Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. (Jan–Mar 2019) 89(1):283–290
https://doi.org/10.1007/s40011-017-0942-0

RESEARCH ARTICLE

Comparative Study on Structure of Exopolysaccharide and Capsular Polysaccharide Produced by Southern Ocean Origin *Pseudoalteromonas* sp. MB-16

Siddhartha Sengupta[1,2] · Aparna Banerjee[3] · Urmi Halder[3] · Pratibha Gupta[1,4] · Chiranjib Banerjee[1,5] · Rajib Bandopadhyay[1,3]

Received: 19 September 2016 / Revised: 24 August 2017 / Accepted: 22 November 2017 / Published online: 30 November 2017

Abstract A comparative study on the basic structural characteristics of exopolysaccharide (EPS) and capsular polysaccharide (CPS) produced by the Southern Ocean (Indian Sector) origin *Pseudoalteromonas* sp. MB-16 has been conducted. It has a higher production rate of EPS as compared to that of CPS and the maximum production rate is 0.7 g/l when glucose is the carbon source. Elemental analysis and infrared spectroscopic study have given a brief compositional insight on the EPS and CPS produced by the marine bacterium, whereas scanning electron micrographs have revealed the morphological structure of *Pseudoalteromonas* sp. derived EPS and CPS. Both the polysaccharides show significant thermal stability as confirmed by thermogravimetric analysis. In all the experiments, *Pseudoalteromonas* sp. derived EPS and CPS have given similar results when compared to standard bacterial polysaccharides dextran and guar gum. This present study gave an important preliminary idea about the nature of bacterial polysaccharides produced by the Southern Ocean (Indian Sector) origin microbe.

Keywords Southern Ocean (Indian Sector) · *Pseudoalteromonas* sp. · Exopolysaccharide · Capsular polysaccharide

Significance Statement First report on bacterial EPS/CPS production from Southern Ocean (Indian Sector). Optimization of EPS/CPS production. Preliminary characterization of both EPS and CPS from *Pseudoalteromonas* sp. MB-16.

✉ Rajib Bandopadhyay
rajibindia@gmail.com

[1] Department of Bio-Engineering, Birla Institute of Technology, Mesra, Ranchi, Jharkhand 835215, India

[2] Department of Biochemistry and Medical Biotechnology, Calcutta School of Tropical Medicine, 108 C.R. Avenue, Kolkata, West Bengal 700073, India

[3] Department of Botany, UGC-Center of Advanced Study, The University of Burdwan, Golapbag, Bardhaman, West Bengal 713104, India

[4] ICAR-Indian Institute of Agricultural Biotechnology, Office: PDU Campus, ICAR-IINRG, Namkum, Ranchi, Jharkhand 834010, India

[5] Department of Environmental Science and Engineering, Indian School of Mines, Dhanbad, Jharkhand 826004, India

Introduction

Polysaccharides are secreted by diverse group of microorganisms, from algae to bacteria or fungi. Among the all other polysaccharide production sources, bacterial polysaccharides are particularly gaining its importance because of its bulk culture possibility and comparatively easy downstream processing [1]. Though physiological role of polysaccharides in bacteria is not clear till date, but roughly it is known to help in a number of processes like-pathogenicity, protection from extreme environment, quorum sensing and biofilm formation [2]. Research in bacterial polysaccharides is in its logarithmic phase for the past few years as it is proved to have different industrial applications like- dextran as food additive, emulsifier, thickener in food industry or as a drug carrier in biomedical applications [3, 4], succinoglycan as cosmetic additive, cross linker, emulsifier, and viscosifier. [5–7], xanthan as gum [8] and many others. In fact bacterial polysaccharides are also getting used in green synthesis of metal nanoparticles now a days. Bacterial polysaccharides mediated green synthesized AgNPs are becoming a potent alternative to antimicrobials [9, 10].

In order to discover more possible applications of bacterial polysaccharides, it is necessary to find some novel bacterial sources and subsequently preliminary characterization of the polysaccharide is important before its industrial application or commercialization. *Pseudoalteromonas* sp. is usually a marine microorganism and is an extreme halophilic psychrophile. Microbes under extreme environment produce polysaccharides as a part of their survival strategy. A recent report by Saravanan and Jayachandran [11] has directly correlated EPS production by *Pseudoalteromonas ruthenica* with its biofilm forming capacity. Another study on EPS produced by *Pseudoalteromonas* sp. has revealed the presence of galacturonic acid, glucuronic acid, rhamnose and glucosamine sugar residues as monomeric composition [12]. Study on CPS produced by *Pseudoalteromonas* sp. is yet not explored, thus more research in this particular field is needed to hint some unique utilization that can be exploited commercially further. Also there are a few earlier reports that are available on Southern Ocean (Indian Sector) microorganisms which mainly deal with the microbial diversity studies only [13, 14]. The Southern Ocean (Indian Sector) origin *Pseudoalteromonas* sp. MB-16 strain has been earlier reported to show good antibacterial activity against *Salmonella typhi, Staphylococcus aureus* and *Pseudomonas aeruginosa*. It also produces bioactive molecules; viz. enzymes esterase, lipase, catalase, urease and protease. Though exopolysaccharide production by this marine strain is already known, but capsular polysaccharide composition of this strain is not earlier reported elsewhere. So our present work on EPS/CPS production by *Pseudoalteromonas* sp. MB-16 provides some important preliminary structural and functional insights on the biology of Southern Ocean microorganisms. This is first of a kind approach in which both EPS and CPS production has been studied simultaneously from the same organism.

Material and Methods

Bacterial Strain

The strain *Pseudoalteromonas* sp. MB-16 has been isolated from water sample taken from 1500 m depth of the Southern Ocean (Indian Sector). The methods of sampling and isolating the strain have been described earlier by Gupta et al. [13]. Throughout the study, the strain has been maintained and is routinely grown in Zobell's Marine Broth medium (Himedia) at 20 °C.

Phylogenetic Analysis of PR-MB-16

Phylogenetic analysis based on 16S rDNA gene sequence has already been described by Gupta et al. [11]. Briefly, the 16S rRNA sequence of strain MB-16 (KF019666) has showed 99% similarity with *Pseudoalteromonas espejiana*. Later in 2017, the same 16S rDNA sequence has been BLAST searched using the National Centre of Biotechnology Information database. EzTaxon-e EzBioCloud program (http://www.eztaxon.org/) is used to identify phylogenetic neighbours and to calculate pairwise 16S rDNA sequence identities.

Production of Exopolysaccharide, Capsular Polysaccharide and Its Optimization

Isolation and extraction of exopolysaccharides have been done following the procedure of Reddy et al. [15] with little modifications. Zobell Marine Broth (Himedia) has been used for growth of *Pseudoalteromonas* sp. MB-16. The culture is maintained at 20 °C under 150 rpm shaking for 7 days. The harvest of EPS and CPS is done through centrifuging the culture medium in order to separate the cells at 15,000×*g* for 20 min. The supernatant is collected for extraction of released exopolysaccharides (EPS), whereas the pellet is used for the extraction of capsular polysaccharides (CPS). The pellet is treated with a mixture of 0.6 M NaCl and 0.06 M of EDTA at 50 ± 2 °C for 3 h. The mixture is then centrifuged at 15,000×*g* for 20 min and supernatant has been saved. EPS and CPS both are extracted by vacuum evaporating each to half volume. To the solution, equal volumes of chilled isopropanol is added, mixed, and kept in at − 20 °C for 72 h for precipitation. Precipitates of EPS and CPS formed are collected by centrifugation at 15,000×*g* and 20 min, washed with same chilled solvent for 2–3 times. Both are dried and stored at room temperature. Excess water is removed under vacuum prior to lyophilization, and stored in air tight vials. Subsequently, the entire process has been repeated with methanol, acetone and ethanol as solvent to observe the change in EPS and CPS production.

Four different media containing different carbon sources are tested for their abilities to influence growth and EPS production of *Pseudoalteromonas* sp. MB-16. Each carbon source has been added individually as equivalent to 30 g/L glucose. The different carbon sources used are glucose, fructose, sucrose and lactose supplemented with Zobell marine Broth as the standard (pH 7.5). The inoculated flasks are incubated for 7 days at 20 °C under 150 rpm shaking, followed by same EPS/CPS extraction protocol as mentioned above.

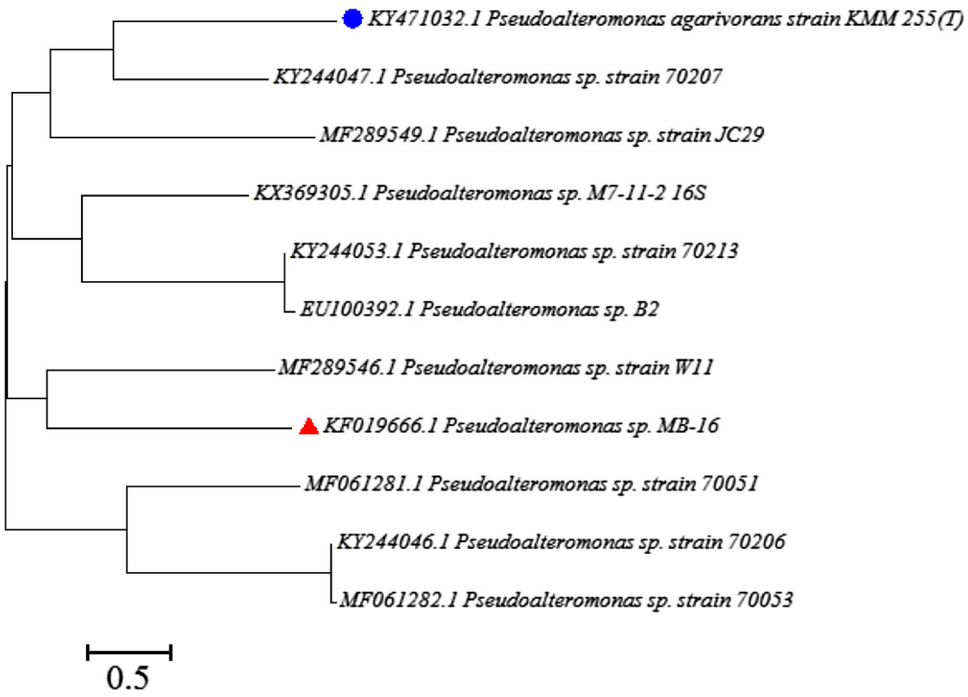

Fig. 1 Phylogenetic tree constructed by using neighbour-joining method with Kimura 2 distance parameter, having bootstrap value of 1000 replicates. The bar represents 0.5 substitutions per alignment. The strain *Pseudoalteromonas* sp. MB-16 is marked with red triangle whereas the highest ANI similarity index showing *Pseudoalteromonas agarivorans* KMM 255(T) is marked in blue circle

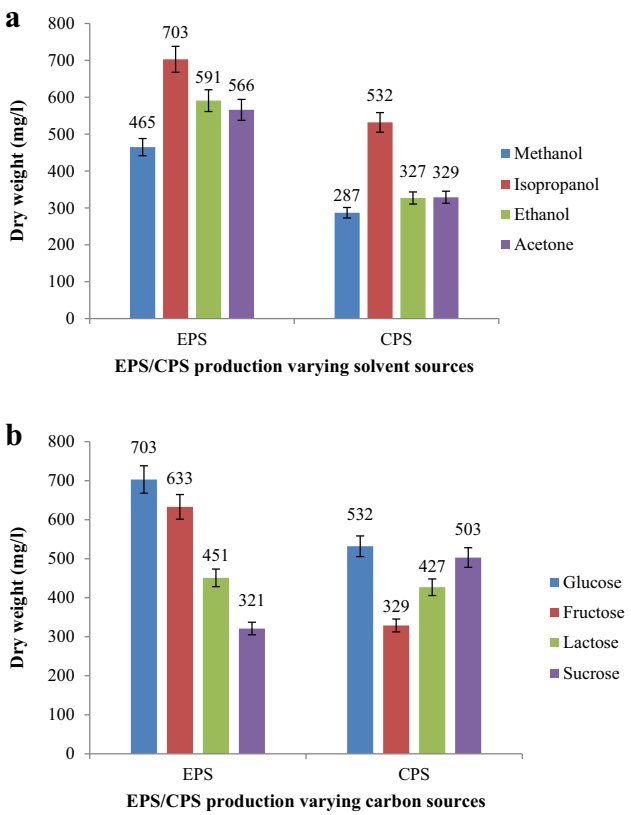

Fig. 2 Comparison of the dry weight between EPS and CPS produced by *Pseudoalteromonas* sp. MB-16; where **a** variation of solvent sources and **b** variation of carbon sources

Elemental Analysis of the Samples

Elemental analysis of total nitrogen, carbon, hydrogen, sulfur and C/N ratio is performed to get an idea about the composition of the organic matter present in the EPS/CPS. It is determined using a CHNS analyzer (Vario EL III, M/s Elementar, Germany). For the analysis, freeze-dried EPS and CPS samples are weighed (5–10 mg) and mixed with vanadium pentoxide [V_2O_5] oxidizer and combusted in a reactor at 1000 °C. Finally, the chromatographic responses are calibrated against pre-analysed standards, and the CHNS elemental contents are reported in weight percent. Eager 200 software is used for post run analysis. The EPS and CPS from the bacterial isolate have been run along with two standards Dextran and Guar Gum.

Scanning Electron Micrograph Analysis

The surface morphology of isolated bacterial EPS and CPS are analysed through SEM (JEOL, JSM-6390LV, Japan) study. The polysaccharides are washed 2–3 times using autoclaved distilled water and fixed with 2.5% glutaraldehyde solution and kept for overnight at 4 °C. It is then dehydrated with series of increasing ethanol concentration and is allowed to get dehydrated completely. The samples are then mounted on a double-faced adhesive carbon tape and through gold sputtering; the bacterial EPS has been made conductive and examined under the SEM. The SEM

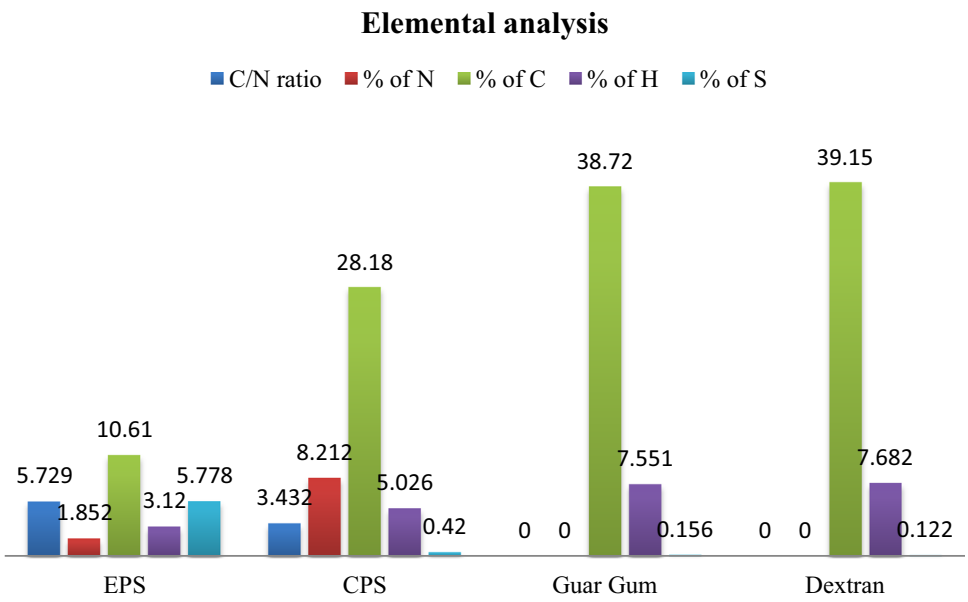

Fig. 3 Elemental analysis result of *Pseudoaltermonas* sp. MB-16 as compared to control guar gum and dextran

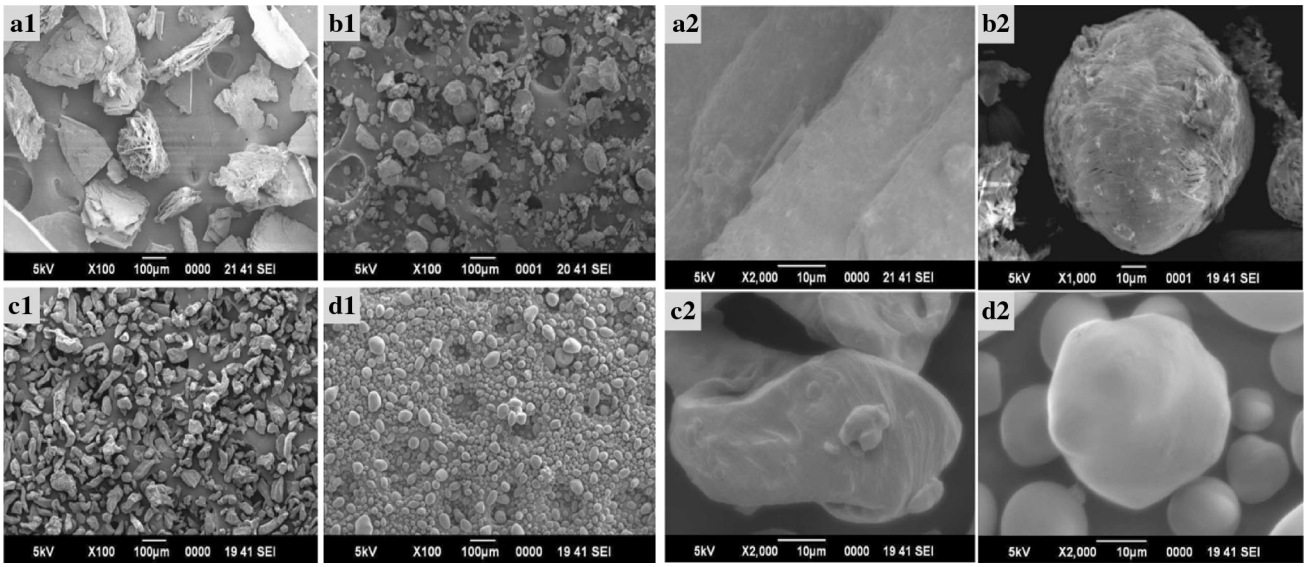

Fig. 4 Scanning electron micrograph of *Pseudoalteromonas* sp. MB-16 EPS ($\mathbf{a_1}$ 100 μm and $\mathbf{a_2}$ 10 μm), CPS ($\mathbf{b_1}$ 100 μm and $\mathbf{b_2}$10 μm), Guar gum ($\mathbf{c_1}$ 100 μm and $\mathbf{c_2}$ 10 μm), Dextran ($\mathbf{d_1}$ 100 μm and $\mathbf{d_2}$ 10 μm)

analysis of the EPS and CPS is also done along with two standard polysaccharides Dextran and Guar Gum. The observations of structural and morphological similarities have been made at different magnifications.

Infrared Spectroscopic Study

The major structural groups are detected using Fourier transformed infrared (FTIR) spectroscopic analysis. The pellet for infrared analysis is prepared carefully by grinding 2 mg dry EPS and CPS with 200 mg dry KBr (1:20 ratio) and pressing it in a mold with two standard polysaccharides Dextran and Guar Gum. The FTIR spectra are recorded in transmittance mode in the region of 400 and 4000 cm^{-1} on an IR-Prestige 21 FTIR instrument (Shimadzu Corporation, Japan).

Determination of Thermal Stability

Thermogravimetric analysis (TGA) is carried out in an inert nitrogen atmosphere and the weight is recorded as a function of increasing temperature. The instrument also records the temperature difference between the specimen and one or more reference pans (differential thermal analysis or DTA). TGA and DTA have been performed on DTG-60 (Shimadzu, Japan). Thermograms for TGA and

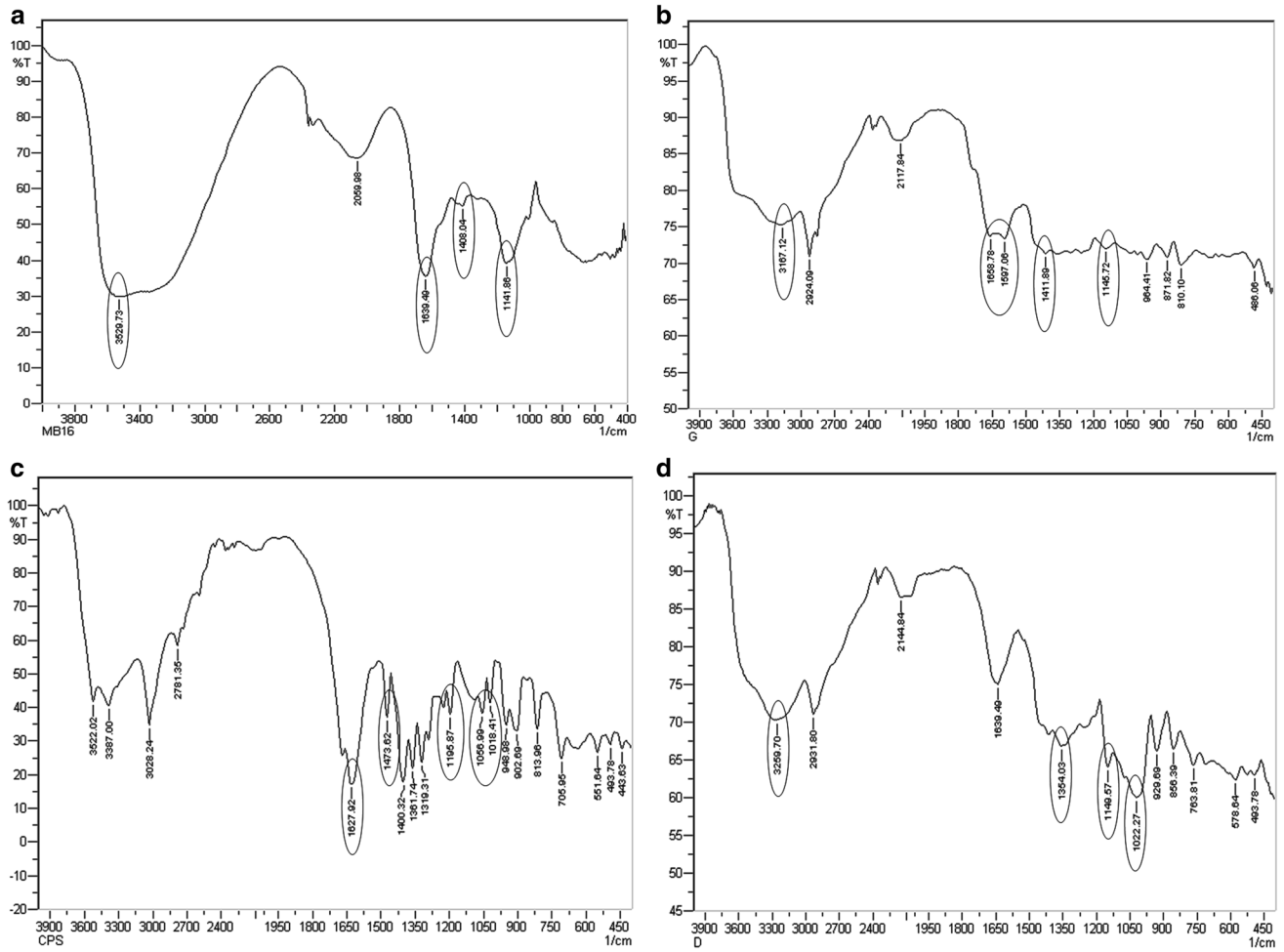

Fig. 5 FT-IR spectrum of *Pseudoalteromonas* sp. MB-16 EPS (**a**) and CPS (**b**); compared to control Guar gum (**c**) and Dextran (**d**)

DSC have been obtained in the range of 30–700 and 50–550 °C respectively to determine the thermal stability of the EPS and CPS as compared to control bacterial polysaccharides dextran and guar gum.

Results and Discussion

Identification of the Strain

EzTaxon analysis of EzBioCloud program shows 100% similarity of PR-MB-16 strain with *Pseudoalteromonas agarivorans* KMM 255(T); which is a probable indication of close phylogenetic and taxonomic relationships among the marine *Pseudoalteromonas* group (Fig. 1).

Production of Exopolysaccharide, Capsular Polysaccharide and Its Optimization

Culture conditions affect the productivity of the bacterial polysaccharides. The results of the present study show that *Pseudoalteromonas* sp. MB-16 produced EPS exhibit maximum production of 703 mg/1000 ml after 21 days of incubation. The EPS and CPS are extracted using four different solvents- acetone, ethanol, methanol and isopropanol to find out which solvent gives the highest productivity (Fig. 2). It is also observed that isopropanol gives the highest productivity while glucose is the best carbon source added in the medium.

Elemental Analysis of the Samples

The elemental analysis has been done to check total content of carbon, hydrogen, nitrogen and sulphur present in the samples and thus performing a comparative study using known polysaccharides like dextran and guar gum with the C/N ratio proving the marine source of the extracted EPS. C/N ratios in the range 4–10:1 is usually from marine sources [16]. Both EPS and CPS produced by *Pseudoalteromonas* sp. MB-16 showed C/N ratios 5.729 and 3.432; which ascertain its marine origin (Fig. 3). The trace amount of sulphur present in the CPS is an indication of

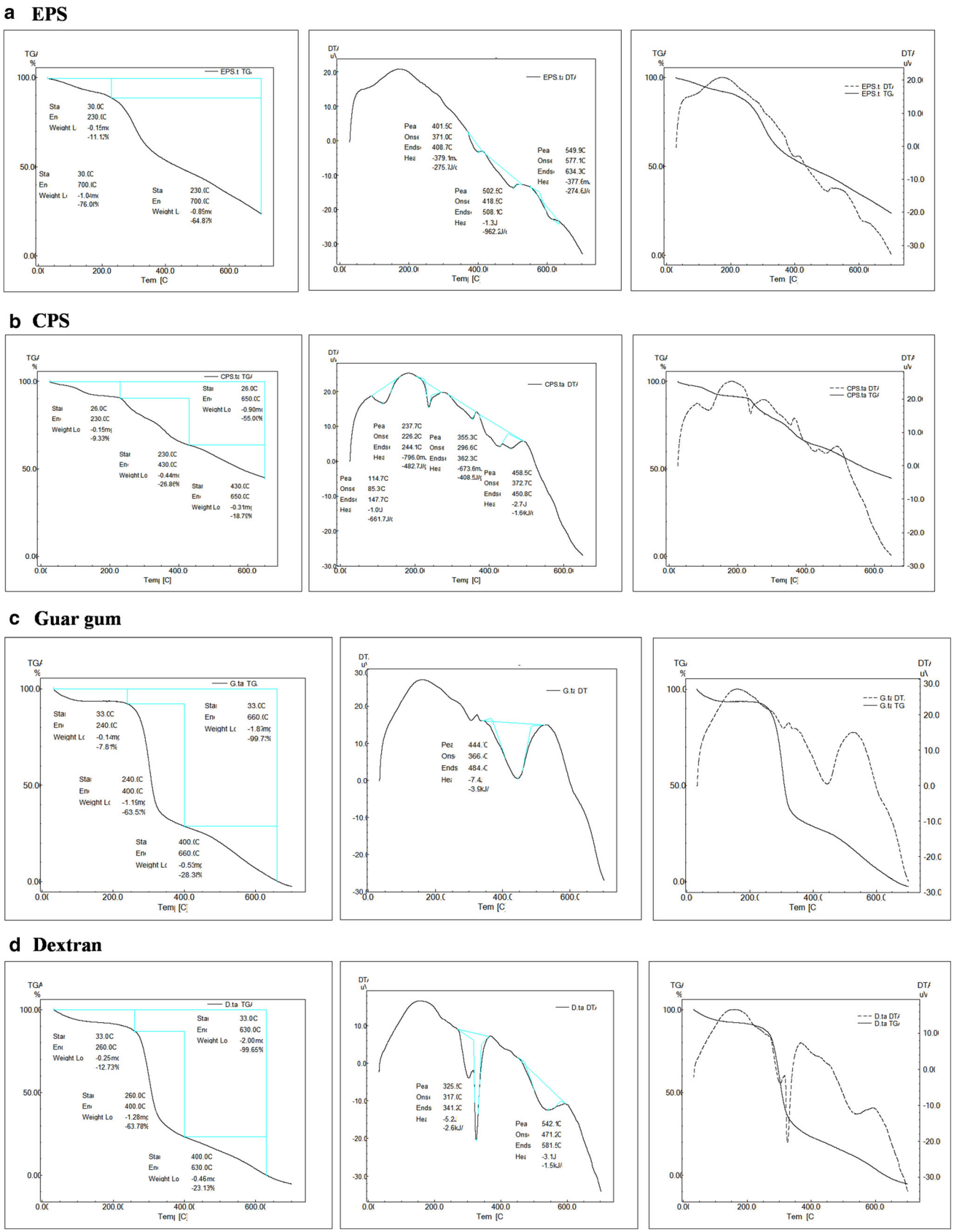

Fig. 6 TGA/DTA analysis of *Pseudoalteromonas* sp. MB-16 derived EPS and CPS as compared to control dextran and guar gum

presence of minute protein contaminant in the CPS. Whereas the high sulphur content of the EPS as compared to standard dextran and guar gum hints the EPS to be a probable sulphated polysaccharide.

Scanning Electron Micrograph Analysis

The SEM image of EPS, CPS and the known polymers have been observed at 100× magnification. The results obtained by SEM on the polysaccharides show a smooth surface, with distinct porous regions of a polymeric material. Both the EPS and CPS showed highly compact, flakes like structures (Fig. 4). This indicates the potential of EPS as viscosifying, as a thickener or as stabilizing agent for novel food products. The SEM analysis of the known polymers also shows similar outputs.

Infrared Spectroscopic Study

Infrared spectra of EPS and CPS extracted from the isolate *Pseudoalteromonas* sp. MB-16 along with two known polysaccharides Guar Gum and Dextran has shown specific absorbance of N–H stretching at 1650–1580 cm^{-1}, C–H stretching vibration of CH_2 at 2915–2935 cm^{-1}, medium C–C stretching at 1400–1500 cm^{-1},C–N stretch at 1250–1020 cm^{-1},C–O–C and C–O stretch at 1000–1200 cm^{-1}, indicating the presence of primary amine group, aromatic compounds, halide group, aliphatic alkyl group and carbohydrates (Fig. 5); a characteristic feature of bacterial polysaccharides.

Determination of Thermal Stability

The TGA of Guar Gum has presented a mass loss of 7.81% between 33 and 240 °C, another loss of 63.53% between 240 and 400 °C and finally a loss of 28.38% between 400 and 660 °C with the DTA curve showing one exothermic peak at 444.7 °C. Whereas, the TGA of Dextran has presented a mass loss of 12.73% between 33 and 260 °C, another loss of 63.78% between 260 and 400 °C and finally a loss of 23.13% between 400 and 630 °C with the DTA curve showing two exothermic peaks at 325.54 and 542.1 °C respectively. Compared to the control dextran and guar gum, TGA of the EPS presents a mass loss of 11.127% between 30 and 230 °C and finally a loss of 64.873% between 230 and 700 °C with the DTA curve showing three exothermic peaks at 401.55, 502.55 and 549.99 °C respectively and TGA of CPS presents a mass loss of 9.33% between 33 and 260 °C, another loss of 26.86% between 260 and 400 °C and finally a loss of 18.79% between 400 and 630 °C with the DTA curve showing four exothermic peaks at 114.7, 237.7, 355.3 and 458.5 °C respectively (Fig. 6).

All the three polymers have shown a mass loss of around 72–75% after getting exposed to an increasing temperature along with exothermic peaks, which confirms the thermal stability of the polymer along with the reaction kinetics.

Conclusion

Microbial EPSs are omnipresent in the extreme marine environment where they are essential for survival. Most of the functions attributed to polysaccharides are of a protective nature and their precise roles are dependent on the ecological niches in which the microorganisms live. EPS and CPS of *Pseudoalteromonas* sp. can play an important role in its biotechnological and industrial application. In this study, the authors have isolated both EPS and CPS from a Southern Ocean (Indian Sector) origin *Pseudoalteromonas* sp. MB-16 under various physical and chemical factors and partially characterized the nature of the polysaccharides; which is probably the first report on production of EPS/CPS from any Southern Ocean (Indian Sector) origin bacteria and also one of a kind work where both EPS and CPS production capacity of any bacterium has been taken under consideration. Bacterial polysaccharides also present a real potential in cell therapy and tissue engineering with an advantage over the polysaccharides from eukaryotes, since they can be produced totally under controlled condition in bioreactors. Thus, EPS and CPS can be a potential factor to allow further research on *Pseudoalteromonas* sp. and make it as a potent candidate for commercial exploitation. Moreover, further studies are required to determine its different biotechnological uses including environmental bioremediation, biomedical applications, food industry additives, in cosmeceuticals etc.

Acknowledgements The authors are thankful for the Expedition support to MoES, New Delhi and NCAOR, Goa (No. MoES/NCAOR/SOS/1/2007PC-I; dated January 4, 2011) and research support to BIT Mesra, Ranchi. One of the authors gratefully acknowledges the financial support as M. Tech fellowship to Centre of Excellence (COE) (Ref No. NPIU/TEQIP II/FIN/31/158, dated April 16, 2013) at the Department of BioEngineering. Another author is thankful to SRF (State Funded) for the financial assistance [Fc (Sc.)/RS/SF/BOT./2014-15/103 (3)]. Urmi Halder is thankful for the financial assistance to JRF-State Fund [Fc (Sc.) /RS/SF/BOT./2017-18/22)].

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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