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ORIGINAL ARTICLE

Characterization of an exopolysaccharide produced by *Enterobacter* sp. YU16-RN5 and its potential to alleviate cadmium induced cytotoxicity in vitro

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Abstract

Natural biopolymers have gained remarkable attention for bioremediation particularly in heavy metal removal and oil degradation due to their non-toxic nature and lack of secondary pollution. The exopolysaccharides (EPS) produced by the bacteria have become an important class of biopolymers that are employed in bioremediation. The bacteria isolated from the rhizospheric soil have higher metal tolerance and their EPS are effective in biosorption of heavy metals. Here, we report the characterization of an EPS (EPS-RN5) isolated from the root nodule-associated bacteria, *Enterobacter cancerogenus* strain YU16-RN5 and its heavy metal biosorption abilities. The bacteria isolated from the West coast of India was cultured in yeast extract mannitol (YEM) medium for EPS extraction and to study the production kinetics on a temporal scale. The biochemical composition, rheological properties and thermostability of EPS-RN5 was characterized by standard methods. The biosorption potential of EPS-RN5 against the selected heavy metals was analyzed by employing the inductively coupled plasma atomic emission spectroscopy (ICP-AES) technique. Further, cell culture experiments were used to test the role of EPS-RN5 in reducing the cytotoxicity exerted by the heavy metals in vitro using a human embryonic kidney cell line (HEK 293T). The bacteria showed good growth in YEM media and the maximum EPS yield was 1800 mg/L at 96 h. The molecular weight of EPS-RN5 was 0.7×10^6 Da and it contained 61.5% total sugars and 14.5% proteins. The monosaccharide composition of the EPS included glucose, sorbose and galactose in the ratio 0.25:0.07:1.0. The EPS-RN5 showed high thermal stability with a degradation temperature of 273 °C. Rheological analysis revealed the non-Newtonian behavior, with pseudoplastic characteristics. The EPS-RN5 efficiently absorbed cadmium and other heavy metals such as mercury, strontium, copper, arsenic, and uranium. In vitro studies revealed significant protective effect against the cadmium-induced cytotoxicity in HEK 293T cells. These results indicate the potential applications of EPS-RN5.

Keywords Exopolysaccharide · *Enterobacter cancerogenus* · Biosorption · Heavy metals · Cytotoxicity

Introduction

Heavy metals are stable in nature and persist in the environment for longer periods due to their nondegradability. The recalcitrant property of the heavy metals leads to the accumulation or biomagnification in the food chain causing serious health and ecological risks (Gutnick and Bach 2000). Heavy metals and metalloids such as cadmium, copper, nickel, cobalt, chromium, zinc and arsenic are toxic to living organisms. For instance, cadmium is known to have numerous undesirable effects on both humans and animals. It exerts toxic effects on multiple organs including the liver, kidney, pancreas and can adversely affect their functions (Zhang et al. 2017). Several strategies such as reverse osmosis, ion exchange, chemical coagulation and ultra filtration among others are adopted for the removal of heavy metal ions from the contaminated environment (Renu and Singh 2017; Sun et al. 2020a, b).

Biological methods such as phytoremediation, bioaccumulation, biocoagulation, bioleaching, biosorption and bioimmobilization are introduced to remove heavy metals from the contaminated sites as they are environmentally safer than the chemical methods (Rahman and Singh 2019;

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Rebello et al. 2021). Natural biopolymers have a greater ability to immobilize heavy metals due to the presence of chelating groups. Biopolymers from the bacterial origin such as exopolysaccharides (EPS) have metal-binding properties (Kaplan et al. 1987; Aryal et al. 2010; Raklami et al. 2020). Some of the important bacterial EPS that facilitate heavy metal biosorption are alginate (*Pseudomonas aeruginosa*, *Azotobacter vinelandii*), gellan (*Sphingomonas paucimobilis*), hyaluronan (*Pseudomonas aeruginosa, Pasteurella multocida*, *Streptococci* attenuated strains), xanthan (*Xanthomonas campestris*), galactopol (*Pseudomonas oleovorans*) and fucopol (*Enterobacter* A47) (Öner 2013). Carboxyl, amino and sulfonate groups in alginate are responsible for the metal ion binding by ion exchange mechanisms (Bertagnolli et al. 2014). The positively charged metal ions are chelated more efficiently by the anionic biopolymers. This is one of the primary mechanisms involved in metal biosorption.

This study reports the potential heavy metal biosorption property of an EPS (EPS-RN5) produced by a coastal rhizosphere-associated bacterium *Enterobacter* sp. strain YU16-RN5. The Inductively coupled plasma atomic emission spectroscopy (ICP-AES) and FT-IR were used to characterize the heavy metal absorption mechanisms of EPS. In addition, using the in vitro cell culture experiments the possible protective role of EPS-RN5 in alleviating the cytotoxicity of cadmium on HEK 293T cells was established.

Materials and methods

Isolation and identification of an exopolysaccharide producing marine bacterial strain

The bacterial strain YU16-RN5 was isolated from the root nodules of a coastal dune plant, *Derris elliptica* from the coastal region of Mangalore (12°47′ 10.3524″ N 74°51′ 12.4344″ E). The root nodules were separated from the roots of the plant, washed with sterile water thoroughly to clean the dirt, immersed in 70% ethanol for 30 s and washed twice with sterile distilled water (Mendes et al. 2007). Later, it was macerated and the suspension obtained was serially diluted (ten-fold) in sterile normal saline (0.9% NaCl). From this 100 µL sample suspensions were inoculated into yeast extract mannitol (YEM) agar plates (Himedia, India) (Reis et al. 2004). The plates were incubated at 37 °C up to 5 days, and colonies showing mucoidal morphology were pure cultured and preserved in 30% (v/v) glycerol at −80 °C (Rosalam and England 2006). As an initial screening test for β-glucan production, the bacterial isolate was streaked in YEM agar plates supplemented with 0.05% aniline blue and incubated at 37 °C for 48 h. The plates were observed for blue-colored colonies which indicate the presence of β-glucan production (Nakanishi et al. 1976). Taxonomic identification was done by 16S rRNA gene sequencing. The sequence data were aligned and compared with available standard sequences of bacterial lineage in the web-based EzTaxon-e server (Kämpfer et al. 2018). The 16S rRNA sequence was submitted to GenBank under the accession number MH191375.

Extraction and purification of EPS

For the EPS production, the isolate was grown in YEM broth at 37 °C for 96 h using a shaker set at 100 rpm. After incubation, the culture broth was centrifuged at 8000 rpm for 10 min to separate the cells and the cell-free supernatant was mixed with thrice its volume with chilled ethanol. The contents were kept overnight at 4 °C and the precipitated EPS was separated by centrifugation at 8000 rpm for 10 min (Quesada et al. 1994). The collected EPS was dissolved in MilliQ water and re-precipitated to remove the remnants of media contaminants. The precipitated EPS was separated by centrifugation and dialyzed using a 12 kDa cut-off dialysis membrane (HiMedia, India) against MilliQ water for 48 h with intermittent changes of water. The dialyzed EPS was lyophilized and the yield was determined (dry weight basis). The EPS obtained was designated as EPS-RN5 and used for further studies.

Biochemical and structural analysis of EPS

Total sugar content in EPS-RN5 was measured by phenol sulfuric acid method (Dubois et al. 1956) and protein content by Lowry's method (Lowry et al. 1951). Uronic acid was estimated by treating the EPS with tetraborate (12 mM) in concentrated sulfuric acid and m-hydroxy diphenyl reagents (Blumenkrantz and Asboe-Hansen 1973). For structural characterization, FT-IR spectral analysis was used by scanning in the wavenumber range of 4000–400 cm^{-1} using an Alpha e-Bruker spectrometer (Bruker Optik GmbH, Ettlingen, Germany).

Estimation of molecular weight by gel permeation chromatography

For the determination of molecular weight, gel permeation chromatography (GPC) technique was used. Briefly, the Sephacryl S-500 HR column (XK 26 mm/100 cm) was eluted with 0.1 M Tris–HCl buffer (pH 7.2). The column was calibrated using a mixture of blue dextran standards (Sigma, USA) with a range of molecular weights (50, 150, 670 and 20,000 kDa). The lyophilized EPS-RN5 solution (1.0 mg/mL) (0.50 mL) was added to the column and eluted with 0.1 M Tris HCl buffer at a flow rate of 1.0 mL/min. Eluted fractions (0.5 mL each) were collected and analyzed for total

sugars using phenol–sulfuric acid method (Dubois et al. 1956). The molecular weight was estimated from the graph plotted using the blue dextran molecular weight standards against the elution volume as previously described elsewhere (Insulkar et al. 2018; Sajna et al. 2013).

Thermogravimetric analysis

Thermogravimetric analysis (TGA) of EPS-RN5 was carried out in a thermal analysis system (TG–DTA/DSC Model: Q600 SDT). For this, about 7 mg of lyophilized EPS sample was used and subjected to a temperature range of 30–700 °C with a temperature increment of 10 °C/min under the flow of nitrogen. The degradation pattern was studied by plotting the weight (percentage) loss and heat flow against temperature (Wang et al. 2010). The graph was constructed using Origin 2017 SR2 software.

Monosaccharide analysis of the EPS-RN5 by Gas chromatography – Mass spectrometry

The monosaccharide composition of the EPS-RN5 was analyzed by Gas chromatography—Mass spectrometry (GC–MS). For this, 1.0 mg purified sample was subjected to methanolysis using methanolic TFA (3 M) for 4 h at 100 °C. The mixture was dried under nitrogen and dissolved in dichloromethane (DCM) and dimethylacetamide. The content was evaporated under nitrogen and re-dissolved in N, O-bis (trimethylsilyl)—trifluoroacetamide-trimethyl chlorosilane (BSTFA-TMCS) in DCM and kept at 60 °C for 1 h. The derivatized sample was analyzed using a GC–MS system (Agilent 7890 GC with 5975 C MS, USA) with DB 5 ms 30 × 0.25 mm column (Agilent Technologies). For MS analysis, 1.0 µL sample was injected using a splitless injector with injector temperature maintained at 260 °C. Oven programming included an initial column temperature of 50 °C for 2 min followed by a ramp at 10 °C/min to 310 °C and held at 310 °C for 7 min. Monosaccharides were identified using a MS detector in full scan mode in the molecular mass range of 350–700 m/z. Monosaccharides were identified by comparing them with the standard mass spectra available in the NIST 11 mass spectral library (Blau and Halket 1993). Inositol was used as an internal standard.

Rheological characterization

To evaluate the rheological properties, EPS-RN5 was dissolved in MilliQ water and diluted to 0.5% (w/v) concentration. The viscosity of the solution was measured over a range of shear rates (96–768 s^{-1}) at 25 °C using a Brookfield DV3T Rheometer (Brookfield, USA) equipped with spindle CP-51. The flow (n) and consistency (K) index were determined using the Power-law model $\tau = K\gamma^n$ where τ is the shear stress (Pa), K is the consistency index (Pa sn), γ is the shear rate (s^{-1}), and n is the flow index (dimensionless) (Steffe 1996). The flow curve was constructed using GraphPad Prism 8.0.2.

Quantification of heavy metal biosorption

Heavy metal biosorption ability of the EPS-RN5 was evaluated against mercury, cadmium, strontium, copper, arsenic and uranium ions. For this, mercuric sulfate, cadmium chloride, strontium chloride, copper chloride, sodium arsenate and uranyl nitrate solutions of 100 mg/L concentration was prepared in MilliQ water. All the chemicals employed in this study were of analytical grade. EPS-RN5 at a concentration of 1% (w/v) was added to metal ion solutions and allowed to react for 30 min. The solution was centrifuged at 8000 rpm for 10 min and the precipitate obtained due to metal interaction with the EPS was digested with nitric acid and diluted with MilliQ water. The concentrations of absorbed metal in the precipitate and metal ions in the supernatant were determined using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (ARCOS, M/s. Spectro, Germany). The metal uptake (q_e) was determined as follows according to previously described methods (Zewail and Yousef 2015; Omorogie et al. 2012; Zhao et al. 2015):

$$qe = \frac{V(Ci - Cf)}{W}$$

where V = the volume of mixture solution (L), Ci = the initial concentration of metal in mixture solution (mg/L), Cf = the equilibrium concentration of metal in mixture solution (mg/L), W = the dry weight of EPS (g), The graph was constructed using GraphPad Prism 8.0.2.

In vitro studies to evaluate the protective effect of EPS-RN5 against the cytotoxicity of cadmium in HEK 293T cells

For in vitro studies, a human embryonic kidney cell line (HEK 293T) obtained from National Centre for Cell Science, Pune, India was used. The cells were maintained in Dulbecco's minimum essential medium (DMEM) containing 1.0 mM sodium pyruvate, L-glutamine, 4.5 g/L glucose and 1.5 g/L sodium bicarbonate supplemented with 10% fetal bovine serum (FBS) in 5% CO_2 atmosphere at 37 °C (Forma STERICYCLE 371, CO_2 incubator, Thermo Scientific, USA). Cells were used for the experiments within three passages. The exponentially growing HEK 293T cells were trypsinized and seeded to 96-well plates at a density of 5000 cells/well and incubated for 24 h in a CO_2 incubator (5% CO_2, 37 °C). To study the effect of EPS-RN5 in reducing the cytotoxic effect, EPS solutions at 25 and

50 µg/mL were sterilized using 0.22 µ syringe-driven filters. These were added to the cells exposed to cadmium (10, 20, and 40 µg/mL). The contents were incubated for 48 h. The wells containing HEK 293T cells with only EPS were maintained as positive control and those with only cadmium was maintained as a negative control. Following incubation, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) at a concentration of 1.0 mg/mL was added to the assay wells and incubated at 37 °C for 4 h in dark. The formazan crystals were solubilized in DMSO and the absorbance was recorded using a multimode plate reader (FLUOstar Omega) at 570 nm (Mosmann 1983). Cytotoxicity was measured using the following equation:

$$\text{Cytotoxicity}(\%) = \frac{\text{Absorbance of the control} - \text{Absorbance of the test}}{\text{Absorbance of the control}} \times 100$$

For visualization of the data, a graph was constructed using GraphPad Prism 8.0.2 and the micrographs obtained were arranged using CorelDraw Technical Suite.

Results and discussion

EPS production by the bacterial isolate

The isolate YU16-RN5, was a gram-negative bacterium identified by 16S rRNA gene sequencing as *Enterobacter* sp. (MH191375) with 100% similarity to *Enterobacter cancerogenus*. The species used for the study was isolated from the urban coastal region of Mangalore (South-West coast of India) that is exposed to heavy metal contamination by various anthropogenic activities. The bacteria isolated from these regions generally have adaptations to such hostile environment and the EPS produced by them act as metal chelating agent rendering a natural survival mechanism against heavy metal exposure. There has been a similar study that reports the heavy metal tolerance by a few bacterial communities associated with the monazite sand of Someshwara beach (Shreedhar et al. 2014). The isolate *Enterobacter* sp. YU16-RN5 grown on YEM agar plates showed copious mucoidal pale white colonies at 37 °C at 96 h of incubation (Fig. 1a). On the YEM agar media supplemented with aniline blue, the bacteria formed blue-colored mucoid colonies indicating the EPS as β-glucan-like biopolymer (Fig. 1b). The β-glucans are industrially important biopolymers with a range of therapeutic applications (Moscovici 2015). The maximum yield of the EPS in the YEM media was 1800 mg/L at 96 h (Fig. 1c). This yield is comparable with the EPS produced by *Enterobacter cloacae* WD7 (1680 mg/L) (Prasertsan et al. 2006) and *Enterobacter* A47 (1810 mg/L) (Marques 2017). Higher EPS yields without the requirement of complex media composition are one of the interesting features of *Enterobacter* strains.

Biochemical and structural characteristics of EPS-RN5

The total sugar content of the EPS-RN5 on a dry weight basis was 61.4 ± 2.3% that is comparable with the EPS produced by *Enterobacter* sp. PRIM-26 (62%) (Priyanka et al. 2015). The EPS-RN5 contained 14.5 ± 2.9% proteins and small amounts of uronic acid (8.3 ± 0.3%). The presence of uronic acid groups in the polysaccharides imparts bioactivity having implications in regenerative medicine, tissue engineering and also act as anti-thrombotic and anti-arthritic agents (Silvi et al. 2013). The isolate *Enterobacter* sp.YU16-RN5 produced EPS with a molecular mass of 0.7×10^6 Da. However, the EPS produced by *Enterobacter* sp.YG4 had a molecular mass of 11.99×10^6 Da (Nagaraj et al. 2016), the EPS of *Enterobacter cloacae* and *Enterobacter* strain A47 had a molecular mass of 1.7×10^6 and 5.8×10^6 Da, respectively (Meade et al. 1994; Freitas et al. 2009). It has to be noted that the genus *Enterbacter* produces high molecular

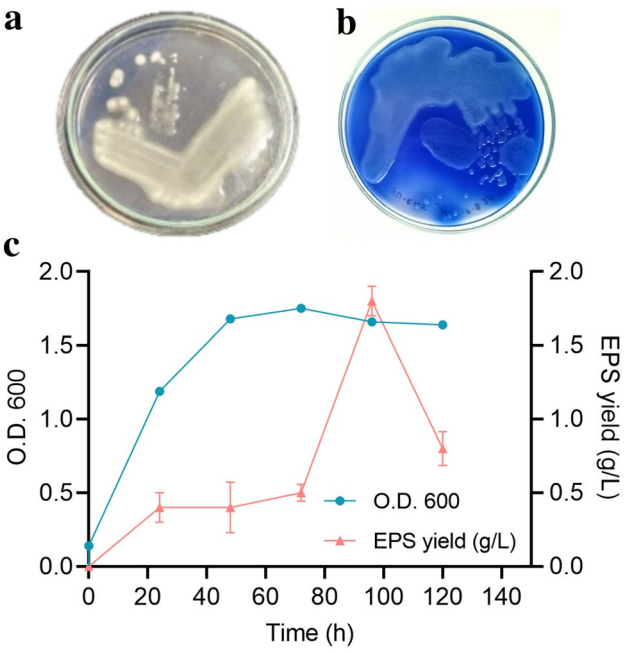

Fig. 1 EPS production by the bacterial isolateYU16-RN5. **a** Mucoid colonies of YU16-RN5 grown on yeast extract mannitol (YEM) agar. **b** Bacteria colonies grown in YEM media with 0.05% aniline blue are stained blue due to the presence of β-glucan. **c** Graph showing the growth pattern of YU16-RN5 in YEM broth and EPS yield at different time points

weight EPS which often leads to increased viscosity and is considered as one of the most desirable rheological features (Herbst et al. 1992).

The FT-IR spectrum of the EPS-RN5 showed a characteristic peak of polysaccharide at 3289–3742 cm^{-1}, representing O–H stretching of hydroxyls (Fig. 2a). The band at 3420 cm^{-1} represents the presence of N–H group. The well-defined peaks found between 1200 and 900 cm^{-1} represents skeletal C–O and C–C vibration of glycosidic bonds and pyranoid rings. The peak at 1250 cm^{-1} may also be attributed to the C–O–C vibration of acyls. The two strong bands around 1607 and 1405 cm^{-1} in the spectrum can be attributed to the asymmetric and symmetric stretching of carboxylates, respectively (Synytsya et al. 2003). The band at 1720 cm^{-1}observed is attributed to the C=O stretching of carbonyls in acyl groups (Alvarez-Manceñido et al. 2008). However, the ionizable functional groups such as carboxy, carbonyl and amide groups can contribute to the binding mechanism of cadmium by EPS (Camacho-Chab et al. 2018). The peak at 894 cm^{-1} is assigned to β-glycosidic linkage and a peak at 1721 cm^{-1} indicates the presence of uronic groups (Wang et al. 2015).

Thermal stability of the EPS-RN5

The thermostable property of the biopolymers plays an essential role in its diverse industrial utilization (Marinho-Soriano and Bourret 2005). The TGA analysis (weight loss versus temperature) showed that EPS-RN5 degrades in two steps wherein the first phase represents the decrease in weight due to loss of water molecules and in the second phase depolymerization occurs (Fig. 2b). In the first step, weight loss of 12% within 153 °C and in the second step depolymerization at 273 °C, with 49% loss was observed. This depolymerization temperature of EPS-RN5 is comparable to that of xanthan gum (278 °C with 30% of weight loss), KF5 EPS (279.6 °C) and locust gum (278.5 °C) (Wang et al. 2010). The stability of EPS-RN5 at higher temperatures makes it a promising additive as a stabilizer, thickener and gelling agent in value-added products.

Monosaccharide composition

From the GC–MS analysis the monosaccharide composition of EPS-RN5 was identified as glucose, sorbose and galactose in the ratio 0.25: 0.07: 1.0 (Fig. 3). The EPS also contained low amounts of mannoonic acid, pentanoic acid, ribitol, tri, octa and hexadecanoic acids. Whereas, EPS of *Enterobacter* sp. A3CK showed mannose, glucose and galactose and that of *E. cloacae* strain P2B was reported to contain mannose, glucose, galactose and xylose (Naik et al. 2012). However, the sugar monomers of EPS of *Enterobacter* sp. MS16 was composed of glucose, galactose, and arabinose (Jhadhav et al. 2011) and that of *Enterobacter* A47 contained glucose, galactose and fucose, (Torres et al. 2014). Thus, it shows that EPS produced by most of the *Enterobacter* sp. possess glucose and galactose monomers in common. Whereas, the other monomers might be strain specific or expressed in different environmental conditions (Lloret et al. 1998). Furthermore, the EPS produced by *Enterobacter* sp. A47 DSM 23139 composed of fucose, glucose, galactose, glucuronic acid, pyruvate, succinate, acetate (Freitas et al. 2011; Huang

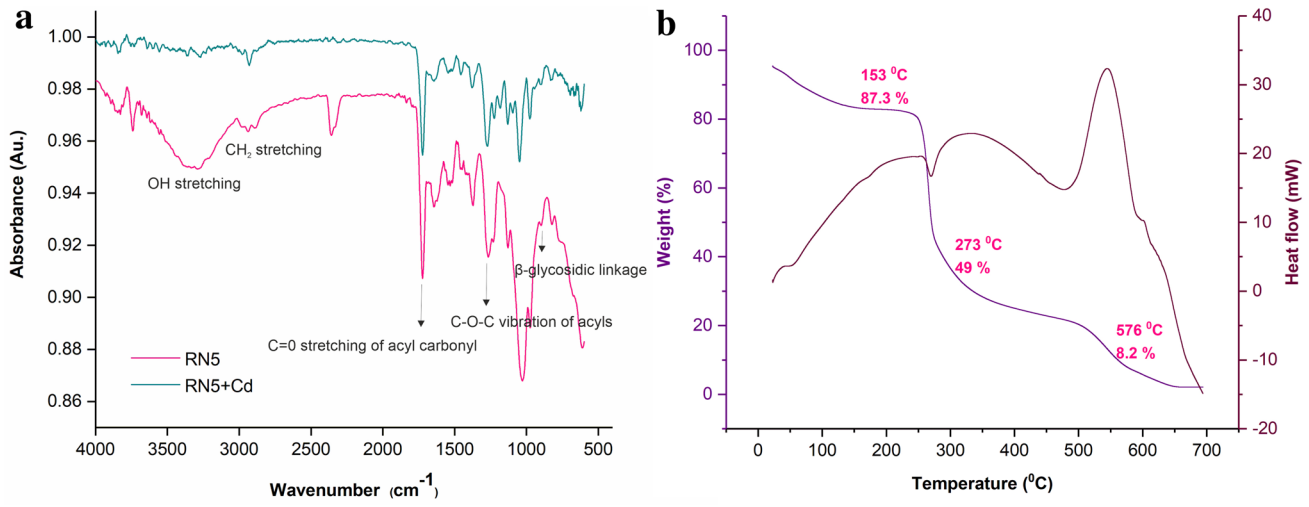

Fig. 2 FT-IR and thermogravimetric analysis of EPS-RN5. **a** FT-IR spectra of EPS-RN5 (pink lines) and EPS-RN5 chelated with cadmium (green lines) showing major peak. **b** Thermogravimetric degradation pattern of EPS-RN5 showing three phases of degradation at 153, 273 and 576 °C

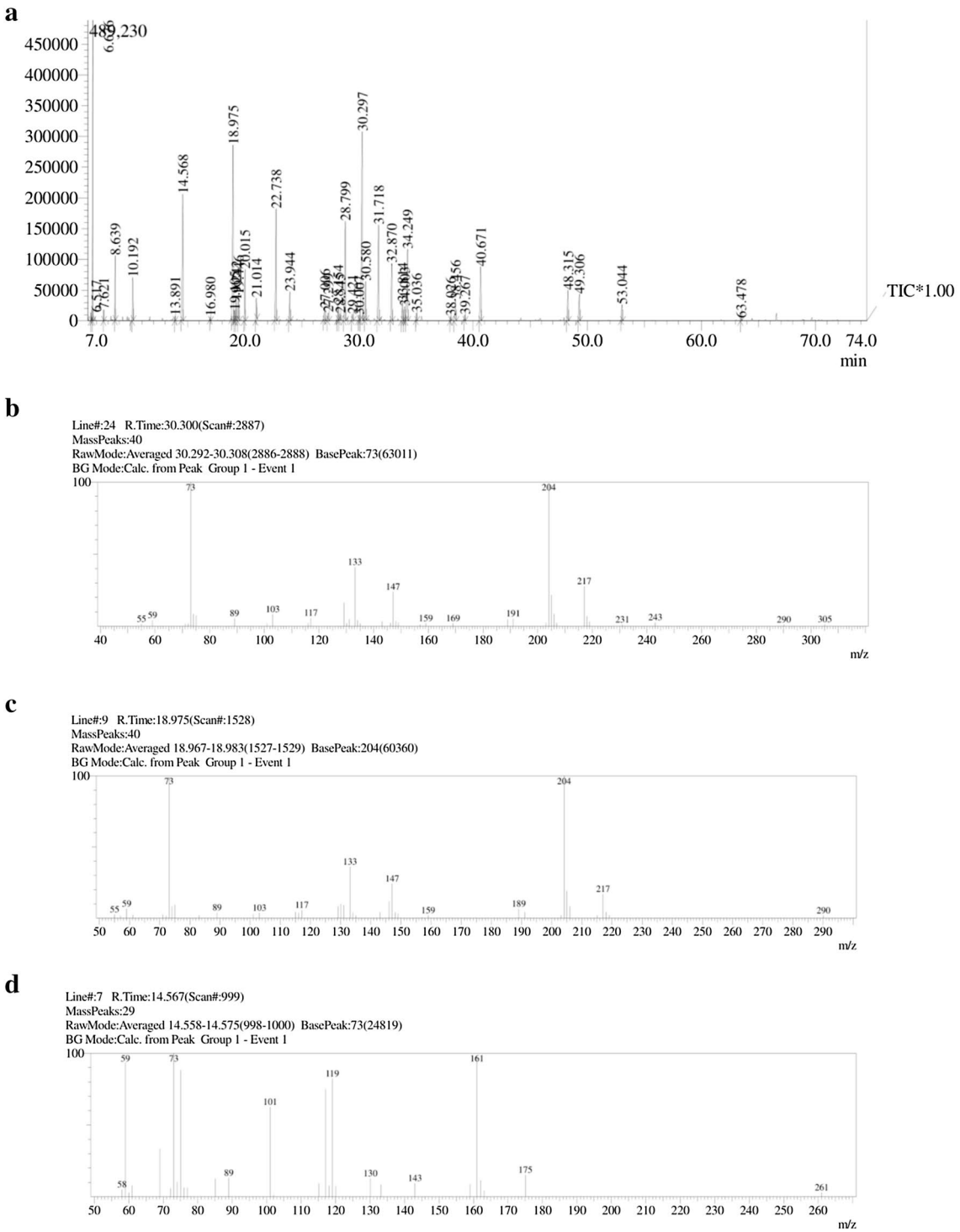

Fig. 3 Gas chromatogram of EPS-RN5 (**a**), the mass spectra of the peaks with retention time 30.297 (**b**), 18.975 (**c**) and 14.568 (**d**)

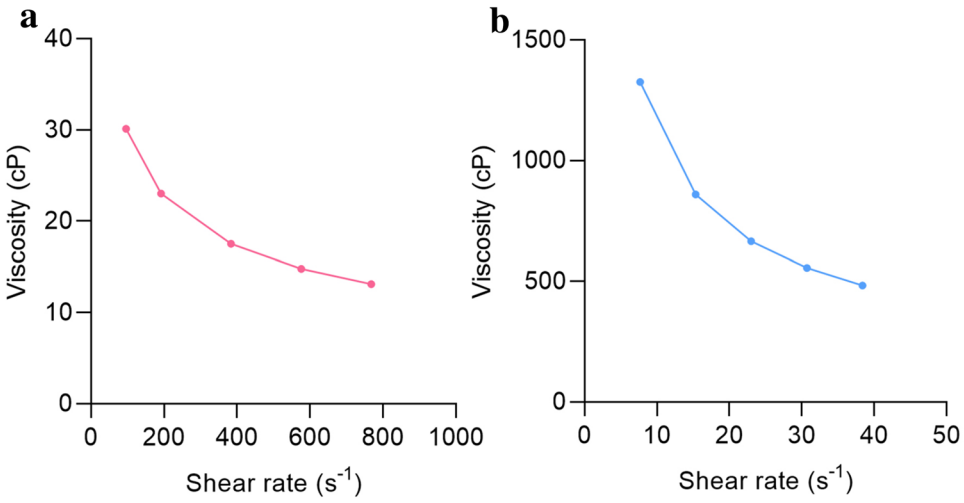

Fig. 4 Rheological behaviour of EPS-RN5 at **a** 0.5% concentration and **b** 0.5% xanthan taken as a positive control

et al.2015) is reported with hypoglycemic and hypolipidemic activities in type 2 diabetic mice (Huang et al. 2015).

Rheological properties of EPS-RN5

To evaluate the rheological properties, the steady flow behavior of an aqueous solution of EPS-RN5 (0.5% w/v) over a range of shear rates were measured. The EPS-RN5 showed a slight decrease in the viscosity at increasing shear rates of 96–768 s^{-1}, indicating its shear-thinning behavior (Fig. 4a). Xanthan gum (0.5%) that shows shear-thinning behavior was used as the control (Fig. 4b). The shear stress versus shear rate data for EPS-RN5 at different concentrations at 25 °C, fitted well to the power-law model. According to the power-law model, the value of the consistency index (K) was 187 cP and flow behavior index (n) was 0.6 for the EPS-RN5 solution. The EPS-RN5 solution also showed thixotropic behavior wherein, the viscosity lost during increased shear stress was regained upon the removal of stress-inducing forces. Shear-thinning phenomenon occurs when the polymer chains align themselves in the direction of flow, reducing the chemical interaction in the chain entanglement (Vardhanabhuti and Ikeda 2006). EPS-RN5 is water soluble and forms non-Newtonian solutions that forms a gel-like substance. Such polysaccharides with unique rheological properties are used as a thickening, gelling, or stabilizing agents in the food industry (Jindal and Khattar 2018). The pseudoplastic property contributes to better sensory qualities of food and also helps in different levels of food processing (Moreno et al. 2000). Many applications in cosmetics,

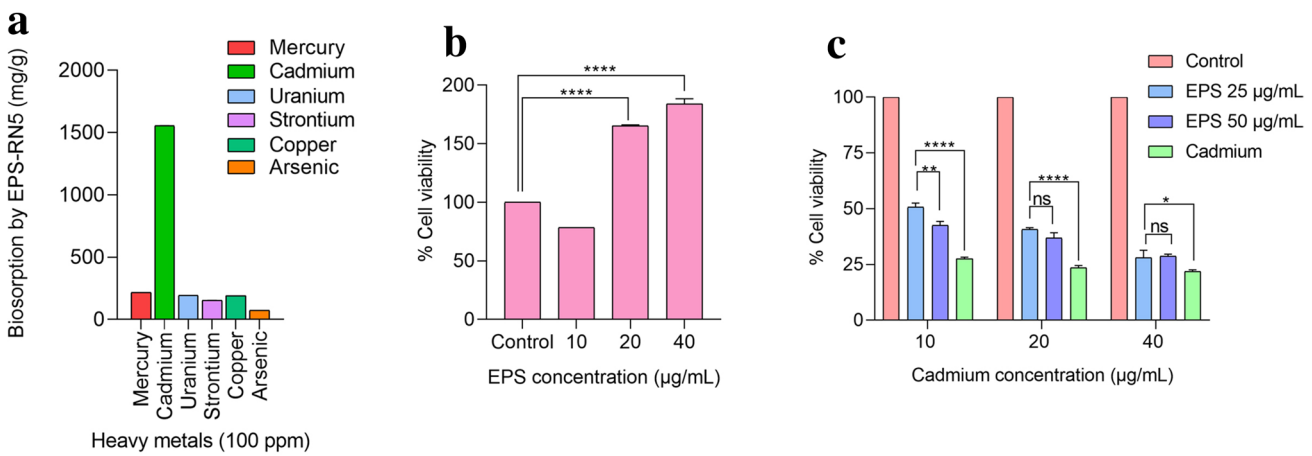

Fig. 5 Quantification of heavy metal biosorption and cell protective effect of EPS-RN5 against cytotoxicity of cadmium.Biosorption of EPS-RN5 at 1.0% concentration (w/v) against selected heavy metals (**a**). Cell proliferative activity of EPS-RN5 tested using MTT assay (**b**). Protective effect of EPS-RN5 against the cadmium-induced cytotoxicity on the HEK cells evaluated using MTT assay (**c**). Data are represented as mean ± SD ($n = 3$). Significance of difference between the groups are indicated by *$p \leq 0.05$; **$p \leq 0.01$; ****$p \leq 0.0001$; ns-non significant ($p > 0.05$)

foodstuffs, or the petroleum industry require the EPS with pseudoplastic qualities.

Metal biosorption

The heavy metal ions of mercuric sulfate, cadmium chloride, strontium chloride, copper chloride, sodium arsenate and uranyl nitrate in an aqueous solution (100 ppm) mixed with EPS-RN5 (1% w/v) resulted in the precipitation. The metal content in the precipitate was measured by ICP-MS and showed that, cadmium absorption was the highest (1555 mg/g) followed by mercury (218 mg/g). EPS-RN5 also showed absorption of strontium, copper, arsenic and uranium ions but at comparatively lower levels (Fig. 5a). The absorption behavior could be attributed to the metal ion acting as a complexation agent of protein or such other compounds (Yang et al. 2015) present in the EPS-RN5. The presence of uronic acids, pyruvate, and inorganic residues such as phosphate or sulfate, acidic amino acids, and phosphate-containing nucleotides along with ionisable functional groups and non-carbohydrate substituents like acetamido groups in the EPS can also render negative charge to the EPS that in turn interact with positively charged metal ions playing an important role in metal binding property (Mohite et al. 2017). The amino acid and protein-like substances in the EPS is also believed to assist in heavy metal absorption mechanism (Cui et al. 2020). The chelation was confirmed by comparing the metal concentration in the supernatant that showed a relative decrease due to its precipitation from the solution. The comparison of IR spectrum of the EPS-RN5 and metal-bearing EPS-RN5 from the precipitate showed marked changes in the peak positions and peak intensity. The EPS-RN5 containing cadmium showed a sharp peak shift at 2934 cm^{-1}, representing the –CH stretching vibration and 3400 cm^{-1} of –OH vibration. Involvement of such groups in the complexation of cadmium has earlier been reported (Zeng et al. 2020). There are also reports indicating the contribution of hydroxyl (O–H) and carbonyl (C = O) groups of the polymer in metal chelation (Shuhong et al. 2014). Moreover, the absorption rate may also be dependent on the metal ion concentration and/or absorbent concentration (Feng et al. 2012; Chakraborty et al. 2018)and the shift in FT-IR peaks correspond to the metal-binding process occurring on the surface of the biosorbent (Pavasant et al.2006). The formation of metal cation complex by the EPS results in metal immobilization within the EPS (Gupta and Diwan 2017). In view of these properties there is a great interest in the application of EPS as bioflocculants, bioabsorbents, drug delivery and heavy metal removal agents (Salehizadeh and Yan 2014). The EPS from cadmium-tolerant bacteria *E. ludwigii* LY6 and *Enterobacter* sp. DNB-S2 have shown a similar biosorption mechanism of the heavy metals (Biswas et al. 2020; Sun et al. 2020a, b). The EPS-RN5 exhibited excellent cadmium binding activity and removed 88.9% of cadmium from the solution and this could be related to its distinct degree of specificity and affinity. The electro-negativity and radius of metal ion also influence its affinity for the EPS binding sites (Can and Jianlong 2007).

Role of EPS-RN5 against cytotoxicity induced by cadmium

Toxicity of cadmium can cause damage to different organs including kidney, lung, liver and bone. However, the primary target organ of cadmium toxicity is the kidney. Moreover, cadmium is considered as a model cumulative nephrotoxicant (Klaassen et al. 2009). Hence, HEK 293T (human embryonic kidney cells) cell lines were chosen for the study. Cadmium tested at different concentrations on HEK 293T cells showed a dose-dependent toxicity with more than 80% cell killing at 40 µg/mL. On the other hand, the β-glucan-like EPS-RN5 showed the highest biocompatibility and exhibited the cell proliferative activity up to 40 µg/mL (Fig. 5b). Based on these results, the EPS-RN5 was tested against cadmium-induced cytotoxicity. The EPS-RN5 treatment reduced the cell killing effect of the cadmium significantly and the cell viability of the cadmium exposed (10 µg/mL) HEK 293T cells showed 50.7% cell viability at 25 µg/mL from 27.7% (Fig. 5c). However, the EPS at higher concentration (50 µg/mL) showed a lower cytoprotective effect. This may be due to the higher chelation of metal ion by the EPS changing the cell microenvironment due to the higher viscosity of the culture media. At higher cadmium concentration (40 µg/mL), no significant difference in the cell viabilities between the EPS-RN5 concentrations was observed. The capacity of EPS containing β-glucans to reduce heavy metal-induced cytotoxicity under in vitro conditions has been reported in *Enterobacter* sp. YG4 (Nagaraj et al. 2018). From our study, it is evident that the treatment of HEK 293T cells with EPS-RN5 could reduce the cytotoxicity of cadmium chloride. A few studies have also attributed these effects to the antioxidant activity of the EPS (Nagaraj et al. 2016) as well as the ability to neutralize the reactive oxygen species thereby protecting the cells against oxidative stress and preventing apoptosis (Nagaraj et al. 2018). Further studies on understanding the exact mechanisms of metal chelation will provide opportunities for utilizing the EPS-RN5 for specific applications in cadmium toxicity and remediation.

Conclusion

The EPS-RN5 produced by *E. cancerogenus* strain YU-16RN5 showed prominent shear thinning, thermostable, cell proliferative and metal chelation properties. These

characteristics of EPS-RN5 could prove it to be an ideal candidate for food, pharmaceutical and biomedical industries. The presence of anionic functional groups plays an important role in the biosorption of heavy metals particularly cadmium ions. The cytoprotective role of EPS-RN5 against cadmium-induced cytotoxicity in kidney cells in vitro can provide applications in addressing cadmium toxicity.

Author contributions DBE and PDR contributed to the study conception and design. Material preparation, data collection and analysis were performed by DBE, Athmika. The first draft of the manuscript was written by DBE. The manuscript was reviewed and edited by DBE, Athmika and PDR. The work was supervised by PDR. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. The 16S rRNA gene sequence has been deposited in the GenBank with the accession number MH191375.

Declarations

Conflict of interest The authors declare no competing interests.

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