Biotechnology Journal International

Volume 27, Issue 2, Page 1-14, 2023; Article no.BJI.98544
ISSN: 2456-7051
(Past name: British Biotechnology Journal, Past ISSN: 2231–2927, NLM ID: 101616695)

Structural and Rheological Properties of Exopolysaccharides Produced by Some Lactic Acid Bacterial Strains Isolated from Palm Wine

O. L. Adamu-Governor [a,b,c*], T. A. Shittu [c], O. R. Afolabi [d], A. I. Elijah [e], C. N. Opara [f] and S. V. A. Uzochukwu [g]

[a] Department of Biological Science, Yaba College of Technology, Nigeria.
[b] College Central Research Laboratory, Yaba College of Technology, Nigeria.
[c] Department of Food Science and Technology, Federal University of Agriculture, Abeokuta, Nigeria.
[d] Department of Microbiology, Federal University of Agriculture, Abeokuta, Nigeria.
[e] Department of Food Science and Technology, University of Uyo, Uyo, Akwa Ibom State, Nigeria.
[f] Department of Microbiology, Federal University, Otueke, Bayelsa State, Nigeria.
[g] Department of Plant Science and Biotechnology, Federal University, Oye-Ekiti, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJI/2023/v27i2674

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/98544

Original Research Article

Received: 12/02/2023
Accepted: 15/04/2023
Published: 24/04/2023

ABSTRACT

Aims: This study evaluated the physical, chemical and rheological properties of exopolysaccharides (EPSs) produced by Lactic Acid Bacteria (LAB) isolated from palm wine.
Materials and Methods: EPSs from palm wine LAB strains were produced on 6% sucrose broth, purified and freeze-dried prior to analyses. Molecular weights (MW), rheological and structural composition (functional groups) of the EPSs were determined using standard methods and Fourier transform infrared spectroscopy (FTIR).

**Corresponding author: E-mail: unileonard@yahoo.com, leonard.adamu@yabatech.edu.ng;*

Results: The average MW of the EPSs ranged from 2.02×10^6 to 6.53×10^6 Da while the flow index (n) values ranged from 0.03-3.13 at 0.2%, 0.06-1.51 at 0.4%, 0.38 - 1.85 at 0.6%, 0.14 - 2.26 at 0.8% and 0.55 - 6.42 at 1% concentrations at elevated temperatures for EPS solutions from the ten LAB species. The FTIR spectrum revealed prominent peaks of various groups of OH (3420 cm^{-1}) and CH_3 bending (2090 cm^{-1}) in all the EPSs corresponding to both hydroxyl and amine groups, and aliphatic C-H bonds, respectively. EPS synthesized by *Leuconostoc lactis*, *Lactococcus lactis* subsp *lactis* and *Lactiplantibacillus plantarum* showed weak absorption peaks (1148 – 1145 cm^{-1}) indicating the C-O-C and C-O bonds, while absorption peaks of *Lactobacillus lactis*, *Lactobacillus acidophilus* and *Lactiplantibacillus plantarum* (1267 – 1253 cm^{-1}) indicated O- acetyl ester and other non-sugar components.
Conclusions: The FTIR spectra, rheological properties and molecular weight of EPSs synthesized by the ten LAB strains indicated potentials that could be exploited in different industrial applications, and as stabilizers in food industries

Keywords: Exopolysaccharides; molecular weight; lactic acid bacteria; fourier transform infrared spectroscopy; palm-wine; viscosity.

1. INTRODUCTION

"Microorganisms, including lactic acid bacteria (LAB), have been reported to produce polysaccharides that are potentially useful as thickeners, stabilizers, emulsifiers, bodying agents, gelling agents, or fat replacers in several food products" [1-4]. Some LAB species are well known as polysaccharide/gums producers, and gum (dextran) from *Leuconostoc mesenteroides* has been exploited commercially [5,6]. The biodegradable and high molecular weight features of these polymers biosynthesized by a wide range of bacteria, yeasts and moulds have been documented [6-8]. The rheological properties of gums produced by LAB which are influenced by the structure, molecular mass and amount of gum produced, have been shown to markedly improve the texture and consistency of fermented products [3,9,10].

"LAB are food grade organisms, generally recognized as safe (GRAS). During the fermentation of palm sap, certain strains of LAB produce exopolysaccharides (largely dextrans and levans), which are responsible for the consistency and soluble white colouration of the palm wine" [6,11,12]. "The exopolysaccharide (EPS) from LAB has potential applications in improvement of rheology, texture and mouth-feel of fermented products" [3,13]. The utility of such LAB-derived EPS is demonstrated by its biopolymer nature and physico-chemical properties suitable for wide range of technological applications. Generally, industrial applications of EPS and other polymers are determined by their molecular weight and/or the functional groups content of the molecular chains [2,14,15]

Molecular mass of EPS has been shown to influence their functionality as food additive. To this end, the required molecular mass of EPS for meaningful result particularly dextran on sourdough bread should range from 2×10^6 to 4×10^6 Da (U.S. Patent 6, 399, 119) [16]. Studies have shown that ability of EPS to confer viscosity in aqueous solutions or food products is largely determined by the molecular parameters [17,18]. The molar masses of EPS produced by lactic acid bacteria varied according to strains and depend on the polymer [18]. Ruas-Madiedo et al. [19] reported the molar mass of EPS produced by two strains of *Streptococcus thermophilus* (designated as Sts and Rs), and that Sts had higher molecular weight (3.7×10^6 Da) and consequently more viscous than Rs with lower molecular weight (2.6×10^6 Da). To obtain a high viscosity in a certain product, the application of EPS with higher molar mass with relative stiffness is desirable [14]. Earlier, the role of stiffness as one of the ingredients in viscosifying properties of EPS has been reported [20].

EPS has been shown to have desirable rheological properties such as high specific viscosity and tolerance to extremes in pH and temperature [15,21]. Generally, the rheological properties of hydrocolloids/EPSs are of special importance in process design, evaluation and modelling, and for texture attribute modification. However, viscosities of hydrocolloids are affected by concentrations, temperatures, pressure, shear rate and time of shearing. The effect of concentration on apparent viscosity of hydrocolloids is usually expressed by either a power relationship or an exponential, while the effect of temperature on the apparent viscosity at

a specified shear rate has been described by an Arrhenius-type model [22,23].

Flow behaviour of hydrocolloid solutions has been described using several models including power law (Ostwald-deWaele), Casson models, linear (Newtonian or Bingham) and power law with a yield stress (Herschel-Bulkley) [23,24]. "However, the power law model is perhaps the most widely employed model for non-Newtonian liquids and is used extensively to describe the flow properties of liquids in theoretical analysis as well as in practical engineering applications" [22,25].

"Further, Fourier transform-infrared spectroscopy has been a useful tool in monitoring structural changes in biopolymers" [26]. "The use of Fourier transform-infrared spectroscopy (FTIR) in the field of microbiology has proved to be a promising technique. FTIR spectroscopy simultaneously measures the vibration of functional groups of different cell component in multi-component mixtures. Admittedly, polysaccharides contain a significant number of hydroxyl groups, which exhibit an intense broad stretching peak. To determine the molecular mass of an EPS, exist different methods; the chromatography using refractive index (RI) detection [18], gel permeation chromatography in an HPLC [10,27] or fast protein liquid chromatography (FPLC) system and formula derivation. The use of formula for determination of the molecular weight of EPS developed by Banks and Greenwood has been reported" [15,28].

In recent years, much attention has been given to a large variety of exopolysaccharide-producing LAB from different sources including grains, dairy, meat products, fermenting vegetables and fermented foods [6,14,29,30]. "Such LAB exopolysaccharides are considered to be safe and possess the possibility of replacing stabilizers and thickeners currently produced commercially by non-food grade bacteria" [3,4,10,31,32]. Low yields of polysaccharide production by the majority of LAB species remains the main reason for their non-commercial exploitation. Though some LAB species have showed high yields EPSs production potential [16,33]. The functional properties of EPSs are influenced by their primary structure [34], and structural analysis combined with rheological studies revealed that there is considerable variation among the different EPSs [17,19]. In this study, the physical, chemical and rheological properties of EPSs produced by some LAB species isolated from palm sap were evaluated with a view to establishing potential applications for commercial exploitation.

2. MATERIALS AND METHODS

2.1 Source of Isolates

Ten strains of exopolysaccharide-producing lactic acid bacteria (*Leuconostoc lactis*; AB023968, *Lactobacillus fermentum*; AF477498.1, *Lactobacillus lactis*; AY675257.1, *Lactococcus lactis subsp lactis;* AY920468, *Lactobacillus delbrueckii*; X52654.1, *Lactobacillus acidophilus*; FJ556999.1, *Lactobacillus plantarum*; EU121673, *Lactobacillus crispatus*; AB008206.1, *Leuconostoc mesenteroides*; AB023243, and *Lactobacillus plantarum*; EU148598) were used in this study. The LAB species were obtained from fresh palm wine as previously described by Adamu-Governor et al. [35] and identified based on 16S rRNA gene analysis. The identified LAB strains were stored in 6% sucrose agar slants at 4 °C.

2.2 Microbial Gum Production

Cultivation was performed in basal medium (v\v, 6% sucrose, 0.5% peptone, 0.05% K_2HPO_4, 0.025% $MgSO_4$) in 1.5 L flasks with 1 L working volume according to the method described by Adamu-Governor et al. [6]. Growth was monitored by absorbance measurement at a wavelength of 650 nm using a spectrophotometer (Spectrum lab S23A, Globe Medical, City, England).

2.2.1 Isolation and quantification of EPS

Isolation and quantification of EPS were carried out according to the method described by Adamu-Governor et al. [6]. The cells suspension was stirred with glass rod, heated at 80 °C (Heating block) to extract EPS associated with bacteria cells and cells were harvested from the fermented culture broth by centrifugation at 11,000 x g for 30 min in a pre-weighed tube. EPS were precipitated with 3 volumes of chilled ethanol (95%, v/v) and kept overnight at 4 ^{o}C for complete precipitation. The precipitated crude EPS was collected by centrifugation at 10,000 x g, 4 °C (J2-HS, Beckman, USA) for 30 min, and EPS pellets were dried in an oven at 105 °C to a constant weight. The EPS pellets were dissolved in 50 ml distilled water, precipitated twice with isopropanol and then freeze-dried (Telster,

Cryodos-8mode, Spain). Quantification of EPS was done using dry weight method [6].

2.2.2 Physical analysis of exopolysaccharides

The functional groups of the freeze-dried EPS were determined by Fourier transformed-infrared (FT-IR) spectroscopy [36]. The EPS pellets for FT-IR analysis was obtained by grinding a mixture of 1.2 mg EPS and 150 mg of dry potassium bromide powder in a mortar, followed by pressing the mixture into a mould. The Fourier transformed-infrared spectra were recorded on a FT-IR (Bruker IFS 66v/s, Germany) equipped with OPUS version 3.1 software for windows in the region of 4000-40 cm^{-1}, and at a resolution of 4 cm^{-1}.

2.3 Specific Viscosity

The apparent viscosity (Pa.s) of the EPS solution (0.2 - 1.0% in distilled water, pH 7.0, 32 ± 2 °C, 45, 60 and 90 °C) was measured using Brookfield viscometer, (DV-E viscometer, spindle number 2; Brookfield engineering Laboratory, Inc II commerce, Middleboro) according to Kaur et al. [37] with modifications. EPS solutions of 0.2, 0.4, 0.6, 0.8 and 1% w/v were prepared by dissolving 0.4, 0.8, 1.2, 1.6 and 2.0 g of freeze-dried EPS powder in 200 ml water, respectively. The solution was stirred with a sterile glass rod until a particle suspension-free solution was obtained and the pH adjusted to 7 with the addition of either 0.1 M sodium hydroxide or 0.1 M hydrochloric acid as required. The viscosities at different concentrations of the EPS solutions were measured at various shear rates (10.6, 12.72 and 21.2 per second). All viscosity (cP) measurements were repeated thrice using solutions freshly prepared. The specific viscosity of the EPS solutions was calculated by employing the formula:

$$\eta_{sp} = \frac{\eta}{\eta_o} - 1 \quad (1)$$

Where η_{sp}, specific viscosity; η, viscosity of EPS solution; $\eta_{0,}$ viscosity of solvent.

2.3.1 Molecular weight of exopolysaccharides

The molecular weight of EPS was calculated following the formula developed by Banks W and Greenwood CT [28] as follows:

$$M_w = 0.9(\eta_{sp}) \times 10^6 \quad (2)$$

Where, $M_{w,}$ the molecular weight of EPS; $\eta_{sp,}$ the specific viscosity of EPS solution at 0% concentration.

2.3.2 Rheological analysis

Power law modelling analysis was used to find a rheological model that can be employed to fit the experimental measurements.

$$\tau = K\gamma^n \quad (3)$$

Where τ, shear stress; K, consistency index; γ, shear rate; n, flow index.

2.4 Statistical Analysis

Molecular weight data were generated in triplicates, subjected to statistical analyses using a one-way Analysis of Variance test (ANOVA) and data were reported as mean value ± standard deviation with the aid of SPSS (IBM SPSS Inc 26 Chicago, IL, USA)

3. RESULTS

3.1 Fourier Transform Infrared Analysis

The Fourier transform infrared (FT-IR) spectrum of the crude EPSs synthesized by lactic acid bacteria isolated from palm wine is presented in Figs. 1a–, 2a–d) and 3a – b). Generally, the IR spectra of the partially purified selected LAB species/strain EPSs showed more complex pattern of peaks from 3500 to 1200 cm^{-l}. The results showed that spectrum of the EPS displayed a broad stretching intense peak at around 3420 cm^{-1} characteristics for hydroxyl and amine groups, and a peak around 2090 cm^{-1} indicating aliphatic C-H bonds (Figs. 1-3). Polysaccharides contain a significant number of hydroxyl groups which exhibit an intense broad stretching peak around 3450 cm^{-l}. The absorption in that region showed a typical trait of hydroxyl groups which strongly suggested that the substance was a polysaccharide.

In addition, a peak was also observed around 2090 cm^{-1} corresponding to methyl groups as well as methylene groups (Figs. 1-3). Further, a strong absorption was observed at around 1643 cm^{-1} which corresponds to primary amide (1^o amide) > C = O stretch and C-N bending of protein and peptide amines. Similarly, a weak symmetrical peak was noticed near 1416 – 1404 cm^{-1} (Figs. 1-3), suggesting the presence of carboxyl groups. The peak within range 1416 – 1404 cm^{-1}could be assigned to > C = O stretch of the COO^- groups and C – O bond from COO^- groups has been established. In addition, the spectra also showed bands around 1000, 1200, 1400, 1500 and 1600 cm indicating conclusively

that it was (1, 3) - glucan linkages. Further, a weak absorption near 1148 – 1145 cm^{-1} particularly in EPS synthesized by *Leuconostoc lactis*, *Lactococcus lactis* subsp *lactis* and *Lactiplantibacillus plantarum showed* C-O-C and C-O bonds (Figs. 1a, 1d and 2c). This weak absorption indicated C-O-C and C-O bonds corresponding to the presence of carbohydrates.

Furthermore, EPS synthesized by *Lactobacillus lactis*, *Lactobacillus acidophilus* and *Lactiplantibacillus plantarum* (Figs. 1c, 2b and 3b) showed spectra with a peak near 1267 – 1253 cm^{-1}. Noticeably, these EPSs differed from EPS synthesized by other LAB species selected in this study due to the presence of peak around 1267 – 1253 cm^{-1} in the spectra suggesting the presence of O-acetyl ester and other non-sugar components (Figs. 1c, 2b and 3b). In general, a strong absorption was observed near 1025 – 1020 cm^{-1} in the spectra of all EPS produced by the ten LAB species in this study and this suggest that all EPS synthesized were undoubtedly polysaccharides. In the same vein, the absorption bands in the region 983-1200 cm^{-1} in this study suggested the presence of sugar monomers such as glucose, galactose and mannose in the EPS of LAB species.

The FTIR spectra EPSs in this study revealed characteristic functional groups, such as a broad stretching -OH at 3420 cm^{-1}, a peak C-H at around 2090 cm^{-1}, strong absorption 1 > C = O stretch and C-N bending of protein and peptide amines at around 1643 cm^{-1} and a weak COOH peak at around 1416 – 1404 cm^{-1} (Figs. 1-3). Further, strong absorption near 1025-1020 cm^{-1} corresponding to the presence of carbohydrate was also revealed in the spectra. These functional groups were common to all the ten LAB species EPS.

3.2 Viscosity Measurement of EPSs Solution

The mean values of apparent viscosities (η) of EPSs solution synthesized by LAB isolated from oil and raphia palm sap differed between species and varied within species (data not shown). As expected, the mean values of apparent viscosities showed an increase at lower shear rate (Ƭ) and a decrease at higher shear rate. The decrease in apparent viscosities of EPSs solution with increasing shear stress in this study revealed a non-Newtonian shear thinning behaviour. The apparent viscosities mean values also showed high viscosities at all shear rates by the LAB EPSs compared with xanthan gum.

3.3 Power Law Model of EPS Solution

The power law mathematical model values of EPSs synthesized by LAB species in this study showed consistency index (k) values ranging from 0.02 – 320.67 for 0.2% (w/v) concentration; 42.98 – 40000.26, 0.35 – 402823.27, 0.06 – 6059.23 and 0.00 – 14953.67 for 0.4, 0.6 0.8 and 1%, respectively (Table 1). Similarly, the flow index (n) obtained ranged from 0.03 – 3.13 for 2%; 0.06 – 0.82 for 0.4%; 0.38 – 1.85 for 0.6%; 0.14 – 2.26 for 0.8%; 0.12 – 6.42 for 1%. The flow index value 'n' of EPSs solution for all LAB species in this study exhibited either shear-thinning or shear-thickening properties at elevated constant temperature and at different concentrations.

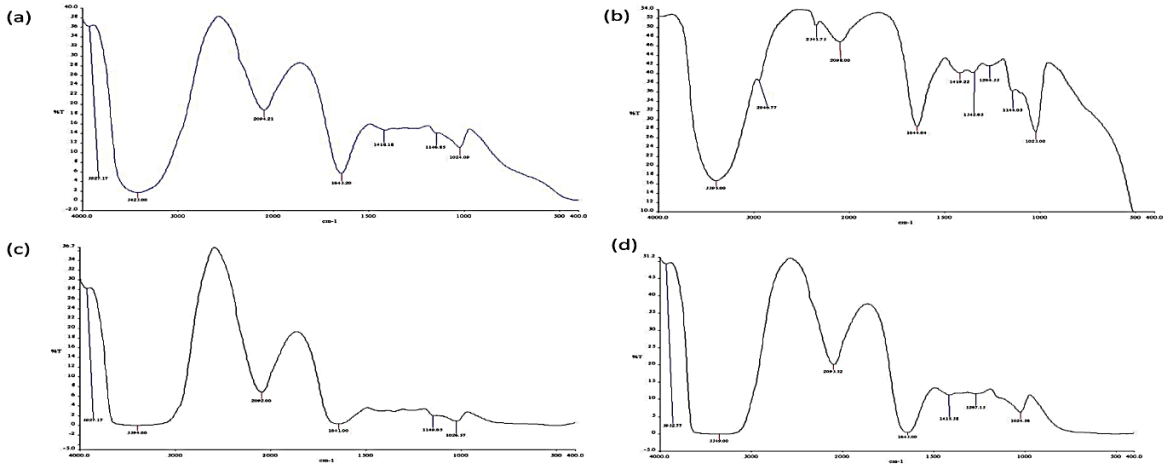

Fig. 1. FT-IR spectrum of the exopolysaccharide produced by (a) *Leuconostoc lactis* (b) *Lactobacillus fermentum* (c) *Lactobacillus lactis* (d) *Lactococcus lactis* subspecies *lactis*

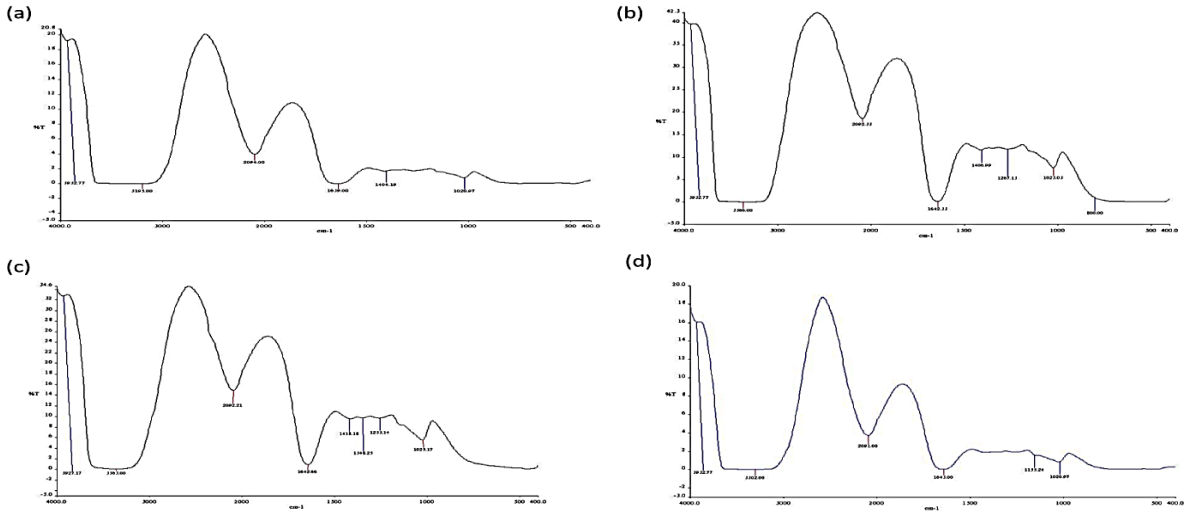

Fig. 2. FT-IR spectrum of the exopolysaccharide produced by (a) *Lactobacillus delbrueckii* (b) *Lactobacillus acidophilus* (c) *Lactiplantibacillus plantarum* (d) *Lactobacillus crispatus*

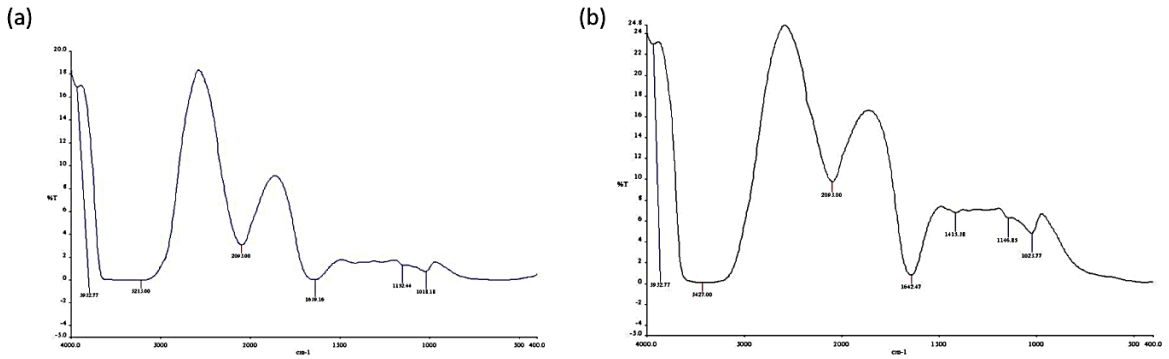

Fig. 3. FT-IR spectrum of the exopolysaccharide produced by (a) *Leuconostoc mesenteroides* (b) *Lactiplantibacillus plantarum*

The flow index values (n) showed that as the concentrations of EPS increased in the solution, the EPS tends to exhibit more of shear-thickening properties as shown in EPSs synthesized by *Leuconostoc lactis, L. delbrueckii, L. crispatus, and Leuconostoc mesenteroides* given that their index values were greater than one (Table 1). Contrarily, the flow index values 'n' of EPS synthesized by *Lactiplantibacillus plantarum* was less than one showing shear-thinning properties as the concentration of EPS increased in the solution. In addition, at 0.2% concentration, EPS synthesized by Lactobacillus *lactis*, *Lactococcus lactis* subsp *lactis*, *Lactobacillus acidophilus* and *Lactiplantibacillus plantarum* strains (1.27 – 1.92) had flow index values greater than one (1), while *Leuconostoc lactis, Lactobacillus fermentum, Lactobacillus delbrueckii, Lactobacillus crispatus* and *Leuconostoc mesenteroides* had flow index less than 1. Generally, the flow index of EPSs of all the LAB species at 0.4% concentration was less than 1 (0.06 – 0.96) and thus, exhibiting shear-thinning properties. Furthermore, only *Lactobacillus fermentum, Lactobacillus lactis, Lactobacillus crispatus* and *Leuconostoc mesenteroides* had flow index greater than 1 (1.15 – 1.85) at 0.6% concentrations. Similarly at 0.8% concentration, all the LAB species/strains EPS showed flow index values greater than 1 (1.03 – 2.26) except *Lactobacillus crispatus, Leuconostoc mesenteroides* and *Lactiplantibacillus plantarum*. Finally, at 1% concentration, six of the LAB species; *Leuconostoc lactis, Lactococcus lactis* subspecies *lactis, Lactobacillus delbrueckii, Lactobacillus acidophilus, Lactobacillus crispatus* and Leuconostoc *mesenteroides* EPSs had flow index values greater than 1 (1.50 – 6.42). Generally, *Leuconostoc lactis* and *Lactobacillus delbrueckii* EPS showed increase in flow index values (0.11 – 6.24; 0.25 – 1.87) as percentage

concentration of EPS in a solvent (w/v) increased. Similar trend was observed with *Lactobacillus crispatus* EPS (0.10 – 3.02). *Lactiplantibacillus plantarum* EPS had a steady flow index value of less than 1 across all concentrations accept at 0.2% concentration.

3.4 Molecular Weight Determination of EPS

The molecular weights of crude EPSs synthesized by EPS-producing lactic acid bacteria isolated from palm wine are presented in Table 2. The apparent molecular weight of the EPS from the ten LAB species ranged from 2.02 × 10^6 Da synthesized by *Lactobacillus fermentum* to 6.53 × 10^6 Da synthesized by *Lactobacillus crispatus*. As expected, the molecular weight of EPSs synthesized by lactic acid bacteria isolated from palm wine differed between species and varied within strains. The molecular weight of EPSs synthesized by two strains of *Lactiplantibacillus plantarum* ranged from 2.13 × 10^6 to 3.21 × 10^6 Da. The molecular weights of the EPSs obtained in this study were derived by viscosity measurements at various concentrations of each EPS.

4. DISCUSSION

Functional groups of the partially purified EPS synthesized by the ten LAB species were detected using FTIR spectrum. Studies have shown that FTIR spectra offer estimate of components/functional groups in the exopolysaccharide (EPS) [15,38,39]. This result is in consonance with previous reports that the presence of –OH groups at (2800 $cm^{-1}$to 3600 cm^{-1}) position of IR spectra of EPS of bacteria indicated polysaccharide [38,40,41]. The range of values for the region where the intense broad stretching peak occurred in this study was within the range reported by Wang et al. [36] that the IR spectra of *Lactobacillus plantarum* KF5 EPS showed the presence of –OH group at around 3307 cm^{-1}. [42] revealed that the IR spectrum of biofilm forming marine bacterium EPS showed the presence of –OH group with a broad stretching peak at 3415.31 cm^{-1} position. "Further, the presence of intense broad stretching peak occurring at 2800 $cm^{-1}$to 3600 $cm^{-1}$is the characteristics absorption band of carbohydrate ring and is responsible for the water solubility of EPS" [36,38,43].

In addition, the spectra also revealed the presence of carboxyl groups. This observation is in agreement with the earlier findings of Helm D and Naumann D [44] and Haxaire et al. [45] who observed a peak at 1404 cm^{-1} in IR spectra of some bacteria cell components and hydration of polysaccharide hyaluronan. Similarly, Wang et al. [36] and Kumar et al. [38] reported a peak near 1447 – 1380 cm^{-1} IR spectra suggesting the presence of carboxyl groups in EPS of Haloalkalophilic *bacillus* species I-450 isolated from heavily polluted soil samples of the Korean Yellow sea and *Lactobacillus plantarum* KF5 isolated from Tiber kefir. [8] also reported that the IR spectrum of crude *Bacillus subtilis* (MTCC 121) polysaccharide sample showed bands at 1000-1500 cm^{-l} which the authors inferred as characteristic to glucan. According to Černá et al. [46] and Čopíková et al. [47], the wave number region from 1200 to 800 wave cm^{-1} is finger print region and can be used to characterize different polysaccharides. Earlier, Mishra A and Jha B [48] reported a broad stretch of C-O-C, C-O at 1000 – 1200 cm^{-1} indicating the presence of carbohydrate in biofilm. Similarly, Pawar et al. [49] also observed a peak at 11000 cm^{-1} for EPS obtained from saline soil bacterium.

"The presence of O-acetyl ester and other non-sugar components at spectra peak around 1267-1253 cm^{-1} has been established" [36,50]. Kazy et al. [50] reported that "the spectra of EPS produced by *Leuconostoc* species CFR 2181 and algal polysaccharide showed an additional peak at around 1240 cm^{-1} region due to the presence of O-acetyl ester". "These reports were comparable with the FT IR spectra obtained in this study. Many researchers have reported that the adherence of non-sugar components such as uronic acid, pyruvate and hexosamine to EPS greatly influences physical properties such as filterability, thermally induced conformational transition [51,52] and viscosity" [52,53]. "Previous reports showed that the strongest absorption band at 1075 cm^{-1} was attributed to polysaccharide" [54]. Similarly, Wang et al. [36] reported that EPS of *Lactobacillus plantarum* isolated from Tibet kefir showed absorption band at the region of ~1075 cm^{-1} which the authors reported as polysaccharide. [55] reported that the monosaccharide constituents of pectic and hemicellulosic polysaccharides such as galactose, mannose and glucose showed the strongest FT IR bands at 1078 cm^{-1}, 1070 $cm^{-1}$and 1035 cm^{-1} respectively. On the contrary, Pawar et al. [49] reported a peak at 1038.92 cm^{-1} of EPS obtained from saline soil bacterium which corresponds to stretching of C-O, alcohol, ether and phenol groups.

Table 1. Power law parameters of microbial gum from LAB isolated from palm wine at 60 °C

	0.2 g/l			0.4 g/l			0.6 g/l			0.8 g/l			1.0 g/l		
	k	**N**	**R^2**	**K**	**n**	**R^2**	**k**	**n**	**R^2**	**k**	**N**	**R^2**	**k**	**n**	**R^2**
Control	113.88	0.03	0.97	7743.37	0.39	1.00	1.70	1.53	1.00	0.29	1.85	0.77	0.00	3.19	0.74
1	320.67	0.11	1.00	3405.51	0.24	1.00	46.50	0.70	1.00	0.58	1.80	0.94	1.57	6.42	0.93
2	26.60	0.96	0.81	912.58	0.06	0.99	1.87	1.58	0.95	0.06	2.26	0.97	315.76	0.12	0.80
3	1.74	1.70	1.00	40000.26	1.51	1.00	8.76	1.17	0.75	2.44	1.44	1.00	61.46	0.59	1.00
4	0.02	3.13	1.00	7412.17	0.46	1.00	4141.71	0.38	1.00	14.95	1.03	0.58	2.37	1.50	1.00
5	106.98	0.26	1.00	372.86	0.25	1.00	21.16	0.96	0.71	12.22	1.07	0.72	0.44	1.87	1.00
6	2.78	1.61	0.91	42.98	0.82	0.92	104.72	0.62	0.74	1.21	1.59	0.99	0.16	2.03	1.00
7	1.01	1.92	1.00	49.73	0.79	0.64	40282.27	0.65	1.00	116.00	0.53	1.00	48.06	0.73	0.81
8	33.32	0.63	0.48	2075.08	0.10	0.95	0.35	1.85	0.73	2.51	1.47	0.78	0.00	3.02	0.96
9	103.57	0.28	0.93	98.68	0.61	0.75	9.57	1.15	1.00	673.52	0.14	1.00	9.99	3.64	0.97
10	8.74	1.27	0.87	37734.44	0.96	1.00	203.20	0.39	1.00	6059.23	0.44	1.00	14953.67	0.55	0.94

Values of apparent viscosity are recorded in triplicate. k, consistency index; n, flow index; n =1, the fluid behaviour shows Newtonian profile; n ≤ 1, the fluid exhibits non-Newtonian of shear- thinning properties; n ≥ .1, the fluid shows non-Newtonian of shear-thickening properties. Control; xanthan gum, 1; Leuconostoc lactis, 2; Lactobacillus fermentum, 3; Lactobacillus lactis, 4; Lactococcus lactis subsp lactis, 5; Lactobacillus delbrueckii, 6; Lactobacillus acidophilus, 7; Lactiplantibacillus plantarum, 8; Lactobacillus crispatus, 9; Leuconostoc mesenteroides, 10; Lactiplantibacillus plantarum

Table 2. Molecular weight of microbial gums from bacteria isolated from palm wine

S/n	Gum producing lactic acid bacterial gums	Molecular weight (Da)
1	*Leuconostoc lactis*	$5.00 \pm 0.00 \times 10^6$
2	*Lactobacillus fermentum*	$2.02 \pm 0.12 \times 10^6$
3	*Lactobacillus lactis*	$2.23 \pm 0.10 \times 10^6$
4	*Lactococcus lactis* subsp *lactis*	$3.78 \pm 0.08 \times 10^6$
5	*Lactobacillus delbrueckii*	$4.67 \pm 0.02 \times 10^6$
6	*Lactobacillus acidophilus*	$3.57 \pm 0.05 \times 10^6$
7	*Lactiplantibacillus plantarum*	$3.21 \pm 0.00 \times 10^6$
8	*Lactobacillus crispatus*	$6.53 \pm 0.21 \times 10^6$
9	*Leuconostoc mesenteroides*	$4.67 \pm 0.10 \times 10^6$
10	*Lactiplantibacillus plantarum*	$2.13 \pm 0.02 \times 10^6$

Values are recorded in triplicate. Values are recorded as mean value ± standard deviation

"In addition, the presence of carboxyl group in EPS may serve as binding sites for divalent cations" [56]. "The carboxyl group may also work as functional moieties to generate new and/or modified polymer variants using different approaches like synthetic polymers or novel formulation designing by linking this polysaccharide with starch" [40]. "Also, the carboxyl and hydroxyl groups in spectra are preferred groups for flocculation processes similar to polyelectrolyte characteristics [36,57]. Besides, presence of several hydroxyl (-OH) groups markedly increases their affinity for binding water molecules thereby rendering EPS hydrophilic" [24].

Exopolysaccharides from the ten LAB species in this study showed high viscosities at all shear rates. LAB EPS showing shear thinning properties with higher viscosities at all shear rates compared with xanthan gum has been established [20,39,58,59]. These authors reported the pseudoplastic nature of LAB EPSs and attributed this property to the breakdown of structural units in EPS generated during shear by hydrodynamic forces. Similarly, other studies have also reported pseudoplastic properties of EPSs from non-lactic bacteria [15,38,60,61]. These authors observed that the viscosity of the EPSs solution decreased with increase in the shear rate. Ismail B and Nampoothiri KM [62] reported that EPS from *Lactobacillus plantarum* MTCC 9510 incorporated into wheat starch had higher viscosity than starch-carboxymethylcellulose as the control at the same concentration. The author also observed that the dispersions of EPS and wheat starch exhibited a non-Newtonian and pseudoplastic behaviour. Structural composition of EPS and molecular weight are responsible for high viscosity and pseudoplastic behaviour of EPS [63]. Shear thinning properties and higher viscosity are desirable qualities for viscosifying agent used in food products [64]. "Pseudoplastic characteristics of biogums enhances sensory qualities such as flavour release and mouthfeel in food products, and guarantees a high degree of mixability and pourability. Industrially, important EPSs such as xanthan gum exhibit high viscosity with pseudoplastic behaviour which makes it an effective thickener and stabilizer in the food industry" [61,65].

"The effect of concentration on apparent viscosity of hydrocolloids is generally described by either an exponential or a power relationship" [23]. This observation is in agreement with the studies of Van den Berg DJC et al. [20] and Ricciardi et al. [59] who reported that results of good shear-thinning property in aqueous solutions of EPS produced by *Lactobacillus sake* 0-1 and *Streptococcus thermophilus* with high viscosities at all shear rates compared with xanthan gum. For most non-Newtonian fluids, at constant temperature and pressure, the viscosity decreases with an increase in shear rate, giving rise to what is known as pseudoplasticity or shear-thinning behaviour [20,38,62].

The flow index (n) of EPSs solution in this study exhibited both shear-thinning and shear-thickening properties at constant concentration and varying temperature. These changes in 'n' values of EPSs synthesized by LAB exhibited at different concentrations and at constant temperature observed in this study may be attributed to structural composition and molecular weight of the EPSs [62,63]. "Temperature has an important influence on the flow behaviour of hydrocolloid solutions" [66]. "Since different temperatures are usually encountered during processing of hydrocolloids, their rheological properties are studied as a function of temperature" [63]. Concentration of EPS in a

solution is known to influence the flow behaviour of the solution. Vicente-García et al. [67] reported that "EPS solution of *Phormidium* 94a isolated from an arid zone of Mexico showed shear thinning property with increasing shear rate and this effect became more pronounced as polymer concentration increased". Marcotte et al. [22] earlier reported that "co-solutes like sucrose, concentration of hydrocolloid, shear rate and temperature are also important variables that influence the rheological status of hydrocolloid gels". "The author also observed a consistent change in apparent viscosity with increasing temperature at 1% concentration xanthan gum. The properties exhibited by the EPS could be attributed to the particular structure composition of the EPS comprising glucose and mannose linked by α and β (1, 3) linkages and its molecular weight" [62]. In addition, Marcotte et al. [22] showed that "gum solutions with a high value of 'n' tend to feel slimy in the mouth". "When high viscosity and good mouthfeel characteristics are desired, the choice should be a gum system having a low 'n' value. Earlier studies have reported that for a given gum type, the value of flow behaviour index (n or 'n') and its change with concentration are highly dependent on the molecular size" [22,68].

The apparent molecular weight of EPSs synthesized by LAB species in this study exceed 10^5 Da. This result agreed with the findings of Minervini et al. [16] who reported that the apparent molecular weight of EPS from *Lactobacillus curvature* DPPMA10 exceeded 10^5 Da. [20] earlier reported that EPS produced by *Lactobacillus sake* 0-1 was 6.0×10^6 Da. Similarly, Muralidharan J and Jayachandran S [15] reported that the molecular weight of EPS from *Vibrio alginolyticus* was 6.39×10^6 Da. The occurrence of two polymers with different molecular weights which occurs in some *Lactococcus lactis* subspecies *cremoris* [69] and *Lactobacillus delbrueckii* subspecies *bulgaricus* [70] strains, have been reported. Also, the occurrence of high ($1\text{-}6 \times 10^6$ Da) and low ($0.1\text{-}1 \times 10^5$ Da) molecular weight fractions in EPS produced by LAB is well documented [64,71]. However, [14] observed that some of the values of molecular weight reported in literatures were over-estimated due to the presence of aggregates in aqueous solutions. The reason for using viscosity measurements method for obtaining the molecular weight of the EPSs was to eliminate the presence of aggregates in aqueous solution [15]. The average molecular weight of non-aggregated molecules of the dextran produced by *Leuconostoc mesenteroides* NRRL B-512F varied between 6.2 and 7.1×10^6 Da [14,72]. In addition, the molecular mass distribution of all polymers is highly dependent on the viscosity of the polymer [20].

In general, it is also known that the molecular weight and the functional groups in the molecular chains of the polymer are important determinants for the flocculating activity [36,38]. In case of protein bio-flocculants, the amino and carboxyl groups are responsible and effective flocculation groups [38,73]. The average molecular weight of EPS produced by *Lactobacillus sake* 0-1 (6×10^6 Da) was in the same order of magnitude as that of xanthan gum (4×10^6 Da to 9×10^6 Da) [14,20].

5. CONCLUSION

The physical, chemical and rheological properties of EPSs synthesized by ten LAB strains from palm wine in this study demonstrated potential industrial applications. The EPSs revealed high molecular weight as revealed by *Lactobacillus crispatus*, *Leuconostoc lactis, Leuconostoc mesenteroides* and *Lactobacillus delbrueckii* that could be exploited in different industrial applications, while the excellent rheological property of EPS synthesized by *Lactococcus lactis* subsp *lactis*, *Lactobacillus acidophilus* and *Lactiplantibacillus plantarum* as revealed by power law modelling analysis can be exploited as thickening agent in food industry. Also, the FTIR spectra of the EPSs revealed the presence of hydroxyl groups, amines groups, methyl and methylene groups, carboxyl groups, glucan linkages, carbohydrates, O-acetyl ester and non-sugar components which are desirable groups for increasing affinity for binding water molecules, binding sites for divalent cations and flocculation processes.

ACKNOWLEDGEMENTS

I would like to thank my Higher National Diploma (HND) project's students especially Omolara, Ayomide, Rukayat, Mujidat, Aminat, Rukayat, Giwa, and Jessica for their assistance in palm wine samples collection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chawla PR, Bajaj IB, Survase SA, Singhal RS. Microbial cellulose: Fermentative production and applications. Food Technology and Biotechnology. 2009;47: 107-124.
2. Badel S, Bernardi T, Michaud P. New perspectives for lactobacilli exopolysaccharides. Biotechnol Adv. 2011; 29:54–66.
3. Adamu-governor OL, Okolie P, Brai R. Effect of gum producing lactic acid bacteria from palm sap on the quality attributes of yoghurt. Food Stab. 2020a;3:77–92.
4. Adamu-Governor OL, Okolie PN, Ikegwu EM, Obeten IH, Abiona CO, Uzochukwu SVA. Comparative study of commercial starter and gum-producing lactic acid bacteria isolated from palm-sap on yoghurt. Asian Food Sci J. 2020b;19:28–39.
5. Zhao X, Liang Q. EPS-producing *Lactobacillus plantarum* MC5 as a compound starter improves rheology, texture and antioxidant activity of yoghurt during storage. Foods. 2022;11:1660. Available:https://doi.org/10.3390/foods11111660
6. Adamu-Governor OL, Shittu TA, Afolabi OR, Uzochukwu SVA. Screening for gum-producing lactic acid bacteria in oil palm (*Elaeis guineensis*) and raphia palm (*Raphia regalis*) sap from South-West Nigeria. Food Sci Nutr. 2018;6:2047–2055.
7. Haque E, Riyaz MAB, Shankar S, Hassan S. Physio-chemical and structural characterization of biosurfactant produced by halophilic *Pseudomonas aeruginosa* EN014 isolated from seawater . Int Pharm Invistigation. 2020;10(4):437-444.
8. Vijayabaskar P, Babinastarlin S, Shankar T, Sivakumar T, Anandapandian KTT. Quantification and characterization of exopolysaccharides from *Bacillus subtilis* (MTCC 121). Advan Biol Res. 2011;5(2):71–76.
9. Dinc M, Pecikoza U, Djokie J. Expoloysaccharide produced by probiotic strain *Lactobacillus paraplantarum* BGCG11 reduces inflammatory hyperalgesia in rat. Front Pharmacol. 2018;9:1.
10. Hussein AS, Ibrahim GS, Asker MMS, Mahmoud MG. Exopolysaccharide from *Lactobacillus helveticus*: Identification of chemical structure and effect on biscuit quality. Czech J Food Sci. 2010;28:225–232.
11. Uzochukwu SVA, Ngoddy PO, Balough E. Standard pure culture inoculum of natural and formulated palm sap fermentaion. Niger Food J. 1991;9:67–77.
12. Uzochukwu SVA, Balogh E, Loefler RT, Ngoddy PO. Structural analysis by 13C-nuclear magnetic resonance spectroscopy of glucan extracted from natural palm wine. Food Chem Elsevier. 2002;76:287–291.
13. Welman AD, Maddox IS. Exopolysaccharides from lactic acid bacteria: Perspectives and challenges. Trends Biotechnol. 2003;21:269–274.
14. Ruas-Madiedo P, Hugenholtz J, Zoon P. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. Int Dairy J. 2002a;12:163–171.
15. Muralidharan J, Jayachandran S. Physicochemical analyses of the exopolysaccharides produced by a marine biofouling bacterium, *Vibrio alginolyticus*. Process Biochem. 2003;38:841–847.
16. Minervini F, De Angelis M, Surico RF, Di Cagno R, Gänzle M, Gobbetti M. Highly efficient synthesis of exopolysaccharides by *Lactobacillus curvatus* DPPMA10 during growth in hydrolyzed wheat flour agar. Int J Food Microbiol. 2010;141:130–135.
17. Pachekrepapo U, Lucey JA, Gony Y. Characterization of the chemical structures and physical properties of exopolysaccharies produced by various *Streptococcus thermophilus* strains. J Dairy Sc. 2017;100:3424-3435.
18. Ruas-Madiedo P, De Los Reyes-Gavilán CG. Invited review: Methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria. J Dairy Sci. 2005;88:843–856.
19. Ruas-Madiedo P, Tuinier R, Kanning M, Zoon P. Role of exopolysaccharides produced by *Lactococcus lactis* subsp. *cremoris* on the viscosity of fermented milks. Int Dairy J. 2002b;12:689–695.
20. Van den Berg DJC, Robijn GW, Janssen AC, Giuseppin MLF, Vreeker R, Kamerling JP, Vliegenthart JFG, Ledeboer AM, Verrips CT. Production of a novel extracellular polysaccharide by *Lactobacillus sake* 0-1 and characterization of the polysaccharide.

Appl Environ Microbiol. 1995;61:2840–2844.

21. Boyle CD, Reade AE. Characterization of two extracellular polysaccharides from marine bacteria. Appl Environ Microbiol. 1983;46:392–399.
22. Marcotte M, Hoshahili ART, Ramaswamy HS. Rheological properties of selected hydrocolloids as a function of concentration and temperature. Food Res Int. 2001;34:695–703.
23. Ghannam MT, Abu-Jdayil B, Esmail N. Experimental investigation of crude oil–xanthan emulsions Flow Behavior. J Pet Sci Res. 2014;3:1.
24. Saha D, Bhattacharya S. Hydrocolloids as thickening and gelling agents in food: A critical review. J Food Sci Technol. 2010;47:587–597.
25. Barnes HA, Hutton JF, Walters K. Viscosity in: An introduction to rheology. Amsterdam, Elsevier Science Publishers; 1989.
26. Wilson RH, Goodfellow BJ, Belton PS. Fourier transfer infrared spectroscopy for the study of food biohydrocolloids. Food Hydrocoll. 1998;2:167–178.
27. Valepyn E, Berezina N, Paquot M. Optimization of production and preliminary characterization of new exopolysaccharides from *Gluconacetobacter hansenii* LMG1524. Adv Microbiol. 2012;02:488–496.
28. Banks W, Greenwood CT. Physical properties of solutions of polysaccharides. Adv Carbohydr Chem. 1963;18:357–398
29. Hempel S, Newberry SJ, Maher AR, Wang Z, Miles JNV, Shanman R, Johnsen B, Shekelle PG, Probiotics for the prevention and treatment of antibiotic-associated diarrhea: A systematic review and meta-analysis. J Am Med Assoc. 2012;307:1959–1969.
30. Tham CS-C, Peh K-K, Bhat R, Liong M-T. Probiotic properties of bifidobacteria and lactobacilli isolated from local dairy products. Ann Microbiol. 2011;62:1079–1087.
31. Adesulu-Dahunsi AT, Sanni AI, Jeyaram K. Production, characterization and *in vitro* antioxidant activities of exopolysaccharide from *Weissella cibaria* GA44. LWT - Food Sci Technol. 2018a;87:432–442.
32. Adesulu-Dahunsi AT, Sanni AI, Jeyaram K, Ojediran JO, Ogunsakin AO, Banwo K. Extracellular polysaccharide from *Weissella confusa* OF126: Production, optimization, and characterization. Int J Biol Macromol. 2018b;111:514–525.
33. Kaditzky S, Vogel RF. Optimization of exopolysaccharide yields in sourdoughs fermented by lactobacilli. Eur Food Res Technol. 2008;228:291–299.
34. Sutherland IW. Structure-function relationships in microbial exopolysaccharides. Biotechnol Adv. 1994; 12:393–448.
35. Adamu-Governor L, Shittu T, Afolabi R, Uzochukwu S. Comparative identification approach for gum-producing Lactobacilli in palm wine. Bacterial Empire; 2023. Available:https://doi.org/10.36547/be.508
36. Wang Y, Li C, Liu P, Ahmed Z, Xiao P, Bai X. Physical characterization of exopolysaccharide produced by *Lactobacillus plantarum* KF5 isolated from Tibet Kefir. Carbohydr Polym. 2010;82: 895–903.
37. Kaur V, Bera MB, Panesar PS. Modeling for exopolysaccharide production by *Alcaligenes faecalis* B14. Int J Eng Res Technol. 2013;2:120–130.
38. Kumar CG, Joo HS, Choi JW, Koo YM, Chang CS. Purification and characterization of an extracellular polysaccharide from *haloalkalophilic Bacillus* sp. I-450. Enzyme Microbial and Technology. 2004;34:673-681.
39. Kavita M, Mishra A, Jha B. Isolation and Physico-chemical characterization of extracellular polymeric substances produced by the marine bacterium *Vibrio parahaemolyticus*. Biofueling. 2011;27(3):309 – 317.
40. Ha YW, Stack RJ, Hespell RB, Gordon SH, Bothast RJ. Some chemical and physical properties of extracellular polysaccharides produced by *Butyrivibrio fibrisolvens* strains. Appl Environ Microbiol. 1991;57:2016–2020.
41. Howe KJ, Ishida KP, Clark MM. Use of ATR/FTIR spectrometry to study fouling of microfiltration membranes by natural waters. Desalination. 2002;147:251–255.
42. Kumar MA, Anandapandian KTK, Parthiban K. Production and characterization of exopolysaccharides (EPS) from biofilm forming marine bacterium. Brazilian Arch Biol Technol. 2011;54:259–265.
43. Karbowiak T, Ferret E, Debeaufort F, Voilley A, Cayot P. Investigation of water transfer across thin layer biopolymer films

by infrared spectroscopy. J Memb Sci. 2011;370:82–90.
44. Helm D, Naumann D. Identification of some bacterial cell components by FT-IR spectroscopy. FEMS Microbiol Lett. 1995;126:75–79.
45. Haxaire K, Maréchal Y, Milas M, Rinaudo M. Hydration of polysaccharide hyaluronan observed by IR spectrometry. I. Preliminary experiments and band assignments. Biopolym - Biospectroscopy Sect. 2003;72:10–20.
46. Černá M, Barros AS, Nunes A, Rocha SM, Delgadillo I, Čopíková J, Coimbra MA. Use of FT-IR spectroscopy as a tool for the analysis of polysaccharide food additives. Carbohydr Polym. 2003;51:383–389.
47. Čopíková J, Barros AS, Šmídová I, Černá M, Teixeira DH, Delgadillo I, Synytsya A, Coimbra MA. Influence of hydration of food additive polysaccharides on FT-IR spectra distinction. Carbohydr Polym. 2006;63: 355–359.
48. Mishra A, Jha B. Isolation and characterization of extracellular polymeric substances from micro-algae Dunaliella salina under salt stress. Bioresour Technol 2009;100:3382–3386.
49. Pawar ST, Bhosale AA, Gawade TB, Nale TR. Isolation, screening and optimization of exopolysaccharide producing bacterium from saline soil. J Microbiol Biotech Res. 2013;3(3):24–31.
50. Kazy SK, Sar P, Singh SP, Sen AK, D'Souza SF. Extracellular polysaccharides of a copper-sensitive and a copper-resistant *Pseudomonas aeruginosa* strain: Synthesis, chemical nature and copper binding. World J Microbiol Biotechnol. 2004;18:583–588.
51. Smith IH, Symes KC, Lawson CJ, Morris ER. Influence of the pyruvate content of xanthan on macromolecular association in solution. Int J Biol Macromol. 1981;3:129–134.
52. Saravanan P, Jayachandran S. Preliminary characterization of exopolysaccharides produced by a marine biofilm-forming bacterium *Pseudoalteromonas ruthenica* (SBT 033). Lett Appl Microbiol. 2008;46:1–6.
53. Sandford PA, Pittsley JE, Knutson CA, Watson PR, Cadmus MC, Jeanes A. Variation in *Xanthomonas campestris* NRRL B-1459: Characterization of xanthan products of differing pyruvic acid content. In: P. A. Sandford & A. Laskin eds. *Extracellular Microbial Polysaccharides*. Washington, D.C, American Chemistry Society; 1977.
54. Nataraj S, Schomäcker R, Kraume M, Mishra IMM, Drews A. Analyses of polysaccharide fouling mechanisms during crossflow membrane filtration. J Memb Sci. 2008;308:152–161.
55. Kacuráková M, Capek P, Sasinková V, Wellner N, Ebringerová A. FT-IR study of plant cell wall model compounds: Pectic polysaccharides and hemicelluloses. Carbohydr Polym. 2000;43(2):195-203.
56. Bramhachari PV, Kishor PB, Ramadevi R, Kumar R, Rao BR, Dubey SK. Isolation and characterization of mucous exopolysaccharide (EPS) produced by Vibrio furnissi strain VBOS3. J Microbiol Biotechnol. 2007;17:44–51.
57. Zajic J, Knitting E. *Development in industrial microbiology*. Washington, D.C., American Institute of Biological Science; 1970.
58. Bubb WA, Urashima T, Fujiwara R, Shinnai T, Ariga H. Structural characterisation of the exocellulas polysaccharide produced by *Streptococcus thermophilus* OR 901. Carbohydr Res. 1997;301:41–50.
59. Ricciardi A, Parente E, Crudele MA, Zanetti F, Scolari G, Mannazzu I. Exopolysaccharide production by *Streptococcus thermophilus* SY: Production and preliminary characterization of the polymer. J Appl Microbiol. 2002;92:297–306.
60. Seo WT, Kahng GG, Nam SH, Choi S, Do-Suh, HH, Kim SW, Park YH. Isolation and characterization of a novel exopolysaccharide-producing *Paenibacillus* sp. WN9 KCTC 8951P. J Microbiol Biotechnol. 1999;9:820–825.
61. Sajna KV, Sukumaran RK, Gottumukkala LD, Jayamurthy H, Dhar KS and Pandey A. Studies on structural and physical characteristics of a novel exopolysaccharide from *Pseudozyma* sp. NII 08165. Int J Biol Macromol. 2013;59 :84–89.
62. Ismail B, Nampoothiri KM. Molecular characterization of an exopolysaccharide from a probiotic *Lactobacillus plantarum* MTCC 9510 and its efficacy to improve the texture of starchy food. J Food Sci Technol. 2014;51:4012–4018.
63. Ismail B, Nampoothiri KM. Production, purification and structural characterization

of an exopolysaccharide produced by a probiotic *Lactobacillus plantarum* MTCC 9510. Arch Microbiol. 2010a;192:1049–1057.
64. De Vuyst L, Degeest B. Heteropolysaccharides from lactic acid bacteria. FEMS Microbiol Rev. 1999;23: 153–177.
65. Katzbauer B. Properties and applications of xanthan gum. Polym Degrad Stab. 1998;59:81–84.
66. Abid Y, Azabou S, Blecker C, Gharsallaoui A, Corsaro MM, Besbes S, Attia H. Rheological and emulsifying properties of an exopolysaccharide produced by potential probiotic *Leuconostoc citreum*-BMS strain. Carbohydr Polym. 2021;256.
67. Vicente-García V, Ríos-Leal E, Calderón-Domínguez G, Cañizares-Villanueva RO, Olvera-Ramírez R. Detection, isolation, and characterization of exopolysaccharide produced by a strain of *Phormidium* 94a isolated from an arid zone of Mexico. Biotechnol Bioeng. 2004;85:306–310.
68. Hamza-Chaffai A. Effect of Manufacturing Conditions on Rheology of Banana Gelified Milk: Optimization of the Technology. J. Food Sci. 1990;55:1630-1633.
69. Marshall VM, Cowieft EN, Moreton R. Analysis and production of two exopolysaccharides from *Lactococcus lactis* subsp. *cremoris* LC330. J Dairy Res. 1995;62:621–628.
70. Grobben GJ, Van Casteren WHM, Schols HA, Oosterveld A, Sala G, Smith MR, Sikkema J, De Bont JAM. Analysis of the exopolysaccharides produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 grown in continous culture on glucose and fructose. Appl Microbiol Biotechnol. 1997;48:516–521.
71. Ricciardi A, Clementi F. Exopolysaccharides from lactic acid bacteria: Structure, production and technological applications. Italian Journal of Food Science. 2000;12:23-45.
72. Cerning J. Exocellular polysaccharides produced by lactic acid bacteria. FEMS Microbiol Lett. 1990;87:113–130.
73. Kurane R, Hatamochi K, Kakuno T, Kiyohara M, Kawaguchi K, Mizuno Y, Hirano M, Taniguchi Y. Purification and characterization of lipid bioflocculant produced by *Rhodococcus erythropolis*. Biosci Biotechnol Biochem. 1994;58:1977–1982.

© 2023 Adamu-Governor et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/98544