



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Biomedical and therapeutic potential of exopolysaccharides by *Lactobacillus paracasei* isolated from sauerkraut: Screening and characterizationThiruvengadam Shankar^a, Selvam Palpperumal^b, Durairaj Kathiresan^b, Subbiah Sankaralingam^{b,*}, Chellapandian Balachandran^{c,d}, Kathirvelu Baskar^{e,**}, Abeer Hashem^{f,g}, Abdulaziz A. Alqarawi^h, Elsayed Fathi Abd_Allah^h^a Vivekanandha College of Arts and Sciences for Women, Elayampalayam, Tamil Nadu, India^b Saraswathi Narayanan College, Madurai Kamaraj University, Tamil Nadu, India^c Department of Biotechnology, Thiagarajar College, Madurai-625 009, Tamil Nadu, India^d National Centre of Excellence (MHRD), Thiagarajar College, Madurai-625 009, Tamil Nadu, India^e Department of Ecotoxicology Ross Lifescience, Pvt., Ltd, Bhosari, Pune, India^f Botany and Microbiology Department, College of Science, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia^g Mycology and Plant Disease Survey Department, Plant Pathology Research Institute, ARC, Giza 12511, Egypt^h Plant Production Department, College of Food and Agricultural Sciences, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:

Received 23 December 2020

Revised 4 February 2021

Accepted 7 February 2021

Available online 17 February 2021

Keywords:

Exopolysaccharides (EPS)

Lactobacillus paracasei

FTIR

HPLC

Total antioxidant capacity (TAC)

ABSTRACT

The intention of the study was evaluated for purification and characterization of exopolysaccharides from *Lactobacillus paracasei*; was isolated from homemade Sauerkraut sample collected from Sivakasi, Tamil Nadu, India, confirmed by biochemical and gene sequencing (16S rRNA). The purification and characterization of exopolysaccharides from candidate bacterium were studied on appearance, solubility of the EPS, carbohydrate estimation, emulsifying activity, sulphate, protein, uronic acid content, FTIR, HPLC and GC-MS analysis. The percentage of elemental carbon, (54.36%) hydrogen (21.74%), nitrogen (9.63%) and sulphur content (18.03%) were recorded in exopolysaccharides. The emulsification index (E24) of EPS was higher in toluene (79.20) and benzene (78.867) supplemented medium. FTIR spectrum of the candidate bacterial EPS confirmed presence of sulphate compounds, carboxyl group, and hydrogen bonded compounds etc. EPS exhibited 76.34% of Total Antioxidant Capacity (TAC), 71.15% of reducing power, 68.65% of Hydrogen Peroxide scavenging activity and also 60.31% DPPH radical scavenging activity. The potential antioxidant properties observed in exopolysaccharides from *Lactobacillus paracasei* is considered as valuable drugs.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Past few years, water soluble exopolysaccharides from microbes have attracted more interest, because of various indus-

trial appliances (Bueno and Garcia-Cruz, 2006). Exopolysaccharides from microbial origin are the long chain compounds composed of various kinds of sugar units for their growth and development. These substances are structurally & functionally diversified on all taxa and secreted by bacteria into the external environment (Dogsa et al., 2005). Bacterial exopolysaccharides are the mixture of complex macromolecular polyelectrolyte substances with various molecular structural. They may be either homopolymeric or heteropolymeric in composition and are diversified with high molecular weight (Nwodo et al., 2012).

Microbial exopolysaccharides possess an important ecological and physiological function in order to protect the microorganisms from the unfavourable conditions. These substances are protect the candidate bacterial cells against antimicrobial substances, desicca-

* Corresponding author.

** Corresponding author.

E-mail addresses: biosankaralingam@yahoo.co.in (S. Sankaralingam), suribaskar@hotmail.com (K. Baskar).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

<https://doi.org/10.1016/j.sjbs.2021.02.030>

1319-562X/© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

tion, bacteriophages, osmotic stress, antibodies and also it is necessary to stick on the solid surface for biofilm formation (Vuyst et al., 1998; Mata et al., 2006). Some of the previous literatures have revealed the bacterial EPS are the best substances instead of gums from plants origin because they are easily biodegradable, vulnerable to climatic conditions and also to reduce the pollution from the environment. In addition, bacterial EPS are frequently utilized as a significant material for the processed foods as well as pharmaceutical applications due to the presence of rheological (Philippis et al., 1991; Roseiro et al., 1992).

The medicinal value of (antioxidant, anti-inflammatory and antitumor) exopolysaccharides has been studied by various bacterial strains. Thus, the therapeutic value of these bacterial exopolysaccharide is more significant in the field of medicine to find fine solutions to the various aspects (El-Newary et al., 2017). Several literatures have revealed that the bacterial exopolysaccharides (EPS) contribute to large number of biological active principles like antioxidant activity (Luo and Fang, 2008), immune stimulating (Xu et al., 2008), antitumor (Tong et al., 2009), and antiviral activity (Wang et al., 2007). The actual mechanism of relationship between the structure as well as function of EPS from the Lactic Acid Bacteria origin, during the fermentation period is still uncertain. Considering the importance of the above, the present work is aimed to carry out the biomedical as well as therapeutic application of exopolysaccharides from *Lactobacillus paracasei*.

2. Methods

2.1. Isolation and identification of bacteria

The homemade sauerkraut sample was collected from Sivakasi, Tamil Nadu, India for isolation of the exopolysaccharide producing candidate bacterial strain. The sample was processed within 30 min to plating in the De Man, Rogosa and Sharpe agar (MRS agar media) in order to evaluate the EPS producing ability. Formation of mucus like substances secreted by the candidate bacterial strain indicate EPS positive signal after incubation and strain was single streaked in the De Man, Rogosa and Sharpe agar (MRS agar plate) for further experimental analysis. The ability of the candidate bacterial strain to spread in MRS media was noted for the release of polysaccharide.

2.2. Molecular identification of candidate strain

The InstaGene™ Matrix genomic DNA isolation kit was used to isolate the genomic DNA from the candidate EPS producing strain. After the PCR amplification using specific primers, PCR products were cleaned using PCR clean-up kit (Millipore). Then sequencing was carried out by specific primers with the help of ABI 3730xl sequencer (Applied Biosystems). Analysed genome deposited to NCBI.

2.3. Characterization of exopolysaccharides (EPS)

2.3.1. Estimation of carbohydrate

Different concentrations of test solutions (EPS) and 2 mg/ml of D-glucose was employed as the standard. At 0.5 ml of phenol was added along with 2.5 ml of 96% H₂SO₄ then incubated for 10 min. After that it was kept in water bath (30 °C) for 20 min and OD was measured at 490 nm (Dubois et al., 1956).

2.3.2. Determination of sulphate and protein

The crude extract of EPS was used to analyse the sulphate content. At 2 mg/ml of potassium sulphate was used as standard. The solution made with 0.2 ml of HCl solution,

3.8 ml of trichloroacetic acid and 1.0 ml of barium chloride gelatin solution were applied at room temperature and determined OD at 360 nm (Lloyd et al., 1961). Simultaneously, the quantity of protein in bacterial extract (EPS) was estimated (Lowry et al. 1951).

2.3.3. Determination of uronic acid content

The carbazole reaction is the most satisfactory method for estimating uronic acid quantification and glucuronolactone was used as a standard (Bitter and Muir, 1962).

2.3.4. Emulsifying activity

Emulsification index of exopolysaccharide was quantified by the method of Cooper and Goldenberg (1987). Bacterial exopolysaccharides containing aqueous phase is mixed with oil or hydrocarbon (In the hydrocarbon ratio: exopolysaccharide 3:2, v/v) and mixed with cyclomixer for emulsifying action for 2 min. At 24 hrs once analyze was performed for oil, emulsion and aqueous layers and emulsification index (EI) was determined following formula EI = height of the emulsion layer/total height × 100.

2.4. Fourier transform infrared spectroscopy analysis (FTIR)

FT-IR spectrum was measured on a Perkin Elmer 6X spectrometer. About 100 mg of optical-grade KBr pellet was ground well using mortar and pestle, from that known quantity of sample was used. The sample was scanned for 16 times and collected the scanning resolution of ±4 cm⁻¹ for data.

2.5. HPLC analysis

The EPS extract was injected to HPLC (C18 column-LC-10VP Shimadzu), it was eluted with dis-H₂O at a flow rate of 1.0 ml/min at 20 °C.

2.6. Gas Chromatographic-Mass spectrophotometric analysis (GC)

The partly filtered EPS extract was hydrolyzed with 4 N HCl for 2 hrs at 100 °C in nitrogen-flushed ampules prior to sealing. The sample was concentrated by under reduced pressure at 40 °C. GC was used to release sugar components from the hydrolysis of bacterial exopolysaccharides as their alditol acetates.

2.7. In vitro antioxidant activities

(i) Determination of TAC (Total Antioxidant Capacity) of EPS

At 0.3 ml of EPS extract with 3.0 ml reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) mixed and kept at 95 °C for 90 min. After incubation, the mixture was read at 695 nm (Prieto et al., 1999).

(ii) Determination of reducing power

At 4 ml of the reaction mixture (consisting of EPS extract with phosphate buffer and potassium ferricyanide 1% w/v) was incubated at 50 °C for 20 min. TCA was then applied to bring an end to the reaction (10% w/v). Subsequently, dis-H₂O was applied to the reaction mixture along with ferric chloride (0.1 percent w/v) then read at 700 nm (Yamaguchi et al., 1998).

(iii) Hydrogen peroxide scavenging assay

At 2 ml (10 mM) hydrogen peroxide solution was made by using the PBS at pH 7.4. Different concentrations of (1 ml) samples viz., 100, 250, 500, 750 and 1000 µg was rapidly mixed with 2 ml of H₂O₂, then incubate at 37 °C for 10 min and read at 230 nm (Gulcin et al., 2004).

(iv) DPPH free radical scavenging assay

At 3 ml of various concentrations of EPS extract (100, 250, 500, 750 and 1000 μg) was added into the 0.1 $\text{mmol}\cdot\text{L}^{-1}$ DPPH solution, methanol and incubated for 10 min; then the absorbance was carried out in 517 nm (Blois and Marsden, 1958).

3. Results

Based on the morphological, biochemical and molecular characters, the candidate bacterium was identified as *Lactobacillus paracasei* and it was submitted in gene bank at NCBI (Accession Number MH553152).

3.1. Characterization of EPS

At 226 mg of EPS from 100 ml of optional medium and while, optimized medium showed 258 mg of EPS in 100 ml. Crude EPS was successfully purified in DEAE cellulose using 1 M NaCl as eluting agent. The quantity of carbohydrate, protein, sulfate and uronic acid were found to be $96.10 \pm 0.2\%$, $58.3 \pm 0.02\%$, $79.1 \pm 0.5\%$ and $31 \pm 0.48\%$ respectively from the EPS extract of candidate species (Fig. 1).

3.2. Emulsification activity

Emulsification index (E24) of EPS extracts was higher in toluene (79.20 ± 0.4) and benzene (78.86 ± 0.7) supplemented medium from the candidate isolate *L. paracasei*. In addition, moderate value was obtained in triton X 100 included medium (52 ± 7.2) followed by coconut oil (51.33 ± 9.01). Least emulsifying activity was observed in petrol (18.66 ± 4.16) substituted medium (Fig. 2).

3.3. FT-IR spectral analysis

FT-IR spectrum of exopolysaccharides from *L. paracasei* was presented (Fig. 3). The strong peak at 3441.00 cm^{-1} explained O–H stretching vibration (alkyl group) and it shows alcohols, phenols even the signal at 2995.45 and 2650.19 cm^{-1} cleared the C–H stretching vibration (Alkynes). The signal at 1645.28 , 1581.63 and 1512.19 cm^{-1} contributed H–C medium stretching vibration. The signal at 1274.95 cm^{-1} concluded $\alpha\text{-CH}_3$ bending. The signals at 796.60 , 754.17 and 719.45 cm^{-1} revealed –OH bending in out of plan. The signal at 675.09 cm^{-1} concerns CH– deformation while the signals 597.93 , 572.86 and 543.93 cm^{-1} designed ring formation for C–C stretching, that showed alkyl halides.

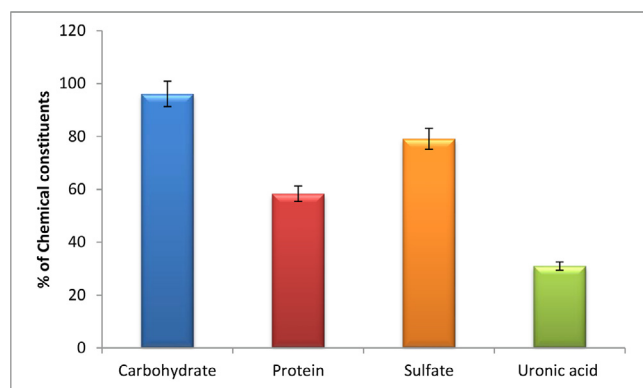


Fig. 1. Estimation of chemical constituents in EPS extract-Lactobacillus paracasei.

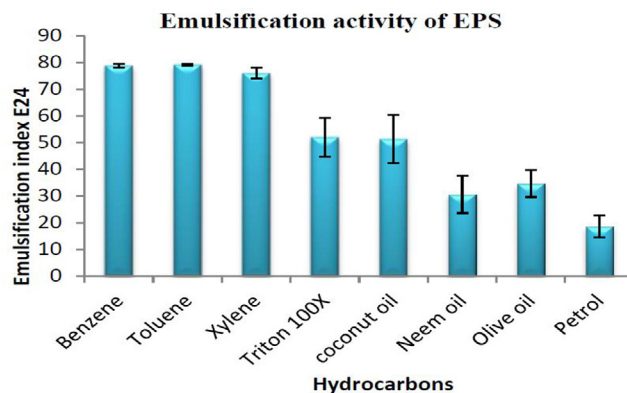


Fig. 2. Emulsification activity of *Lactobacillus paracasei*.

3.4. HPLC analysis

EPS extract was studied in HPLC (C18 column) (Fig. 4). EPS extract was subjected to determine the relative molecular mass of candidate bacterial EPS with 5–10 min retention time. The registered peaks were 2.333 & 6.433 specified that neutral sugars and monosaccharide (mannose, glucosamine, lyxose, rhamnose, ribose, erythrose, glucuronic acid, galacturonic acid, glucose, galactose, xylose and fucose).

3.5. GC-MS analysis

The purified EPS extract was subjected to GC-MS analysis (Fig. 5). GC-MS of bacterial exopolysaccharide peaks were corresponding to the presence of alpha-D-galactose (21.51%) and it was recognized as a valuable product for the food industry.

3.6. Anti-oxidant assays

In the present study, the TAC of candidate bacterial EPS was found to be $76.34 \pm 0.12\%$ (Fig. 6). During the period of study, increasing the scavenging activity was registered while enhancing the concentration of sample. At the same time, the reducing power of the EPS increases when increasing concentration of the sample and the highest obtained activity was $71.15 \pm 0.18\%$ (Fig. 7). The hydrogen peroxide scavenging impact of the bacterial EPS was $68.65 \pm 0.22\%$ (Fig. 8). Concurrently, the DPPH scavenging activity of bacterial EPS was $60.31 \pm 0.09\%$ (Fig. 9).

3.7. Elemental analysis EPS

The chemical composition of EPS in *L. paracasei* was studied. The percentage of elemental carbon, hydrogen, nitrogen and sulfur content in EPS are presented in Table 1.

4. Discussion

In fermentation industry, lactic acid bacteria play a vital role particularly they contribute flavour, texture, preservation of fermented items and dairy products. The exopolysaccharide from bacteria (lactic acid) received attention in biomedical applications. Quite a lot of references have revealed the exopolysaccharides from bacterial origin displayed bioactive principles for the protection purpose in various industries (Ohno et al., 2000; Vimala and Lalithakumar, 2003; Bhaskar and Bhosle, 2005; Rawal et al., 2016).

In this report, polysaccharide producing bacteria was carried out from the fermented sauerkraut to find out the biomedical as well as therapeutic value. The MRS agar media was used to obtain

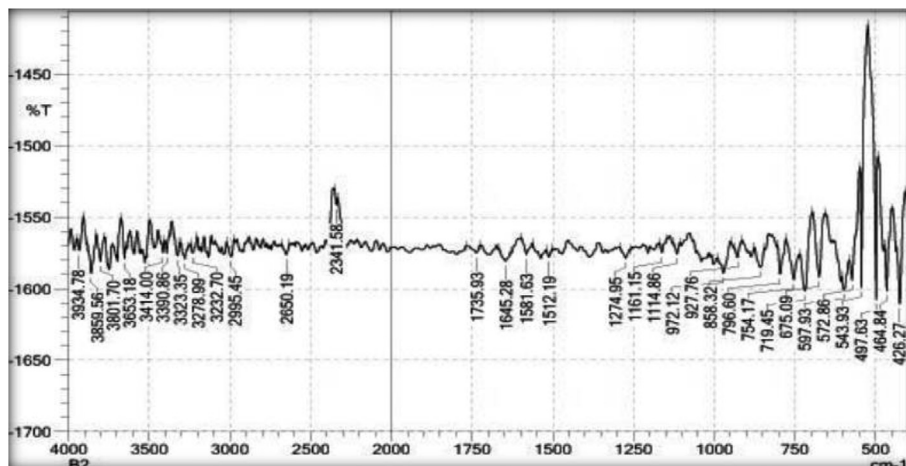


Fig. 3. FT-IR analysis of exopolysaccharides – *Lactobacillus paracasei*.

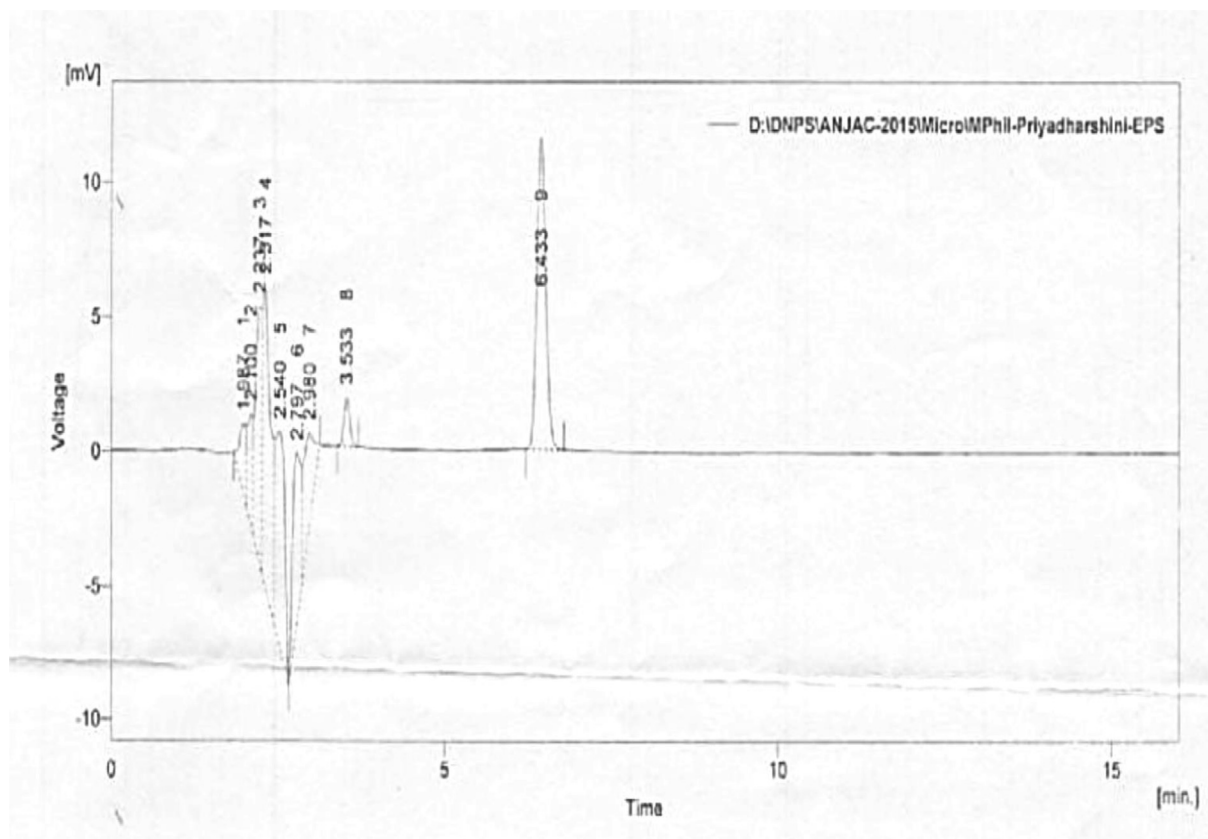


Fig. 4. HPLC spectrum of EPS extract – *Lactobacillus paracasei*.

the selected mucoid colonies that possess Gram negative rod shape motile bacterium. The molecular identification (16S rRNA gene sequencing) of candidate bacterium was done and confirmed as *Lactobacillus paracasei*; it was submitted in gene bank at NCBI (Accession Number MH553152). Correspondingly, [Bragadeeswaran et al. \(2011\)](#) collected biofilm samples from stainless steel test panels for the separation of EPS producing bacterial strain and were screened for presence of colony appearance as well as morphological parameters ([Levined, 1974](#)). The screened bacterial strains were identified by their colony morphology on nutrient agar plate and completely characterized with HPLC. In the same vein, [Ozlem \(2015\)](#) also reported that exopolysaccharide is a com-

plex mixture of sugars that are expelled by bacteria to the outer environment.

About 96.10 ± 0.2 mg/ml of carbohydrate content was found in EPS extract of *L. paracasei*. The present study is in accordance to the earlier report of [Karuppiyah et al. \(2014\)](#), who found 78% of carbohydrate content (neutral sugars) from *Exiguobacterium* sp.. Furthermore, [De Philippis and Vincenzini \(1998\)](#) noticed that cyanobacterial strain from natural sources produced the exopolysaccharide (carbohydrate content). Because the carbohydrate is the major element of the exopolysaccharide, it exhibits more beneficial properties in medicine and food industry.

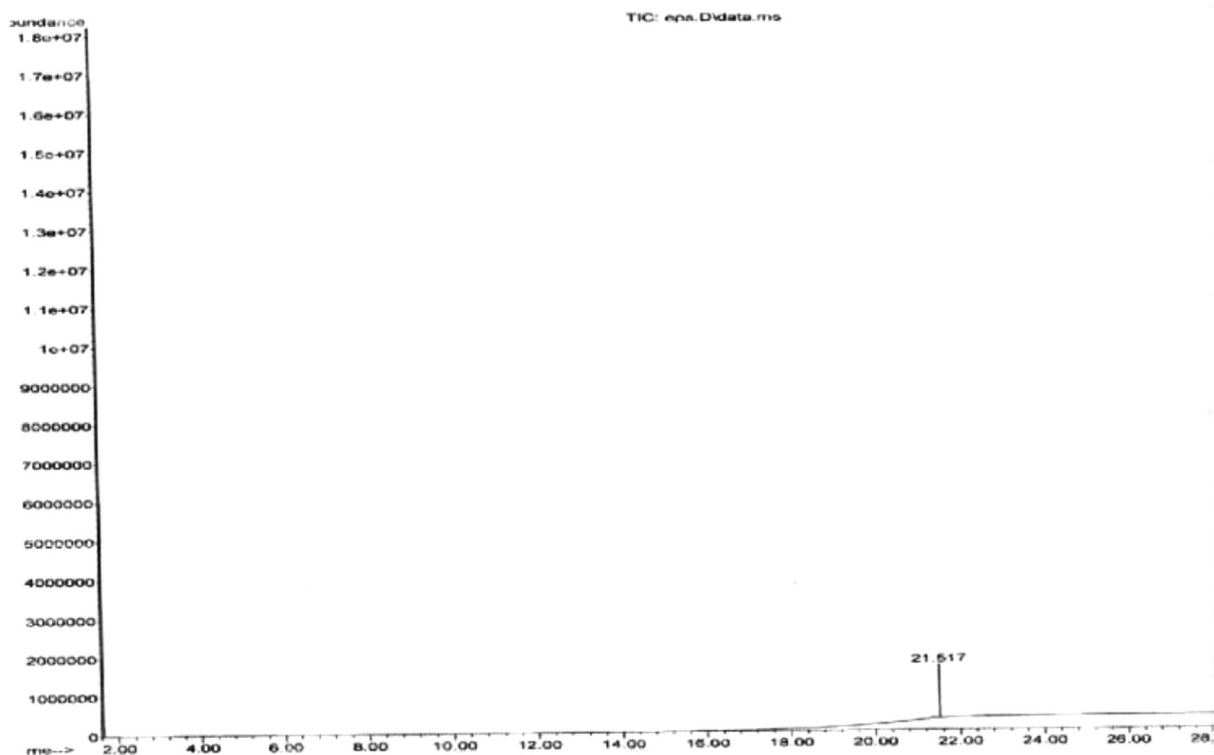


Fig. 5. GC-MS spectrum of EPS extract – Lactobacillus paracasei.

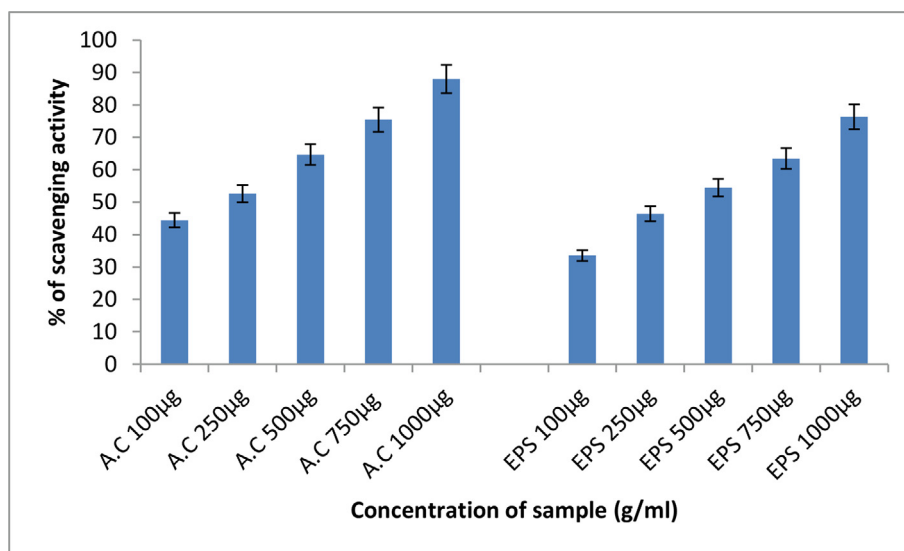


Fig. 6. Total antioxidant capacity of EPS – Lactobacillus paracasei.

In the present investigation, protein content of EPS extract of *L. paracasei* was 58.3 ± 0.2 mg/ml at 1 ml concentration. This report is endorsed by the earlier report of Vijayabaskar et al. (2011) who stated the protein content of exopolysaccharide from *Bacillus subtilis* was higher in malt supplemented medium. Several literatures have quoted the functions and the metabolic activity of the EPS is varied due to the occurrence of chemistry as well as its composition. When compared to proteins, the bacterial EPS has demonstrated small amount of carbohydrate (TCHO) content as well as C:N ratios of the polymer (Bejar et al., 1998; Guezennec et al., 1998; Majumdar et al., 1999).

The quantity of sulphate content (79.16 ± 0.5 mg/ml) in exopolysaccharide was observed in *L. paracasei*. Similarly, Bramhachari and Dubey (2006) who reported total sulfate was lower than detection limits of carbohydrates present in the crude extracts of *Vibrio harveyi* strain VB23. Also earlier report of Anita et al. (2005) stated the 7% sulphate content in EPS was recorded from *Enterobacter cloacae*. Bramhachari et al. (2007) quoted 2.3% & 2.7% sulphate content in EPS from *Vibrio furnissi* and *Marinobacter* sp. respectively.

In our study, uronic acid ($31 \pm 0.48\%$) was found in EPS from the candidate isolate. The present study is in accordance with the previous studies of Anita et al. (2005) who record the 9.23% of uronic

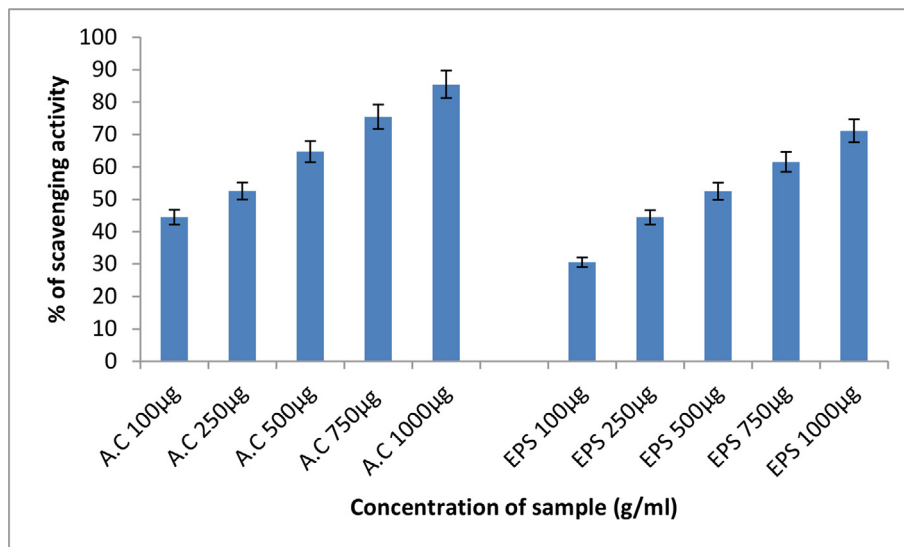


Fig. 7. Reducing power of EPS -Lactobacillus paracasei.

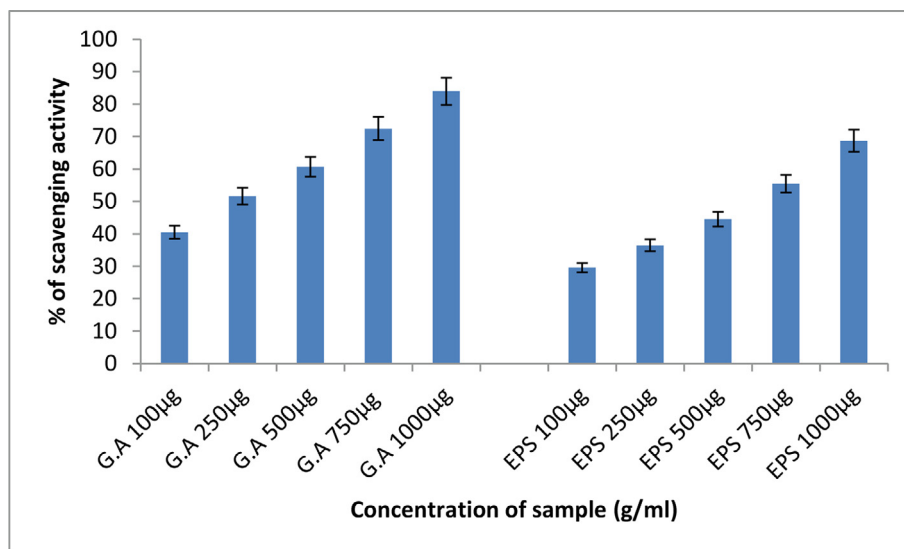


Fig. 8. Hydrogen peroxide scavenging assay of EPS - Lactobacillus paracasei.

content in EPS from *Enterobacter cloacae*. [Bramhachari et al. \(2007\)](#) also stated the 16.5 & 17.6% uronic acid content was found in EPS from *Vibrio furnissi* and *Marinobacter* sp. respectively. [Gutnick and Bach \(2000\)](#) studied the uronic acid in EPS was used for bioremediation of heavy metals and waste water treatment.

In our results, the emulsification index (E24) of EPS in the candidate bacterium *L. paracasei* was higher in toluene and benzene supplemented medium. The Moderate value was observed in triton X 100 and coconut oil added medium. Results can be verified by [Kodali et al. \(2009\)](#) who studied the emulsifying activity (E24) of exopolysaccharide was utmost in xylene and sunflower oil included medium and the moderate value was obtained in kerosene added medium.

In the present study, FT-IR spectrum of purified exopolysaccharides of *L. paracasei* showed the strong peak at 3441.00 cm^{-1} that explained O–H stretching vibration (alkyl group) and it shows alcohols, phenols even the signal at 2995.45 and 2650.19 cm^{-1} cleared the C–H stretching vibration (Alkynes). The presence of weak signals at 497.63 , 464.84 and 426.27 cm^{-1} was correspond-

ing to ring formation of EPS. The present study is identical to the earlier report of [Chowdhury et al. \(2011\)](#) who reported the FT-IR spectral analysis of EPS showed four bands at 3385 , 2981 , 1648 and 1423 cm^{-1} correspond to the stretching vibrations of –OH, C–H, C–C, and symmetric bending of CH_3 , respectively. The absorbance frequency in the area of 1648 cm^{-1} also represented the carbonyl stretching band of amides.

The present report on purified EPS extract from the candidate isolate *L. paracasei* was analysed by HPLC (C18 column). The recorded peak in HPLC was 2.237 & 6.433 and the peak value indicates presence of neutral sugars and monosaccharide. Similarly, [Zhang et al. \(2008\)](#) stated the polysaccharides content of dissolved EPS from *Amphora* sp. was studied with HPLC for existing neutral sugars from the polysaccharides.

GC-MS report of exopolysaccharide from the candidate isolate was obtained corresponding to the presence of (21.51%) alpha-D-Galactose, recognized as a valuable product for the food industry. Similarly, [Ruas-Madiedo et al. \(2010\)](#) reported that exopolysaccharides from *Lactobacillus* and *Bifidobacterium* strains subjected to

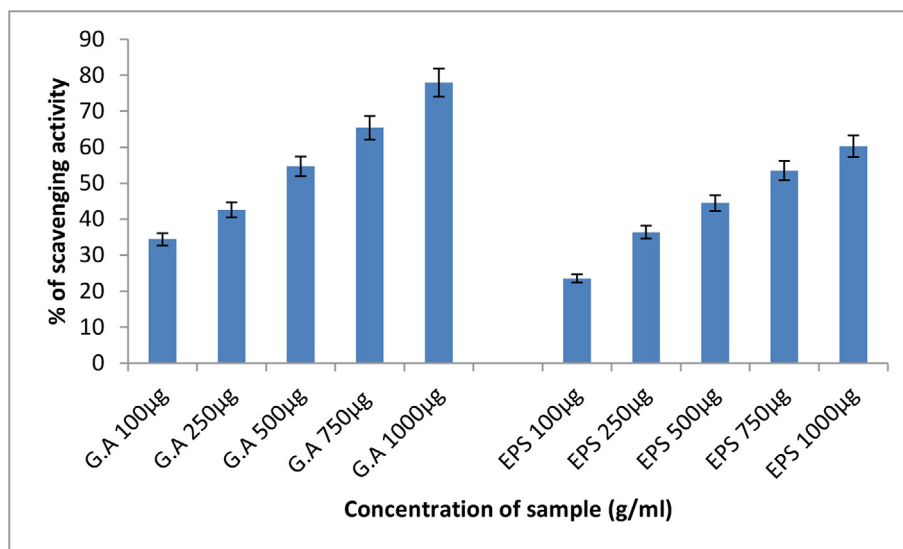


Fig. 9. DPPH free radical scavenging activity of EPS – Lactobacillus paracasei.

Table 1
Elemental analysis of EPS.

Carbon (%)	Hydrogen (%)	Nitrogen (%)	Sulfur (%)
54.36	21.74	9.63	18.03

GC- MS analysis. The peaks showed the presence of glucose, galactose, rhamnose. Mahendran et al. (2013) stated that crude extract of EPS from *Lysinibacillus fusiformis* was subjected to GC-MS analysis and found 1-methyl-2-formylimida-zole (47.95%) was major content. Ibarburu et al. (2006) quoted the sugar composition of crude exopolysaccharide was studied by GC-MS of the TMS methyl glycosides and the major polysaccharide (2-substituted- (1-3)-β-d-glucan).

In our study, the TAC of the exopolysaccharide was calculated and found to be $76.34 \pm 0.12\%$. Likewise, Mahendran et al. (2013) noticed the TAC of EPS in *L. fusiformis* confirmed in malt supplemented medium ($80.13 \pm 0.26\%$). Oktay et al. (2003) suggested that the reducing power of a biochemical matter considerably associate with antioxidant principles. Yang et al. (2015) reported that reducing activity of EPS in *B. amyloliquefaciens* C-1 was 76%. Our report also is similar with the above-mentioned reports which suggest the reducing power of EPS was $71.15 \pm 0.18\%$ when compared with standard compound.

The hydroxyl free radical scavenging results of the exopolysaccharide in the potent isolate of *B. amyloliquefaciens* was $89.75 \pm 2.68\%$. The bacterial exopolysaccharides have free radical scavenging abilities on both DPPH radicals and hydroxyl radicals with various bioactivities viz, prevention of chain initiation, decomposition of peroxides (Zhao et al., 2018; Wang et al., 2017). The previous findings are in accordance with our reports which reveal the H_2O_2 free radical scavenging results of EPS from the candidate isolate was $68.65 \pm 0.22\%$. An evaluation of H_2O_2 scavenging results is one among the significant techniques for determining the results of antioxidants to decrease the level of pro-oxidants (Czochra and Widensk, 2002).

The production of exopolysaccharide exhibited DPPH activity of 63.5% at 10 mg/ml and 48.9% at 4 mg/ml, for *Enterococcus faecium* BDU7 and *Weissella cibaria* GA44 respectively (Abdhal et al., 2014; Adesulu-Dahunsi et al., 2018). The lower molecular mass of the bacterial exopolysaccharide could be a suitable factor for maximum antioxidant activities (Huang et al., 2013). The above-

mentioned result is in accordance with our result which reveals the total antioxidant activity of the candidate isolate ($60.31 \pm 0.09\%$).

5. Conclusion

In this research, exopolysaccharide from *L. paracasei* was purified and characterized. Presence of alkyl group was confirmed through FTIR analysis. Presence of neutral sugars and monosaccharide were confirmed through HPLC analysis. Presence of (21.51%) alpha-D-Galactose was confirmed through GC-MS analysis. Our findings have believed that EPS from the candidate isolate shows a significant bioactive compound used for the development of novel antibiotics which can be an alternative to the existing chemical antibiotic and antioxidant therapy.

Declaration of Competing Interest

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

Acknowledgements

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this research group NO (RG-1435-014).

References

- Abdhal, K., Ganesh, M., Shanmughapriya, S., Kanagavel, M., Anbarasu, K., Natarajaseenivasan, K., 2014. Antioxidant activity of exopolysaccharide from probiotic strain *Enterococcus faecium* (BDU7) from Ngari. Int. J. Biol. Macromol. 70, 450–454. <https://doi.org/10.1016/j.ijbiomac.2014.07.026>.
- Adesulu-Dahunsi, A.T., Jeyaram, K., Sanni, A.I., Banwo, K., 2018. Production of exopolysaccharide by strains of *Lactobacillus paracasei* YO175 and OF101 isolated from traditional fermented cereal beverage. Peer J. 6, 5326. <https://doi.org/10.7717/peerj.5326>.
- Anita, I., Kalpana, M., Bhavanath, J., 2005. Characterization of an exopolysaccharide produced by a marine *Enterobacter cloacae*. Ind. J. Exp. Biol. 43, 467–471.
- Bejar, V., Llamas, I., Calvo, C., Quesada, E., 1998. Characterization of exopolysaccharides produced by 19 halophilic strains of the species *Halomonas eurihalina*. J. Biotechnol. 6 (2), 135–141. [https://doi.org/10.1016/S0168-1656\(98\)00024-8](https://doi.org/10.1016/S0168-1656(98)00024-8).

- Bhaskar, P.V., Bhosle, N.B., 2005. Microbial extracellular polymeric substances in marine biogeochemical processes. *Curr. Sci.* 88 (1), 45–53.
- Bitter, T., Muir, H.M., 1962. A modified uronic acid carboxylic reaction. *Anal. Biochem.* 4, 330–334. [https://doi.org/10.1016/0003-2697\(62\)90095-7](https://doi.org/10.1016/0003-2697(62)90095-7).
- Blois, Marsden, S., 1958. Antioxidant determinations by the use of a stable free radical. *Nature* 181(1647), 1199–1200. doi:10.1038/1811199a0.
- Bragadeeswaran, S., Jeevapriya, R., Prabhu, K., Rani, S.S., Priyadharsini, S., Balasubramanian, T., 2011. Exopolysaccharide production by *Bacillus cereus* GU812900, a fouling marine bacterium. *African J. Microbiol.* 5 (24), 4124–4132. <https://doi.org/10.5897/AJMR11.375>.
- Bramhachari, P.V., Dubey, S.K., 2006. Isolation and characterization of exopolysaccharide produced by *Vibrio harveyi* strain VB23. *Let. Appl. Microbiol.* 43 (5), 571–577. <https://doi.org/10.1111/j.1472-765x.2006.01967.x>.
- Bramhachari, P.V., Kishor, P.B.K., Ramadevi, R., Kumar, R., Rao, B.R., Dubey, S.K., 2007. Isolation and characterization of mucous exopolysaccharide (EPS) produced by *Vibrio furnissii* strain. *J. Microbiol. Biotechnol.* 17 (1), 44–51.
- Bueno, S.M., Garcia-Cruz, C.H., 2006. Optimization of polysaccharides production by bacteria isolated from soil. *Braz. J. Microbiol.* 37 (3), 296–301. <https://doi.org/10.1590/S1517-8382200600300018>.
- Chowdhury, S.R., Basaka, R.K., Sen, R., Adhikari, B., 2011. Characterization and emulsifying property of a carbohydrate polymer produced by *Bacillus pumilus* UW-02 isolated from waste water irrigated agricultural soil. *Int. J. Biol. Macromol.* 48, 705–712. <https://doi.org/10.1016/j.ijbiomac.2011.02.019>.
- Cooper, D.G., Goldenberg, B.C., 1987. Structural composition, emulsifying activity and rheological properties. *Bioresour. Technol.* 98, 3130–3135.
- Czochra, M.P., Widensk, A., 2002. Spectrophotometric determination of hydrogen peroxide scavenging activity. *Anal. Chim. Acta* 452 (2), 177–184. [https://doi.org/10.1016/S0003-2670\(01\)01455-6](https://doi.org/10.1016/S0003-2670(01)01455-6).
- De Philippis, R., Vincenzini, M., 1998. Exocellular polysaccharides from cyanobacteria and their possible applications. *FEMS Microbiol. Rev.* 22 (3), 151–175. <https://doi.org/10.1111/j.1574-6976.1998.tb00365.x>.
- Dogsa, I., Kriechbaum, M., Stopar, D., Lagner, P., 2005. Structure of bacterial extracellular polymeric substances at different pH values as determined by SAXS. *Biophys. J.* 89, 2711–2720. <https://doi.org/10.1529/biophysj.105.061648>.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Pebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugar and related substances. *Anal. Chem.* 28 (3), 350–356. <https://doi.org/10.1021/ac60111a017>.
- El-Newary, S.A., Ibrahim, A.Y., Asker, M.S., Mahmoud, M.G., El Awady, M.E., 2017. Production, characterization and biological activities of acidic exopolysaccharide from marine *Bacillus amyloliquefaciens* 3MS 2017. *Asian Pac. J. Trop. Med.* 10 (7), 652–662. <https://doi.org/10.1016/j.apjtm.2017.07.005>.
- Guezennec, J., Pignet, P., Lijour, Y., Gentric, E., Ratiskol, J., Collic-Jouault, S., 1998. Sulfation and depolymerization of a bacterial exopolysaccharides of hydrothermal origin. *Carbohydr. Poly.* 37 (1), 19–24. [https://doi.org/10.1016/S0144-8617\(98\)00006-X](https://doi.org/10.1016/S0144-8617(98)00006-X).
- Gulcin, I., Küfrevioğlu, O.I., Oktay, M., Büyükkokuroğlu, M.E., 2004. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *J. Ethnopharmacol.* 90 (2–3), 205–215. <https://doi.org/10.1016/j.jep.2003.09.028>.
- Gutnick, D.L., Bach, H., 2000. Engineering bacterial biopolymers for the biosorption of heavy metals; new products and novel formulations. *Appl. Microbiol. Biotechnol.* 54, 451–460. <https://doi.org/10.1007/s002530000438>.
- Huang, Q., Siu, K.C., Wang, W.Q., Cheung, Y.C., Wu, J., 2013. Fractionation, characterization and antioxidant activity of exopolysaccharides from fermentation broth of a *Cordyceps sinensis* fungus. *Process Biochem.* 48 (2), 380–386. <https://doi.org/10.1016/j.procbio.2013.01.001>.
- Ibarburu, I., Soria-Diaz, M.E., Rodriguez-Carvajal, M.A., Velasco, S.E., Tejero-Mateo, P., Gil-Serrano, A.M., Irazorza, A., Duenas, M.T., 2006. Growth and exopolysaccharide (EPS) production by *Oenococcus oeni* 4 and structural characterization of their EPS. *J. Appl. Microbiol.* 103 (2), 477–548. <https://doi.org/10.1111/j.1365-2672.2006.03266.x>.
- Karuppiah, P., Venkatasamy, V., Ramasamy, T., 2014. Isolation and characterization of exopolysaccharide producing bacteria from Pak Bay (Mandapam). *Int. J. Oceanography Marine Ecol.* 3, 1–8. <https://doi.org/10.3923/ijomes.2014.1.8>.
- Kodali, V.P., Das, S., Sen, R., 2009. An exopolysaccharide from a probiotic: Biosynthesis dynamics, composition and emulsifying activity. *Food Res. Inter.* 42 (5–6), 695–699. <https://doi.org/10.1016/j.foodres.2009.02.007>.
- Levined, N.D., 1974. Bergey's manual of determinative bacteriology. In: Buchanan, R.E., Gibbons, N.E. (Eds.) Williams & Wilkins Co. Baltimore, Md. 21202. xxvii+1246 doi.org/10.1111/j.1550-7408.1975.tb00935.x.
- Lloyd, A.G., Dodgson, K.S., Price, R.G., Rose, F.A., 1961. Infrared studies on sulphate esters. I. polysaccharide sulphates. *BBA(BBA)* 1 (46), 108–115. [https://doi.org/10.1016/0006-3002\(61\)90652-7](https://doi.org/10.1016/0006-3002(61)90652-7).
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193, 265–275. <http://www.jbc.org/content/193/1/265.citation.full.html>.
- Luo, D., Fang, B., 2008. Structural identification of ginseng polysaccharides and testing of their antioxidant activities. *Carbohydr. Poly.* 72 (3), 376–381. <https://doi.org/10.1016/j.carbpol.2007.09.006>.
- Mahendran, S., Vijayabaskar, P., Saravanan, S., Anandapandian, K.T.K., Shankar, T., 2013. Structural characterization and biological activity of exopolysaccharide from *Lysinibacillus fusiformis*. *African J. Microbiol. Res.* 7 (37), 4629–4639. <https://doi.org/10.5897/AJMR2013.5639>.
- Majumdar, I., D'Souza, F., Bhosle, N.B., 1999. Microbial exopolysaccharides: effect on corrosion and partial chemical characterization. *J. Ind. Institute Sci.* 79, 539–550.
- Mata, J.A., Bejar, V., Llamas, I., Arias, S., Bressollier, P., Tallon, R., Urdaci, M.C., Quesada, E., 2006. Exopolysaccharides produced by the recently described halophilic bacteria *Halomonas ventosae* and *Halomonas anticariensis*. *Res. Microbiol.* 157, 827–835. <https://doi.org/10.1016/j.resmic.2006.06.004>.
- Nwodo, U., Green, E., Okoh, A., 2012. Bacterial exopolysaccharides: functionality and prospects. *Int. J. Mol. Sci.* 13, 14002–14015. <https://doi.org/10.3390/ijms13114002>.
- Ohno, N., Miura, N., Nakajima, M., Yadomae, T., 2000. Antitumor 1,3-beta-glucan Fromcultured fruit body of *Sparassiscrispa*. *Biol. Pharmaceutical Bull.* 23 (7), 866–872. <https://doi.org/10.1248/bpb.23.866>.
- Oktay, M., Gülçin, I., Küfrevioğlu, O.I., 2003. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *LWT – Food Sci. Technol.* 36 (2), 263–271. [https://doi.org/10.1016/S0023-6438\(02\)00226-8](https://doi.org/10.1016/S0023-6438(02)00226-8).
- Ozlem, A., 2015. Systems biology of microbial exopolysaccharides production. *Front. Bioengineer. Biotechnol.* 3 (200). <https://doi.org/10.3389/fbioe.2015.00200>.
- Philippis, S.D., Sili, C., Tassinato, G., Vincenzini, M., Materassi, R., 1991. Effects of growth conditions on exopolysaccharide production by *Cynospira capsulata*. *Bioresour. Technol.* 38 (2–3), 101–104. [https://doi.org/10.1016/0960-8524\(91\)90138-A](https://doi.org/10.1016/0960-8524(91)90138-A).
- Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphor molybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* 269 (20), 337–341. <https://doi.org/10.1006/abio.1999.4019>.
- Rawal, P.M., Chauhan, P.B., Prajapati, H., Gahlout, M., 2016. Evaluation of cultivation condition for enhanced production of exopolysaccharide by bacterial isolate P 11 under submerged culture condition. *Int. J. Adv. Res. Biol. Sci.* 3 (5), 183–190. <http://s-o-l.org/1.15/ijarbs-2016-3-5-26>.
- Roseiro, J.C., Esgalhado, M.E., Amaral-Collaco, M.T., Emery, A.N., 1992. Medium development for xanthan production. *Process Biochem.* 27 (3), 167–175. [https://doi.org/10.1016/0032-9592\(92\)87005-2](https://doi.org/10.1016/0032-9592(92)87005-2).
- Ruas-Madiedo, P., Medrano, M., Salazar, N., Reyes-Gavilán, D.L., Pérez, P.F., Abraham, A.G., 2010. Exopolysaccharides produced by *Lactobacillus* and *Bifidobacterium* strains abrogate *in vitro* the cytotoxic effect of bacterial toxins on eukaryotic cells. *J. Appl. Microbiol.* 109 (6), 2079–2086. <https://doi.org/10.1111/j.1365-2672.2010.04839.x>.
- Tong, H., Xia, F., Feng, K., Sun, G., Gao, X., 2009. Structural characterization and *in vitro* antitumor activity of a novel polysaccharide isolated from the fruiting bodies of *Pleurotus ostreatus*. *Bioresour. Technol.* 100 (4), 1682–1686. <https://doi.org/10.1016/j.biortech.2008.09.004>.
- Vijayabaskar, P., Babinastarlin, S., Shankar, T., Sivakumar, T., Anandapandian, K.T.K., 2011. Quantification and characterization of exopolysaccharides from *Bacillus subtilis* (MTCC 121). *Adv. Biol. Res.* 5 (2), 71–76.
- Vimala, P., Lalithakumar, D., 2003. Characterization of exopolysaccharide (EPS) produced by *Leuconostoc* sp. V 41. *Asian J. Microbiol. Biotechnol. Environ. Sci.* 5 (2), 161–165.
- Vuyst, L.D., Vanderveken, F., de Ven, S.V., Degeest, B., 1998. Production by and isolation of exopolysaccharides from *Streptococcus thermophilus* grown in a milk medium and evidence for their growth-associated biosynthesis. *J. Appl. Microbiol.* 84, 1059–1068. <https://doi.org/10.1046/j.1365-2672.1998.00445.x>.
- Wang, H., Ooi, E., Ang Jr, P.O., 2007. Antiviral polysaccharides isolated from Hong Kong brown seaweed *Hydroclathrus clathratus*. *Sci. China Life Sci.* 50 (5), 611–618. <https://doi.org/10.1007/s11427-007-0086-1>.
- Wang, X., Shao, C., Liu, L., Guo, X., Xu, Y., Lü, X., 2017. Optimization, partial characterization and antioxidant activity of an exopolysaccharide from *Lactobacillus paracasei* KX041. *Int. J. Biol. Macromol.* 103, 1173–1184. <https://doi.org/10.1016/j.ijbiomac.2017.05.118>.
- Xu, W., Zhang, F., Luo, Y., Ma, L., Kou, X., Huang, K., 2008. Antioxidant activity of a water-soluble polysaccharide purified from *Pteridium aquilinum*. *Carbohydr. Res.* 344 (2), 217–222. <https://doi.org/10.1016/j.carres.2008.10.021>.
- Yamaguchi, T., Takamura, H., Matoba, T., Terao, J., 1998. HPLC method for evaluation of the free radical – scavenging activity of foods by using 1, 1-Diphenyl – 2 – Picryl hydrozyl. *Biosci. Biotechnol. Biochem.* 62 (6), 1201–1204. <https://doi.org/10.1271/bbb.62.1201>.
- Yang, H., Deng, J., Fan, D., Zhang, Y., Zhang, R., Han, B., 2015. Two Novel Exopolysaccharides from *Bacillus amyloliquefaciens* C-1: Antioxidation and Effect on Oxidative Stress. *Curr. Microbiol.* 70 (20), 298–306. <https://doi.org/10.1007/s00284-014-0717-2>.
- Zhang, Y., Ye, Z.L., Fang, X.L., Li, Y.H., Cai, W.M., 2008. Production and characteristics of a bioflocculant produced by *Bacillus* sp. F19. *Bioresour. Technol.* 99 (16), 7686–7691. <https://doi.org/10.1016/j.biortech.2008.01.068>.
- Zhao, W., Zhang, J., Jiang, Y.-Y., Zhao, X., Hao, X.-A., Li, L., Yang, Z.-N., 2018. Characterization and antioxidant activity of the Exopolysaccharide produced by *Bacillus amyloliquefaciens* GSBa-1. *J. Microbiol. Biotechnol.* 28 (8), 1282–1292. <https://doi.org/10.4014/jmb.1801.01012>.

Further reading

- Kawai, H., Isobe, Y., Koribe, M., Tokuda, J., Tokuno, I., Endo, K., Kawai, F., 1992. Production of a novel extracellular polysaccharide by a *Bacillus* strain isolated from soil. *Biosci. Biotechnol. Biochem.* 56 (6), 853–857. <https://doi.org/10.1271/bbb.56.853>.