assessed by measuring the diameter of the inhibition zone surrounding the wells. All experiment were repeated in triplicate.

Results

EPS production output

Results demonstrating the EPS yield of *Brevibacillus borstelensis* and *Bacillus licheniformis* strains. EPS production was significantly affected by many physicochemical parameters, including medium, inoculum size, sugar content , pH, temperature, and fermentation duration). The EPS production rate of *Bacillus licheniformis* was higher than that of *Brevibacillus borstelensis* measuring 3,708±0,005 and 3,072±0,005 g/L, respectively

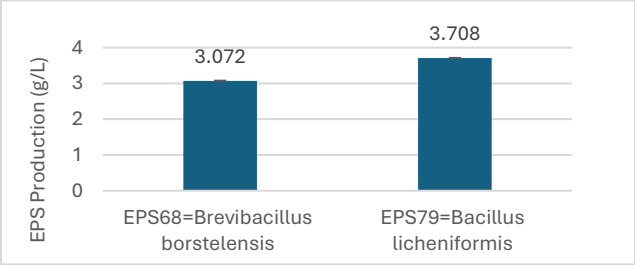

Fig 1. Exopolysaccharide production output by Brevibacillus borstelensis and Bacillus licheniformis

Characterisation of EPS

Thermal degradation

The thermal degradation of EPS was analysed to evaluate its thermal stability, a characteristic for many industrial and biotechnological applications. Figure 2 illustrates the TG curves of the EPS68 and EPS79. These EPSs exhibited three distinct stages of decomposition within the temperature range of 100 °C to 600 °C. The initial phase removed place at temperature ranging from 100 and 150 °C, leading to a 20% reduction in weight primary due to water evaporation. The second phase occurs between 150°C and 500°C, leading to a 74% reduction in weight for EPS 68 and a 69% reduction for EPS79 as a result of pyrolytic decomposition. The third phase occurred at a temperature range of 500–600 °C, leading to a weight reduction of 3% for EPS 68 and 13% for EPS79.In total, 99 % of the EPS weight was diminished throughout the thermal reaction procedure.

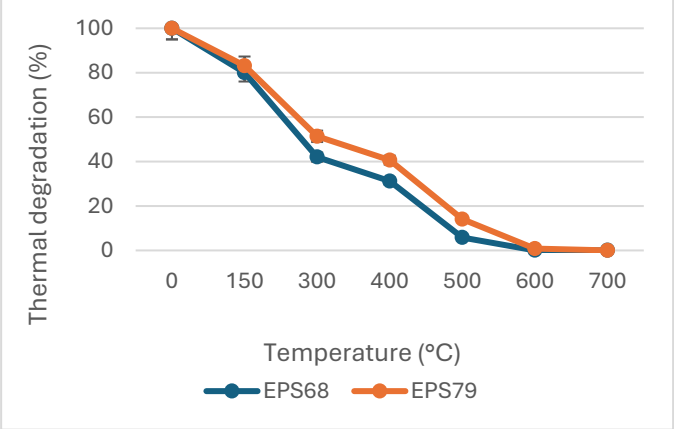

Fig 2. Thermal degradation curve for EPS,

Molecular masses

The reduced viscosity of the exopolysaccharide (EPS) solution was assessed at different concentrations, utilizing acetone as the solvent for EPS68 and toluene as the solvent for EPS79. The intrinsic viscosity [η] was determined by extrapolating the lowered viscosity to a concentration of zero. The viscosity-average molecular weight (Mv) was determined using the Mark-Houwink-Sakurada equation. The resultant molecular mass was 3,680 g/mol for EPS68 and 1,590 g/mol.

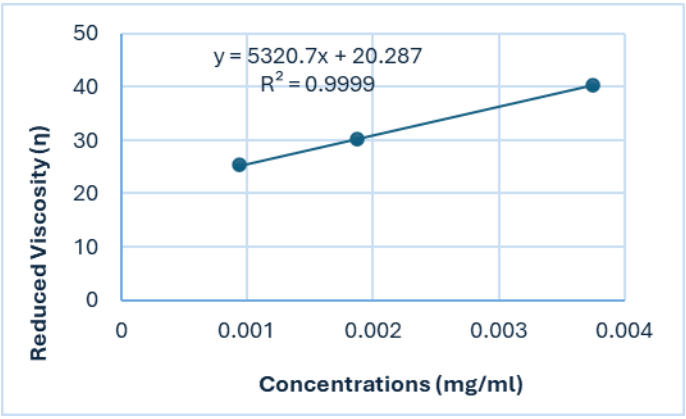

Fig 3. Reduced viscosity versus concentration of EPS68.

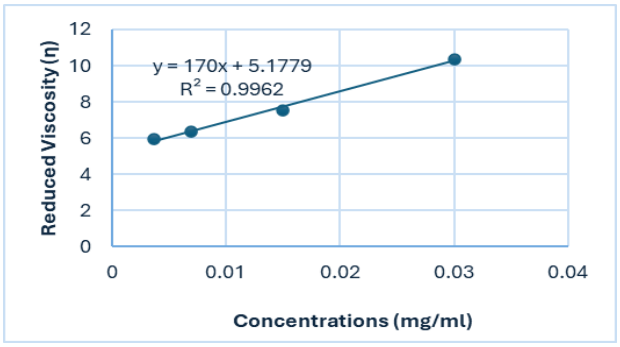

Fig 4. Reduced viscosity versus concentration of EPS79.

FTIR Result

The infrared spectra of the extracted polymers are illustrated in Figures 5 and 6; the IR displays distinctive bands that facilitate the identification of their primary chemical functions. According to the FTIR peaks of EPS68: Sulfated Heteroglycan: Mixed α/β linkages (843, 906 cm^{-1}) and sulfate (1281 cm^{-1}). Analogous to *Porphyridium* EPS (red algal polysaccharides). Anionic EPS containing uronic acids is indicated by COO^- (1625 cm^{-1}) and C=O (1718 cm^{-1}), suggesting the presence of glucuronic or galacturonic acids. The FTIR peaks of EPS79 are as follow: 906 cm^{-1}, 1600 cm^{-1},1650 cm^{-1}, and1718 cm^{-1}indicating a β-linked backbone similar to xanthan, as well as uronic acid/carboxyl groups characteristics of anionic EPS. Likely acetyl/pyruvatesubstitutions (1718 cm^{-1}).

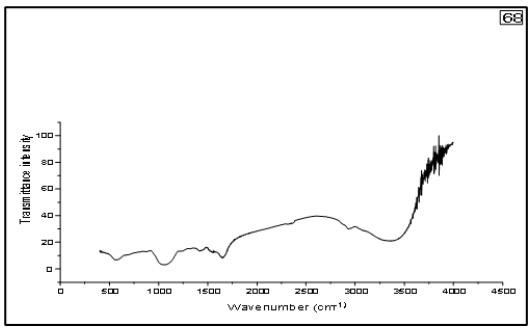

Fig 5. FTIR peaks of EPS68

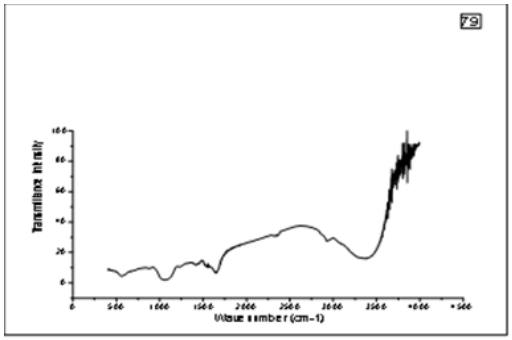

Fig 6. FTIR peaks of EPS79.

Valorisation of exopolysaccharides

Encapsulation of antibiotics

SEM images displayed in Figure 7 were utilized to determine the surface morphology and size distribution. The prepared microspheres exhibit smooth, porous surfaces and spherical shapes with varied diameters. The extraction of the active agent allows the determination of the drug loading in the produced microspheres, which was determined to be approximately 81% for Sp79 and 79% for Sp68 (Table 1).

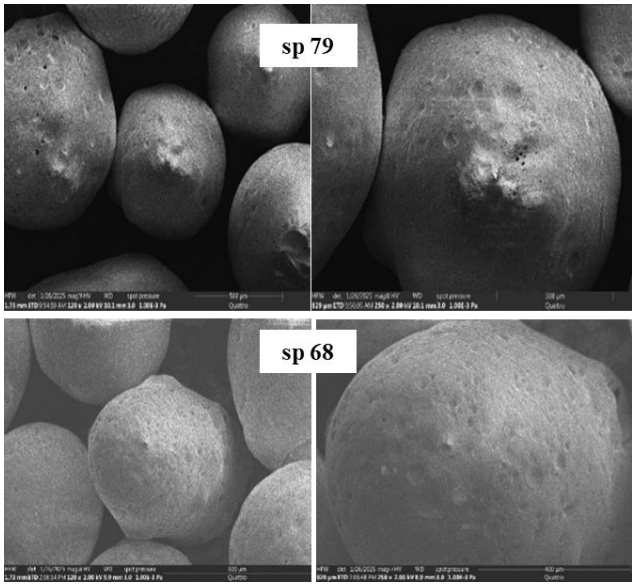

Fig 7. SEM images of prepared microspheres

Data in table (1) summarizes the composition of microspheres containing chloramphenicol with different polymer formulations (79 and 68), alongside microencapsulation results including drug loading (DL), encapsulation efficiency (EE), particle size distribution (d10, d32, d43 in µm), and dispersion. Data are presented as mean ± standard deviation (SD) from many studies.

Optical microscopy was employed to examine the mean diameters of the microspheres utilizing previously established formulas (Kaczmarski & Bellot 2003). The resultant microparticles exhibited a dispersity index of 1.22 and 1.46 with a mean Sauter (d32) diameter of 248.05µm for sp79 and 188.6µm for sp68.

XRD result

X-ray diffraction (XRD) is a flexible, non-destructive analytical technique employed to investigate crystal structure. The extracted polymers were characterised by XRD, and their diffractograms are displayed in Figures 8 and 9. The polymer EPS79 had a

semi-crystalline structure evidenced by low-intensity peaks at 2θ=15°, 29.8°, and 32°; however, the polymer EPS68 displayed amorphous characteristics indicated by the absence of crystallin peaks. The physical states of microspheres were examined utilizing the XRD diffractogram. The produced microspheres (sp79) exhibit a semi-crystalline appearance. The crystal peaks of chloramphenicol were seen at 19°, 22°, and 25°. These peaks appeared less intense than those in the chloramphenicol XRD diffractogram (Kumar et al. 2015). The XRD diffractogram of sp68 exhibits an amorphous structure. Consequently, it may be asserted that chloramphenicol is incorporated inside polymer's amorphous state.

Table 1. Microspheres composition and microencapsulation results. (± SD): Standard deviation; DL: Drug Loading; EE: Encapsulation Efficiency.

Code	Chloramphenicol /Polymer	% DL	%EE	d_{10} (µm)	d_{32} (µm)	d_{43} (µm)	Dispersion
sp79	chloramphenicol/79	21.23±0.7	81.5±0.01	248,4	284,05	303,36	1,22
sp68	chloramphenicol/68	19.83±0.35	79.12±0.11	188.6	257,75	276,25	1.46

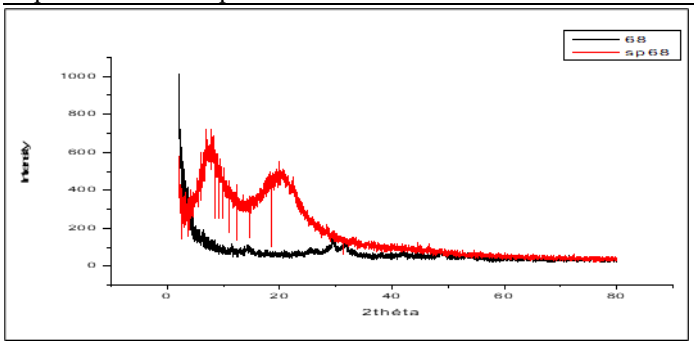

Fig 8. XRD Diffractograms of polymer 68 and their microspheres.

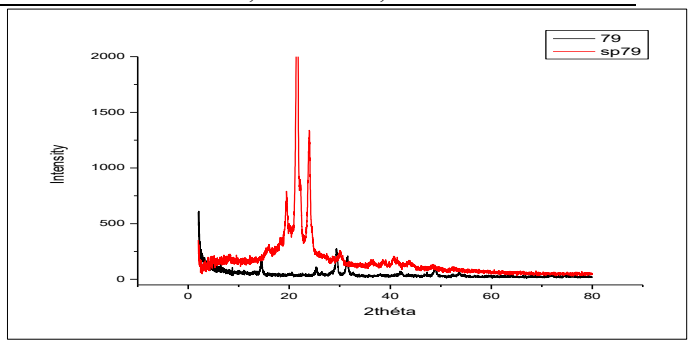

Fig 9. XRD Diffractograms of polymer 79 and their microspheres.

Antibacterial activity

The antibacterial efficacy (measured as the zone of inhibition in mm) of exopolysaccharides (EPS68 and EPS79) both independently and in conjunction with chloramphenicol at different concentrations (100%, 50%, 25%, 13%, and 6%) against susceptible (S) and resistant (R) strains of *E. coli* and resistant *S. aureus* were represented in table (2). Data are presented as mean ± standard deviation.

To evaluate the effectiveness of the antibiotic/exopolysaccharide combination, we conducted antibiotic assays with different doses of EPS (100%, 50%, 25%, 13% and 6%). The results indicates that the integration of EPS with the antibiotic chloramphenicol enhances the antibiotic's efficacy, particularly at a 6% concentration of EPS79 against sensitive *E. coli*, yielding a zone of 35.53±0.57 mm. In contrast, for resistant *E. coli*, the 25% EPS concentration of S68 had the highest efficacy, yielding a zone of inhibition of 35.33±0.57 mm.

Table 2 Zone of inhibition (mm) of EPS68 and EPS79, alone and with chloramphenicol, at different concentrations against susceptible and resistant strains of *E. coli* and resistant *S. aureus* (mean ± SD).

	***E. coli* S (mm)**		***E. coli* R (mm)**		***S. aureus* R (mm)**	
	EPS68	**EPS79**	**EPS68**	**EPS79**	**EPS68**	**EPS79**
Chloramphenicol	32,33 ± 2,51	31,7 ± 0,1	28 ± 0,52	28,26 ± 1,10	22,93 ± 0,90	27,08 ± 0,14
(Eps +ATB)100%	31,37 ± 0,81	31,86 ±1,2	30,56 ± 0,49	31,5 ± 0,5	23,86 ± 0,15	27,76 ± 0,15
(Eps +ATB) 50%	29,26 ± 0,64	32,83 ± 0,28	29,93 ± 0,90	33,33 ± 0,57	24,8 ± 0,1	26,56 ± 0,25
(Eps +ATB) 25%	34,66 ± 0,57	33,26 ± 0,3	35,33 ± 0,57	31,9 ± 0,17	27,8 ± 0,1	27,66 ± 0,20
(Eps +ATB) 13%	32,66 ± 0,57	33,53 ± 0,5	26 ± 1	32,8 ± 0,34	28 ± 1	26,43 ± 0,4
(Eps +ATB) 6%	33,33 ± 1,52	35,53 ± 0,57	28,83 ± 0,20	28 ± 1	23,33 ± 0,57	24 ± 1

To evaluate the efficacy of the antibiotic/exopolysaccharide combination, we conducted antibiotic assays with varying doses of EPS (100%, 50%, 25%, 13% and 6%). The results indicate that the combination of EPS with an antibiotic chloramphenicol enhances the efficacy antibiotic's efficacy, especially for the 6% EPS concentration of S79 against sensitive *E. coli*, achieving a 12%. improvement. In contrast, for resistant. *coli*, the 25% EPS S68 concentration demonstrated the most efficacy achieving 26% effectiveness.

Discussion

Bacteria EPS displays great diversity and functionality, and their production is not constrained by taxa. This variation is evident in the monomeric compositions, linkage bonds, and associated conjugates, while the functions could be categorized as intrinsic and applied (Nwodo et al. 2012). In this study, the two strains, *Brevibacillus borstelensis* strain Gp-1 16S ribosomal RNA gene and *Bacillus licheniformis* strain AS28 16S ribosomal RNA gene, produced EPS at different concentrations (3,072±0,005 g/L and 3,708±0,005, respectively). Asgher et al (2022) reported that the native *Bacillus licheniformis* strain generated 3.4 g/L of EPS biomass. *Bacillus* species are acknowledged for their ability to synthesise significant quantities of exopolysaccharides (EPS), generally between 2 to 12 g/L, depending upon the specific strain and growth conditions (Vasait et al. 2023). *Bacillus subtilis* has exhibited EPS yields of up to 10.3 g/L when cultivated in optimised glucose-rich media (Tuşar et al. 2022). In contrast, *Lactobacillus* species, such as *L. plantarum and L. rhamnosu*ftens yield lower quantities of EPS (0.2–1.5 g/L). Nevertheless, these polymers are esteemed for food texture modification and probiotic applications (Patel et al. 2012).

Other bacteria, including *Pseudomonas aeruginosa*, can produce far higher quantities of EPS, particularly alginate, reaching 15–20 g/L under stress conditions such as nitrogen limitation or elevated osmolarity (Maharani et al. 2018). The industrial application of *Pseudomonas* strains is limited because of its pathogenic nature. In comparison, Bacillus species provides a favourable combination of high EPS yields, non-pathogenicity, and rigorous growth, rendering them more appropriate for food, pharmaceutical, and environmental bioprocessing. The exopolysaccharide (EPS) extracted from *Bacillus* sp. demonstrated a viscosity-average molecular weight (Mv) of 3,680 g/mol, determined using intrinsic viscosity measurements utilizing the Mark-Houwink-Sakurada equation (Figure 3). The moderate molecular mass indicates a polymer of relatively short to medium chain length, consistent with EPSs reported from *Bacillus* strains under comparable culture conditions (Qiang et al. 2019; Zhang et al. 2016). FTIR spectroscopy structural analysis validated the polysaccharide composition of the compound (Figure 1), revealing a broad O–H stretching band at 3,315 cm^{-1}, C–H stretching at 2,922 cm^{-1}, and strong C–O and C–O–C vibrations within the range of 1,040 and 1,140 cm^{-1}, characteristic of glycosidic linkages. A band at 1,636 cm^{-1} signifies the presence of bound water or carboxyl groups, presumably derived from uronic acid residues (Poli et al. 2011, Sutherland 2001). The thermal behaviour evaluated using thermogravimetric analysis (Figure 2) indicated an initial weight reduction of 8.5% below 150 °C, ascribed to moisture, succeeded by a significant degradation between 270 and 340 °C, linked to polymer backbone decomposition. The thermal stability aligns with microbial EPSs and supports the polymer's use in encapsulation systems or as a bioactive component in heat-resistant applications (Wang et al. 2021). The results collectively affirm the EPS's moderate molecular weight, characteristic polysaccharide structure, and adequate thermal stability for diverse biotechnological applications.

The extracted polysaccharide exhibits distinct crystalline forms based on its molecular structure and the presence of various functional groups, which can affect its physical properties and interactions with water (Shikinaka et al. 2016). The level of crystallinity influences the solubility, viscosity, and gel-forming abilities of polysaccharides, making them essential in several applications, including food science to pharmaceuticals. (Patil & Patel 2021). The effective encapsulation of chloramphenicol utilizing bacterial exopolysaccharides (EPS) demonstrate their potential as biodegradable carriers for antimicrobial agents. The SEM images indicates that the synthesized microspheres are spherical, with disparities of 1.22 and 1.46, corresponding to sp79 and sp68, respectively, demonstrating a broad variation in size among the. SEM scans have revealed that polysaccharide microspheres generally exhibit a spherical shape and a diverse range of diameters (Baimark & Srisuwan 2013). The surface texture can differ, with certain specimens displaying smooth surfaces. In contrast, some exhibit roughness, suggesting varying preparation techniques (Fallon et al. 2013) and the size distribution correlates with the other formulation characteristics, such as speed and polymer viscosity.

Polysaccharide microspheres exhibit of high drug loading efficiencies over 80% demonstrating their efficacy as drug carriers (Sharma & Mazumder 2014). These microspheres enhance the solubility and stability

of poorly water-soluble medicines while facilitated regulated release, hence improving therapeutic outcomes and patient compliance (García-González et al. 2015). Polysaccharide microspheres exhibit adaptability beyond drug delivery, they can be tailored to target specific tissues or cells, thereby enhancing their therapeutic efficacy and reducing side effects. This adaptability renders them a viable platform for various biomedical applications, including tissue engineering and regenerative medicine, where the precise distribution of bioactive substances is essential (Nurunnabi et al. 2017). These results align with prior research indicating that the polysaccharide polymer (Ethylcellulose) and solvent-evaporated encapsulation gives comparable results, with particle sizes varying based on the properties of other formulation. (Mouffok et al. 2023)

Exopolysaccharides (EPS) exhibited significant antimicrobial efficacy against Gram-positive and Gram-negative bacterial pathogens, specifically *Staphylococcus aureus* and *Escherichia coli*. The antimicrobial mechanisms of EPS are complex. EPS initially engages with bacterial pathogens, interfering with cell division and modifying cell surface properties, including hydrophobicity and auto-aggregation, thus enhancing the competitive exclusion of pathogens (Abdalla et al. 2021; Wang et al. 2020). Furthermore, EPS impedes biofilm formation by inhibiting quorum sensing signalling pathways and hindering adhesion by covering the surface receptors on pathogens (Liu et al. 2019, Di Perri & Ferlazzo 2022). EPS interacts with both bacterial and eukaryotic cells, functioning as a masking or decoy agent (Medrano et al. 2009). This interaction may obstruct receptors or channels on the outer membrane of gram-negative bacteria, so affecting the bacterial cell envelope structure, especially the peptidoglycan layer, which has been suggested as a potential inhibitory mechanism (Sivasankar et al. 2018). The antimicrobial and antioxidant properties of EPS are ascribed to functional groups including carbonyl, phosphate, and hydroxyl groups (Riaz Rajoka et al. 2020, Salachna et al. 2018) indicated that EPS may enhance the accumulation of secondary metabolites in growth media, negatively impacting both Gram-positive and Gram-negative bacteria. The functional groups in the EPS structure likely interact with bacterial cell envelopes, leading to antimicrobial activity (Zhou et al. 2019). Furthermore, EPS can influence host immunological responses by either stimulating immune cells to eliminate potential pathogens or suppressing inflammation to preserve host health amidst microbial presence (Liu et al. 2019). Modified EPS products, including sulfated variants, exhibit enhanced antimicrobial efficacy, interfere with biofilm's communication and induce ruptures in pathogen cell membranes (Di Perri & Ferlazzo 2022). EPS generally functions extracellularly by signalling to inhibit pathogen adhesion, disrupting biofilm communication, and modulating host immunological responses.

Conclusion

This study emphasized the effective extraction and characterisation of exopolysaccharides (EPS) from thermophilic bacteria, namely *Brevibacillus borstelensis* strain Gp-1 and *Bacillus licheniformis* strain AS28. The results demonstartes that *B. licheniformis* exhibits a higher EPS production rate than *B. borstelensis*, implying possible differences in the metabolic pathways or environmental adaptations between two strains. The comprehensive characterisation of EPS through molecular mass analysis, thermal degradation assessments, and advanced spectroscopic techniques such as FTIR and DRX yielded essential insights into their structural properties, indicating that the generated EPS are likely curdlan-type β-(1,3)-glucans. Identifying low-molecular-weight EPS (<5 kDa) is associated with enhanced bioactivity. This study confirms the potential of these EPS in antibiotic encapsulation, revealing significant synergistic effects that improve antibiotic efficacy against both sensitive and resistant pathogenic bacteria. The unique structural and functional properties of thermophilic bacterial extracellular polymeric substances (EPS) make them highly promising for the development of innovative drug carriers and controlled-release formulations. Further research into their biocompatibility and interactions with diverse therapeutic agents may reveal l novel opportunities for targeted drug delivery. Furthermore, investigating the potential applications of these biopolymers in tissue engineering and regenerative medicine could yield innovative solutions for complex medical challenges that require innovative techniques. The distinctive structural and functional properties of EPS derived from thermophilic bacteria renders them highly promising for the developing of innovative drug carriers and controlled-release formulations. Further investigation of their biocompatibility and interactions with other therapeutic agents may provide a new opportunities for targeted drug delivery. Investigating the potential of these biopolymers in tissue engineering and regenerative medicine could yield innovative solutions for complex medical issues. These findings underscore the necessity of ongoing investigation into the optimisation of extraction techniques and characterisation of the functional properties of EPS, which may yield innovative strategies for addressing antibiotic resistance

and enhancing therapeutic efficacy. These advancements may ultimately facilitate the development of more effective treatment regimens, ensuring that healthcare providers possess strong instruments to address rising bacterial threats.

Conflict of interest

The authors have no conflicts of interest to declare.

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