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Bioprospecting of exopolysaccharide-producing bacteria from different natural ecosystems for biopolymer synthesis from vinasse

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Abstract

Background: Exopolysaccharides (EPSs) belong to a family of organic thickeners or alternative hydrocolloids of microbial origin. Because the chemical structure offers beneficial bioactive functions, biocompatibility and biodegradability, EPSs are used in the chemical, food, pharmaceutical, cosmetics, and packaging industries as well as in agriculture and medicine. In this study, new bacterial strains were selected on the basis of their ability to synthesize EPS from substrate containing vinasse as a nutrient source to identify the best candidate for bio-based polymer production.

Results: Among the 99 newly identified bacterial strains isolated from different natural ecosystem, the strain *Azotobacter chroococcum* 76A was selected as the best biopolymer producer since it synthesized the highest concentration of EPS in all media containing vinasse. The maximum EPS concentration (44.6 ± 0.63 mg/50 mL) was observed at 24 h, corresponding to its sub-stationary growth phase ($7 \times 10^8 \pm 0.29$ CFU/mL). Chemical characterization of the EPS produced showed that carbohydrates representing the principal component, followed by uronic acids and proteins. Interestingly, comparing the IR spectrum of the EPS with alginate by FTIR-ATR analysis revealed an overlap of a peak identified as guluronic acid, a component of alginate.

Conclusions: The potential biotechnological capacity of *A. chroococcum* 76A to synthetize biopolymer from vinasse, inexpensive starting materials, represents a possible alternative to expensive disposal of agri-food waste through its transformation into high value-added products.

Keywords: Bacterial selection, *Azotobacter chroococcum*, Agri-food waste, Vinasse, Exopolysaccharides, Biopolymers

Background

Exopolysaccharides (EPSs) are useful for an extensive range of industrial applications. They are renewable sources of hydrocolloids, which are used in the food, pharmaceutical, agricultural, cosmetics and medical as well as in the chemical industries, where they replace petroleum-based polymers [1–4].

EPSs are produced by bacteria, algae and, in smaller amounts, by yeasts and molds [5–7] to protect the cell from unfavorable, limiting or toxic conditions [8], thereby improving microbial competition in different environments [9]. A great variety of microbial EPSs are known to be synthesized by different microbial genera, such as dextran by *Leuconostoc* and *Lactobacillus*, gellan by *Sphingomonas* and *Aureomonas*, xanthan by *Xanthomonas*, alginates by *Pseudomonas* and *Azotobacter*, succinoglycan by *Alcaligenes* and *Rhizobium*, hyaluronic acid by *Streptococcus*, schizophyllan by *Schizophylum*, levan by *Alcaligenes* and *Zymomonas*, pullulan by *Aureobasidium*,

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cellulose by *Acetobacter*, chitosan by *Mucorales*, galactoglucopolysaccharides and biosurfactant by *Achromobacter*, *Agrobacterium*, *Pseudomonas*, *Rhizobium* and *Zooglea*, scleroglucan by *Sclerotium*, and welan by *Alcaligenes* [9–11]. Due to the wide diversity of structures and functional properties, there is an increasing interest in EPSs synthesized by microorganisms [12–14]. In fact, microbial EPS could represent a good alternative to EPS obtained from plant, animal and seaweed, because they can be produced under controlled conditions [15–17]. However, bacterial EPS represents only a small fraction of the current biopolymer market because of their high cost of production, which is mostly related to substrate cost and recovery [10, 18]. Therefore, the use of cheaper substrates, such as agricultural byproducts and waste materials, could represent a good approach to reduce the production costs for EPS biosynthesis.

Currently, the management and disposal of vinasse, a recalcitrant waste of the sugar-ethanol industry, has emerged as a priority from an environment perspective due to its polluting load, especially Biological Oxygen Demand (BOD) [19, 20]. Agri-food wastes are additional cheap, sustainable and attractive substrate for the production of biopolymers or other high value-added products [21]. Many studies have assessed the recycling and the potential use of agri-food wastes and byproducts or dedicated energy crops for producing polyhydroxyalkanoate (PHA) [22, 23], succinic acid [24–27], biofuels and biogas [19, 28–32] as well as biological hydrogen and volatile fatty acids [33, 34]. Natural environments represent important sources of microbial strains that exert interesting enzymatic activities for biotechnological applications [35].

The aim of this study was to select bacterial strains on the basis of their ability to synthesize EPS from substrate containing vinasse as a nutrient source and identify the best candidate for bio-based polymer production.

Methods

Composition of sugarcane vinasse

Sugarcane vinasse was kindly provided by Agriges S.r.l. (San Salvatore Telesino, Benevento, Italy).

The chemical composition of sugarcane vinasse was determined by high-performance liquid chromatography (HPLC) (Refractive index detector 133; Gilson system; pump 307, column Metacarb 67 h, Varian, with a flow of 0.4 mL/min of 0.01 N H_2SO_4). Chemical oxygen demand (COD) was estimated with an ECO08 thermoreactor (Velp Scientifica, Usmate, Monza Brianza, Italy) and a PF-3 photometer (Velp Scientifica) using NANOCOLOR® kit. BOD_5 was measured with a BOD Sensor System 6 (Velp Scientifica) according to manufacturer's instructions.

Bacterial strains and EPS production on solid media

Ninety-nine bacterial strains isolated from different natural ecosystems (lignocellulosic biomass, soil, compost, bakery products) were used in this study: 27 endo-cellulolytic bacteria, 14 exo-cellulolytic bacteria [36], 3 free-living N_2-fixing bacteria belonging to *Azotobacter chroococcum* species [37], 42 *Bacillus* spp. [38], 3 lactic acid bacteria [39] and 10 *Pseudomonas* spp. [37, 40]. To carry out a preliminary selection, all bacterial strains were first refreshed in a specific nutrient media and incubated for 24–48 h at 30 °C. For EPS detection, all strains were streaked on Salts Agar Base medium (SAB) (composition per liter: 0.15 g KH_2PO_4, 0.5 g K_2HPO_4, 0.2 g $MgSO_4$, 0.1 g NaCl, 18 g Agar, pH 6.8–7) to which mannitol (1%) or sucrose (5%) was added as carbon sources with yeast extract (0.4%). After incubation for 48 h at 30 °C the bacterial strains growth as ropy colonies (data not shown) were further assayed for their ability to produce EPS under specific nutritional conditions. Specifically, the selected strains were streaked onto SAB medium containing a mixture of different concentrations of sucrose (0, 0.5, 1, 1.5, 2 or 5%), sugarcane vinasse (0, 1, 5 or 10%) and with yeast extract (0.4%). Inoculated plates were examined after 48 h of incubation at 30 °C to detect the presence of ropy colonies due to putative production of EPS.

EPS synthesis by selected *Azotobacter chroococcum* strains in liquid medium

On the basis of the capacity to growth and form ropy colonies in the different solid media containing vinasse as carbon source, *A. chroococcum* 76A, AZ1 and 67B were selected to evaluate their ability to produce EPS in liquid medium. These bacterial strains were precultured in 10 mL of Yeast Mannitol (YM) broth and incubated for 24–48 h at 30 °C. After inoculum standardization using a Thoma cell counting chamber (depth 0.02 mm; 1/400 mm^2; Hawksley, United Kingdom), 1 mL of each culture was added to achieve viable counts of approximately 2×10^7 cells/mL in Salts Broth (SB) containing vinasse (1%) and sucrose (5%) as carbon sources. After 24 and 48 h of incubation at 30 °C, tenfold serial dilutions of the cultures were made in Ringer solution (Oxoid, Milan, Italy) and bacterial cells were enumerated by spreading 100 μL of each dilution on YM solid medium plates. EPS was quantified as described by Palomba et al. [16]. Briefly, supernatant containing EPS was precipitated with 2 volumes of chilled 98% (vol/vol) ethanol and incubated overnight at 4 °C. After centrifugation (5200×*g* for 10 min at 4 °C), the recovered pellets were suspended in 1 mL of distilled water, freeze-dried and weighed to obtain the amount of EPS, expressed as polymer dry mass (PDM, mg/30 mL of wet medium).

Monitoring and characterization of EPS produced by *Azotobacter chroococcum* 76A

Monitoring of microbial growth and EPS production

Azotobacter chroococcum 76A was inoculated into 50 mL of liquid substrate composed of vinasse (1%) and sucrose (5%) as carbon sources. Samples were withdrawn immediately after inoculation and after 8, 16, 24, 32 and 48 h of incubation at 30 °C, to determine bacterial growth and EPS concentration performed as described above.

Chemical characterization of EPS

After the inoculum standardization, *A. chroococcum* 76A was inoculated in liquid or solid media containing vinasse (1%) and sucrose (5%). After 24 h at 30 °C, the EPS was recovered from liquid cultures as described above or was taken directly from plates (Fig. 1) by repeatedly washing with distilled water until disappearance of the visible ropy patina, after which the EPS was collected in sterile falcon tubes [16]. EPSs obtained from the liquid or solid media were precipitated by ethanol, freeze-dried and then dissolved in hot water, cooled at room temperature and finally dialyzed against water for 3 days (Visking Dialysis Membrane MWCO 12–14 kDa, GmbH, Germany). Next, the EPS samples were freeze-dried again. Gross chemical composition was determined by assaying the contents of total carbohydrates, proteins and uronic acids. The total carbohydrate contents were quantified according to the phenol–sulfuric acid method [41] using a standard curve with glucose. The protein concentrations were determined using a Bradford protein assay kit (BioRad, Milan, Italy) and Bovine Serum Albumin (BSA) as a standard [42]. The total content of uronic acids was determined according to the method described by Blumenkrantz and Ashoe-Hansen [43] using galacturonic acid for calibration. The monosaccharide composition of the EPS was determined after acid hydrolysis with 2 N trifluoroacetic acid (TFA) at 120 °C for 2 h using a glucose enzymatic assay [44]. The spectral characterization was performed via Attenuated Total Reflection Fourier Transform Infrared (FTIR-ATR) spectroscopy and by ^{1}H-NMR. The infrared spectra of the EPS were recorded at room temperature with a Spectrum 100 FTIR spectrometer (Perkin-Elmer Inc., Norwalk, CT, USA) equipped with a crystal diamond universal ATR sampling accessory. For the ^{1}H-NMR analysis, the samples were dissolved in D_2O (5 mg/mL) and spectra were recorded on a Bruker AMX-600 MHz ^{1}H-NMR at 40 °C [45].

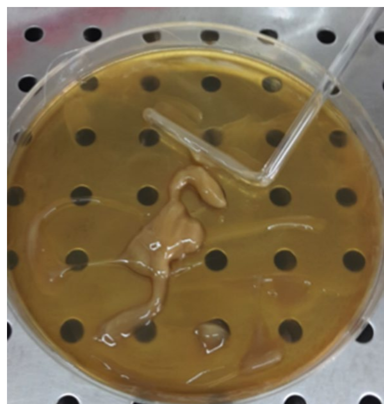

Fig. 1 Collection of EPS produced by *Azotobacter chroococcum* 76A from solid medium containing vinasse (1%) and sucrose (5%) after 24 h of incubation at 30 °C

Statistical analyses

One-way ANOVA followed by Tukey's HSD post hoc test for the pairwise comparison of means (at $P<0.05$) were used to assess the differences in EPS production. Statistical analyses were performed using the SPSS 21.0 statistical software package (SAS Inc., Cary, NC).

Results and discussion

Screening of EPS-producing bacterial strains on solid media containing sugarcane vinasse

Ninety-nine bacterial strains previously isolated from different matrices were tested to identify high EPS-producing strains. The preliminary screening allowed the selection of 14 strains able to grow and form ropy colonies on solid media containing mannitol or sucrose with or without yeast extract (0.4%). Interesting was to observe that the natural soil ecosystem showed the highest occurrence of EPS-producing bacteria (38.5%, Table 1). Hence, studies of the occurrence of bacteria with structural elements such as capsules or polysaccharides that have physiological functions in different natural ecosystems are important to obtain better knowledge of the origin and evolution of bacteria. Moreover, the higher occurrence of EPS production by bacterial strains isolated from soil or lignocellulosic biomass have an ecological meaning since these traits may be used by naturally occurring bacteria to survive

Table 1 Percentage of bacterial strains able to growth and secrete EPS on solid media containing a mixture of different concentrations of mannitol (1%) or sucrose (5%) as carbon sources

Source	Number of tested strains	EPS-producing strains (%)
Soil	13	38.5
Lignocellulosic biomass	41	14.6
Bakery products	45	6.7

and grow in specific habitats enhancing their environmental survival [36, 37, 46].

EPS-producing bacterial strains from the preliminary screening, were further tested on media containing sugarcane vinasse composed of 30.33 g/L of lactic acid, 29.6 g/L of succinic acid, 12.63 g/L of acetic acid, 6.77 g/L of fructose, and 3.26 g/L of ethanol and had a pH of approximately 6.4, COD and BOD values of 556 g/L and 216 g/L, respectively. In particular, *Xanthomonas campestris* CP81 and SBP63, *Sphingobacterium multivorum* CA77, *Labedella gwakjiensis* CP710, *Aurantimonas altamirensis* SBP73 and *Curtobacterium flaccumfaciens* CP77b isolated from lignocellulosic biomass, *A. chroococcum* 76A, *A. chroococcum* 67B, *A. chroococcum* AZ1, *Pseudomonas gessardii* SA33A and *Pseudomonas* sp. SA33B isolated from soil and *Leuconostoc lactis* 95A, *Leuconostoc lactis* 69B and *Lactobacillus curvatus* 69B2 isolated from bakery products, were screened on solid media with different concentrations of sugarcane vinasse and sucrose, as carbon sources. Increasing the vinasse concentration resulted in a reduction of bacterial strains able to grow and produce EPS on the solid media that were tested (Table 2). However, more bacterial strains were able to grow and synthetize EPS from vinasse when sucrose was added to the medium. In fact, by increasing the concentration of sucrose from 0.5 to 2%, the number of bacterial strains that exhibited ropy colonies increased from 7 to 13. In contrast, a higher amount of sugarcane vinasse seemed to result in an inhibitory effect, since only four strains exhibited EPS production on solid media containing 5% of vinasse. Increasing the concentration of vinasse up to 10% resulted in the inhibition of bacterial growth (Table 2). The presence of specific toxic compounds such as furfural, HMF, *p*-hydroxybenzoic aldehyde and vaniline, are known to have inhibitory effects on microbial metabolism, limiting the efficient conversion of fermentable sugars into biochemicals [25, 26, 47].

Similarly, Vermani et al. [48] reported that high concentrations of glucose and lactose in culture media exerted inhibitory effects on the microbial growth and EPS synthesis in *Azotobacter* strains.

Among the fourteen bacterial strains tested, only *A. chroococcum* 76A, *A. chroococcum* 67B and *A. chroococcum* AZ1 were able to produce EPS on media containing 1% or 5% of sugarcane vinasse without added sucrose or yeast extract as additional nutritional sources.

Table 2 Screening of EPS-producing bacterial strains on solid media composed by Salts Agar Base (SAB) with nutrient mixtures of vinasse (1, 5 or 10%) and sucrose (0, 0.5, 1, 1.5, 2 or 5%) as carbon sources with and without yeast extract (0.4%)

Composition of nutrient mixtures added to SAB			Number of positive strains[a]
Vinasse (mL/L)	Sucrose (g/L)	Yeast extract (g/L)	
10	0	0	3
10	0	4	3
10	5	0	7
10	10	0	7
10	15	0	8
10	20	0	10
10	50	0	13
50	0	0	3
50	0	4	3
50	5	0	3
50	10	0	3
50	15	0	3
50	20	0	4
50	50	0	6
100	0	0	0
100	0	4	0
100	5	0	0
100	10	0	0
100	15	0	0
100	20	0	0
100	50	0	0

V: Vinasse; 10 mL/L = 1%, 50 mL/L = 5%, 100 mL/L = 10%

S: Sucrose; 0 g/L = 0%, 5 g/L = 0.5%, 10 g/L = 1%, 15 g/L = 1.5%, 20 g/L = 2%, 50 g/L = 5%

Y: Yeast extract; 0 g/L = 0%, 4 g/L = 0.4%

[a] Number of bacterial strains that grew as ropy colonies on solid media within 48 h of incubation at 30 °C

Growth and EPS production by *A. chroococcum* strains in liquid media containing sugarcane vinasse

Based on the results obtained using the solid media, three selected *Azotobacter* strains were tested in liquid media containing vinasse (1%) with or without sucrose (2% or 5%). All experiments were performed at 30 °C, which was reported as the optimum temperature for EPS production by *Azotobacter* spp. [49, 50]. After a 24-h incubation, only *A. chroococcum* 76A grew in the medium containing vinasse as the sole carbon source, and it produced the highest quantity of EPS (25.3 ± 0.10) in the medium containing 5% of sucrose after 48 h of incubation at 30 °C (Table 3). *A. chroococcum* 67B and AZ1 required 5% of sucrose to grow, although lower EPS concentrations (22.8 ± 0.22 and 21.5 ± 0.26 mg/30 mL, respectively) were detected (Table 3). 1% vinasse and 5% sucrose as carbon sources was the best substrate for stimulating the growth and metabolism of *A. chroococcum* 76A, 67B and AZ1. The ability of some strains belonging to the genus *Azotobacter* to synthesize EPS was widely recognized and reported [50–52] but *A. chroococcum* has been

Table 3 Evaluation of EPS synthesis by different *A. chroococcum* strains in liquid media containing vinasse (1%) without and with 2% or 5% of sucrose after 48 h of incubation at 30 °C

A. chroococcum strains	V (1%)		V (1%) S (2%)		V (1%) S (5%)	
	Growth*	EPS[§]	Growth*	EPS[§]	Growth*	EPS[§,†]
76A	+	− +	+	− +	+	+ 25.3 ± 0.10[a]
67B	–	–	–	–	+	+ 22.8 ± 0.22[b]
AZ1	–	–	–	–	+	+ 21.5 ± 0.26[c]

V vinasse, *S* sucrose

*bacterial growth: − = absence of growth; + = presence of growth (about 10^8 CFU/mL)

[§] EPS synthesis: − = no synthesis of EPS; − + = low synthesis of EPS; + = high synthesis of EPS

[†] mg of EPS/30 mL of medium. The values represent the mean ± S.D. of three replicates of independent experiments. Different letters indicate significant difference ($P \leq 0.01$)

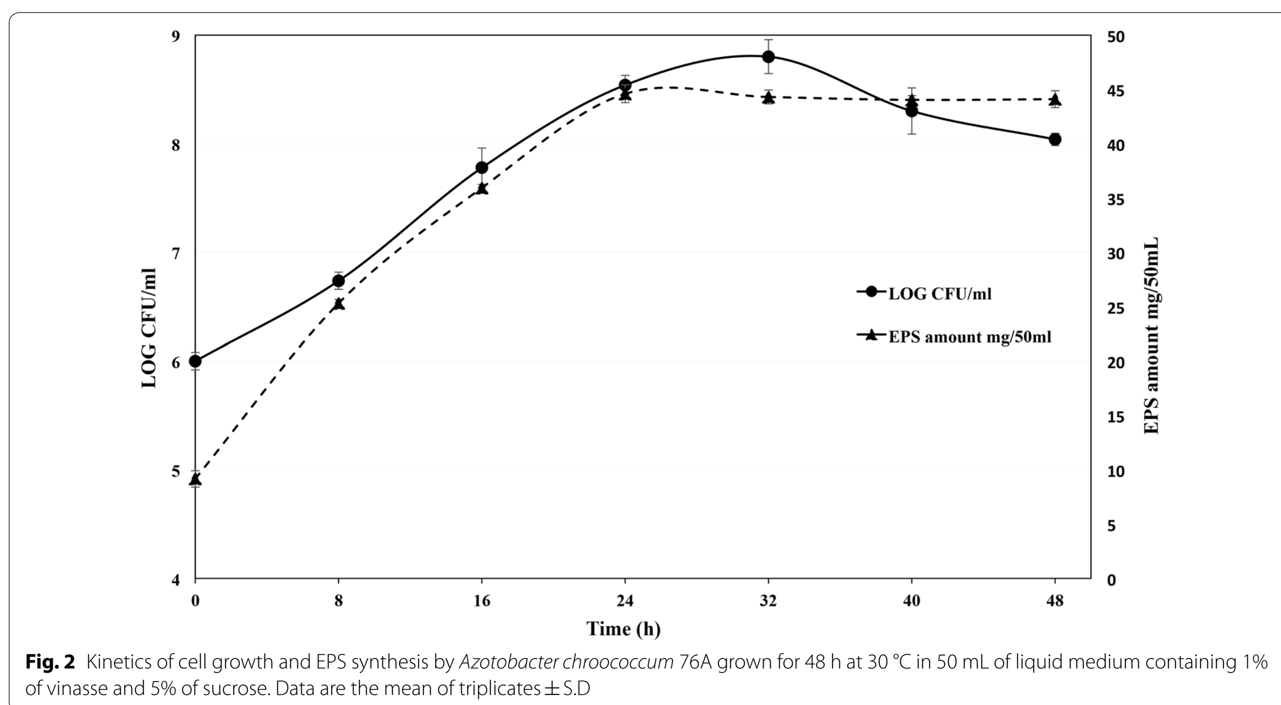

Fig. 2 Kinetics of cell growth and EPS synthesis by *Azotobacter chroococcum* 76A grown for 48 h at 30 °C in 50 mL of liquid medium containing 1% of vinasse and 5% of sucrose. Data are the mean of triplicates ± S.D

extensively especially studied for its ability to promote plant growth under environmental stress conditions due to the synthesis of enzymes and EPS [53–55] but its ability to grow in medium containing vinasse was less investigated [56]. However, to our knowledge, this is the first study that explored the capacity of *A. chroococcum* to synthetize EPS utilizing vinasse as a carbon source. Quagliano and Iyazaki [57] tested the effect of different carbon sources on the production of EPS by *A. chroococcum* 6B. Using pure sugars, such as glucose and fructose, this strain was able to synthesize high amounts of EPS, with poly-β-hydroxybutyrate (PHB) also detected when the strain was grown in the presence of glucose. Utilizing a complex carbon source such as sugarcane molasses, the concomitant production of EPS and PHB was observed [23]. The high amount of EPS obtained in this study using a complex carbon source could be due to the presence of fructose in the sugarcane vinasse. In fact, fructose, especially in nitrogen-limiting condition and an excess of carbon, promotes bacterial growth of diazotrophic bacteria [57], and therefore, EPS production since it is closely coupled with cell growth [58]. Moreover, fructose improves also EPS biosynthesis as fructose-6-phosphate is the first metabolite to be converted to mannose 6-phosphate in the pathway for alginate biosynthesis [57]. In addition, in stress conditions such as nitrogen limitation, *Azotobacter*

spp. cultures grow under nitrogen fixation producing acidification of medium, that it is associated with EPS excretion [59].

Based on its biotechnological capabilities, *A. chroococcum* 76A was selected for further investigations. This strain was extensively characterized and previously selected and investigated as bio-effector in sustainable agricultural systems also under abiotic stressful conditions (drought and salinity) [53, 54]. Therefore, the growth and EPS production of *A. chroococcum* 76A was monitored over a 48-h incubation at 30 °C using medium containing 1% vinasse and 5% sucrose as carbon sources (Fig. 2). The maximum EPS concentration (44.6 ± 0.63 mg/50 mL) was reached at 24 h, corresponding to the sub-stationary growth phase ($7 \times 10^8 \pm 0.29$ CFU/mL), after which no significant differences in EPS synthesis were recorded (Fig. 2). Cell growth and EPS production kinetics by *Paenibacillus jamilae* were previously investigated by Morillo et al. [60]. According to our results, this strain reached the maximum production of EPS at the beginning of the stationary growth phase. However, Gauri et al. [50] reported that in N_2-free basal glucose medium, the strain *Azotobacter* sp. SSB81 reached the stationary phase after 36 h, and the maximal amount of EPS produced was observed at 30 h in exponential phase growth, after which it suddenly decreased.

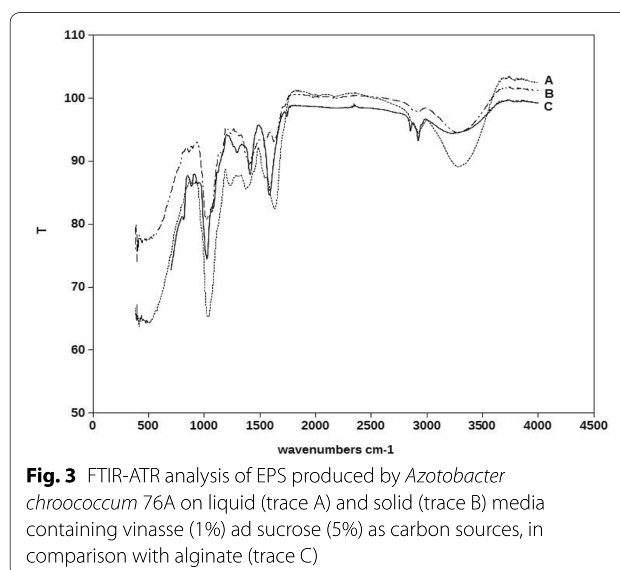

Fig. 3 FTIR-ATR analysis of EPS produced by *Azotobacter chroococcum* 76A on liquid (trace A) and solid (trace B) media containing vinasse (1%) ad sucrose (5%) as carbon sources, in comparison with alginate (trace C)

Chemical characterization of EPS produced by *A. chroococcum* 76A

The EPS produced by the selected strain *A. chroococcum* 76A was recovered from solid or liquid media containing vinasse (1%) and sucrose (5%) after 24 h of incubation ay 30 °C, to assess the gross chemical compositions by determining the total carbohydrate content, protein concentration and uronic acid content. As expected, carbohydrates were the dominant fraction, representing approximately 59 and 52% of the EPS synthetized by *A. chroococcum* 76A on solid and liquid medium, respectively (Table 4). However, the uronic acid content was approximately 23% (w/w) in both samples, although the protein concentration appeared to be higher in EPS recovered from liquid cultures (11.3 ± 5.16%, w/w) than from solid medium (8.3 ± 0.99%, w/w; Table 4). The monomer composition of both EPS samples showed that, in addition to uronic acids, the primary monosaccharide present was glucose (Table 4), suggesting that the isolated biopolymers were a complex mixture of polysaccharides. The heterogeneous composition of the EPSs can be ascribed to the different growth conditions, i.e. in liquid medium and on solid agar that were carried out respectively in the SB and SAB media, that are known to significantly affect the bacterial EPS's nature [61]. This result was also supported by FTIR-ATR and NMR spectral analyses. The IR spectra of EPS were reported in comparison with alginate. It was interesting to note the overlap of signals attributable to guluronic acid in both samples (Fig. 3). In particular, as shown in Fig. 3, the spectrum of the EPS recovered from either the liquid (trace A) or solid (trace B) media were characterized by a large signal at approximately 3300 cm^{-1}, corresponding to the O–H stretching vibrations of α-carboxylic groups that are typical of uronic acids. Moreover, the primary signals observed at approximately 1724 cm^{-1} were particularly intense in the EPS recovered from liquid medium (Fig. 3, trace A), which could be attributed to the vibrations of

Table 4 Carbohydrate, protein and uronic acids contents in the EPS produced by *Azotobacter chroococcum* 76A grown on solid or in liquid media containing vinasse (1%) ad sucrose (5%) as carbon sources

Samples	Carbohydrate (%, w/w)	Protein (%, w/w)	Uronic acids (%, w/w)	Monosaccharides (glucose %, w/w)
EPS agar[a]	58.3 ± 1.62	8.3 ± 0.99	22.7 ± 2.61	80.1 ± 2.11
EPS broth[b]	52.0 ± 2.12	11.3 ± 5.16	22.8 ± 3.76	29.7 ± 1.32

[a] EPS produced by *A. chroococcum* 76A after 24 h of growth on solid medium at 30 °C

[b] EPS produced by *A. chroococcum* 76A after 24 h of growth in liquid medium at 30 °C

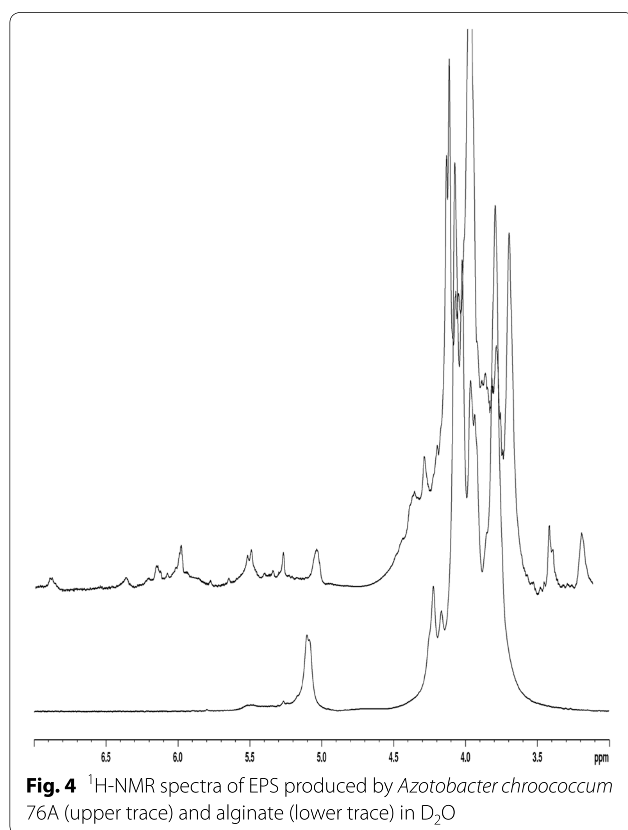

Fig. 4 ^{1}H-NMR spectra of EPS produced by *Azotobacter chroococcum* 76A (upper trace) and alginate (lower trace) in D_2O

ester groups and symmetric and asymmetric stretching vibrations of guluronic acid, one of the two components of alginates (Fig. 3, trace C). The ^{1}H-NMR spectrum analysis of the EPS also suggested that the isolated polymers were a mixture of different polysaccharides in which, in addition to an alginate fraction, a glucan fraction was also present as a primary component (Fig. 4). Indeed, in the anomeric region of the spectrum (from 4.3 to 5.9 ppm), the EPS synthetized on solid medium presented *inter alia* a primary signal at 5.19 ppm (Fig. 4, upper trace) resembling the anomeric signals of alginate (Fig. 4, lower trace), in addition to other signals at approximately 5.46 ppm that were attributable to glucose. A similar pattern of signals was seen in the EPS sample that was synthetized in liquid medium (data not shown). Alginates are polysaccharides composed of D-mannuronic and L-guluronic acids and are of great interest for a wide range of industrial applications, such as the pharmaceutical, agricultural, food, cosmetic, textile and paper industries as well as in water-treatment processes and cell immobilization and encapsulation [9, 62]. Moreover, some degradation products can be employed as anti-inflammatory and immunosuppressive agents in medical field [62]. Although most of the alginate currently produced for commercial purposes is derived from brown seaweeds [63], considering the environmental concerns associated with marine algae harvesting and processing, there is increasing interest in alginates from bacteria, primarily due to their high purity and regular structure [64]. Bacterial alginate synthesis is restricted to bacteria belonging to the genera *Pseudomonas* and *Azotobacter* [65]. However, very few studies have investigated alginate produced by *A. chroococcum* despite the genus *Azotobacter* is considered the best candidate for the industrial production of alginate [66].

Conclusions

Microbial selection allowed the detection of *A. chroococcum* 76A, a new bacterial strain potential able of bio-based alginate synthesis from cheap substrate containing vinasse. This approach represents a possible alternative to expensive disposal of agri-food wastes through their transformation into high value-added products.

Abbreviations

EPS: exopolysaccharide; SAB: Salts Agar Base; YM: Yeast Mannitol; SB: Salts Broth; PDM: polymer dry mass; FTIR-ATR: Attenuated Total Reflection Fourier Transform Infrared; ^{1}H-NMR: Proton Nuclear Magnetic Resonance; PHA: polyhydroxyalkanoate; PHB: poly-β-hydroxybutyrate.

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Authors' contributions

VV participated in the microbial selection and wrote the main manuscript text. BN, PDD and AP performed the chemical characterization of EPS and wrote the manuscript for this part. GP, VI and AR participated in the microbial selection and characterization of sugarcane vinasse. OP conceived the study, participated in its design, and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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