JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

ASSESSMENT OF EXOPOLYSACCHARIDES PRODUCING LACTIC ACID BACTERIA ISOLATED FROM *NUNU* – A LOCALLY FERMENTED MILK IN NIGERIA

Naheem Adekilekun Tijani[1], Kaltume Umar Hambali[1], Saheed Adekunle Akinola[2], Abdullateef Opeyemi Afolabi[1], Kehinde Olusayo Awojobi[3]*

Address(es):
[1] Department of Microbiology and Immunology, Kampala International University, Western Campus, Ishaka, Uganda.
[2] Department of Microbiology and Parasitology, School of Medicine and Pharmacy, College of Medicine and Health Sciences, University of Rwanda, Butare, Rwanda.
[3] Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

*Corresponding author: tijani.naheem@kiu.ac.ug

https://doi.org/10.55251/jmbfs.11906

ARTICLE INFO

Received 6. 10. 2024
Revised 23. 4. 2025
Accepted 19. 5. 2025
Published 1. 8. 2025

Regular article

OPEN ACCESS

ABSTRACT

Exopolysaccharides (EPS) are extracellular polymers made of monomers of carbohydrates, which are important in many biological mechanisms. Several lactic acid bacteria (LAB) can confer desirable properties to specific fermented products when EPSs are produced during dairy fermentation. The present study isolated LAB from a locally fermented milk (*Nunu*) and screened for EPS production. The LAB strain with the highest EPS production potential was selected for EPS bulk production in a modified EPS Selection Medium (mESM). The effects of pH, temperature, incubation time, and carbon source were performed for optimization studies. The obtained EPS were then analysed for their chemical characteristics like total carbohydrate content (TCHO) and monomer composition. In total, three strains of LAB belonging to the genus *Lactobacillus* were isolated from the fermented milk (*Nunu*) sample. The *Lactobacillus* sp. (Nu3) was the best EPS producer among others. Optimization studies revealed starch as the most appropriate carbon source, with an EPS yield of 4.86 g/100mL. Two days (48 h) of incubation time was confirmed to be optimum for producing a maximum yield of 1.02 g/100 mL. The optimum pH and temperature for EPS production were 7.0 and 30ºC, yielding 1.87 g/100 mL and 1.15 g/100 mL, respectively. A maximum EPS yield of 5.31 g/100 mL was recovered under the optimal conditions. The monomer analysis of the EPS under study revealed glucose and galactose are the main monosaccharide components, with fructose and mannose being the minor. Moreover, the EPS-producing *Lactobacillus* sp. (Nu3) was found to be an inventive EPS producer, and its EPS material could be utilized as an additive in the food industry.

Keywords: Exopolysaccharide producing LAB, *Nunu*, Extracellular biopolymer, Microbe-synthesized flavour enhancer

INTRODUCTION

Fermented dairy products are foods that are generally consumed globally. In recent times, the consumption and market trend of dairy products has been on the rise because of the health and nutritious benefits **(García-Burgos *et al.*, 2020)**. Worldwide, several products of fermented milk are essential components of the daily diet of various ethnic groups and are significant to their modern life's nutritional needs**(Granato *et al.*, 2010)**. In Northern Nigeria, predominantly Fulani women sell a naturally fermented skimmed milk product known as *Nunu.* Usually made with cow's milk, but sometimes using goat's milk. It is made by enabling fresh milk to ferment for 24 hours at room temperature in a covered calabash **(Bukar and Salami-Suleiman, 2019)**.
Microorganisms such as bacteria, fungi, actinobacteria, and microalgae naturally produce exopolysaccharides (EPS), which are long-chain, high molecular-weight polymers of carbohydrates, in their metabolic pathways **(Ghareeb *et al.*, 2024)**. Different bacteria species including lactic acid bacteria (LAB), bifidobacteria, and propionibacteria have shown the ability to excrete EPS during growth and metabolism **(Sørensen *et al.*, 2022)**. In the food sector, polysaccharides with valuable health effects are frequently utilized as gelling, stabilizing, emulsifying, or thickening agents **(Dilna *et al.*, 2015)**. Inherently, LABs are Gram-positive bacteria that can produce lactic acid as a byproduct of fermentation via metabolism. They are extensively used in industrial fermentation operations, biotechnology, medicine, and traditional dairy products **(You *et al.*, 2020)**. Generally, LAB are regarded as safe (GRAS), and as such, they are easily incorporated into food and other edible products without labelling **(Peerzada *et al.*, 2023)**. Due to EPS synthesized by probiotic LAB originating from fermented dairy products, intestinal microbiota have managed to improve human health **(Chen *et al.*, 2019)**. LAB produces various metabolites which include bacteriocins, fatty acids, and organic acids, along with EPS **(Lynch *et al.*, 2018)**.
The rising need for the synthesis of microbial polysaccharides for usage in the pharmaceutical and food industries has been extensively studied **(Imran *et al.*, 2016)**. Over recent decades, scientists have gained interest in EPS-producing-LAB strains because of their immense contribution to the fermentation of many dairy products, specifically, milk-based products like yoghurt, curd, sour cream, buttermilk, and cheese where they assist in improving the taste, texture, flavour, stability, and shelf life of fermented products **(Han *et al.*, 2016)**. Owing to their ability to increase viscosity and mouthfeel, EPS-producing LAB are efficient microorganisms in the industrial production of functional food products. They are utilized as starter cultures or coadjutants to produce fermented foods like cheese, yoghurt, kefir, and other cereal-based products **(Zannini *et al.*, 2016)**. They are reported as the most significant bacteria in desirable food fermentations and have been implicated in the fermentation of many native fermented foods in Nigeria **(Adesulu-Dahunsi *et al.*, 2017)**. Among LAB strains, EPSs are commonly produced by *Streptococcus*, *Fructilactobacillus*, *Limosilactobacillus*, *Lentilactobacillus*, *Pediococcus*, *Lactococcus*, *Lacticaseibacillus*, *Lactobacillus*, *Lactiplantibacillus*, *Latilactobacillus*, *Weissella*, and *Leuconostoc* species **(Kaur and Dey, 2023; Nguyen *et al.*, 2020)**. Similarly, **Malang *et al.* (2015)** reported that some Bifidobacteria species and other non-starter LABs have also shown the potential to produce EPSs. Since they are non-toxic and biodegradable, they have continued to receive considerable attention from researchers **(Yadav *et al.*, 2024)**. Recently, it has been revealed that EPS may act as probiotics or as anti-ulcer, immunomodulating, mycotoxin detoxification, anti-biofilm agents and benefit human health by preventing pathogenic bacteria adhesion and tumors growth **(Sørensen *et al.*, 2022)**. Moreover, microbial EPS has demonstrated a wide spectrum of functional effects with a vast array of uses like viscosifiers, bio-thickeners, stabilizing, and emulsifying agents **(Bajpai *et al.*, 2016)**. When EPS consists of similar monosaccharides, it is classified as homopolysaccharide (HoPS) and when dissimilar monosaccharides are present, it is classified as heteropolysaccharide (HePS) **(You *et al.*, 2020)**. HoPS is mostly produced by *Leuconostoc*, *Weissella*, and *Pediococcus*, whereas HePS is primarily produced by *Streptococcus* and *Lactobacillus*. However, HoPS such as galactan can be produced by *Lactobacillus* **(Dertli *et al.*, 2018)**. Therefore, owing to the safe character of EPS produced by LAB, which attracts great interest from food industries, this present study aimed to isolate and identify strains of EPS-producing LAB from fermented milk samples (Nunu) for the microbial production of EPS.

Furthermore, the EPS production from the LAB strain was optimized and characterized for its chemical properties.

MATERIAL AND METHODS

Sample collection

The fermented milk samples (*Nunu*) were purchased from commercial street hawkers at various locations in Ile-Ife, Osun State, Nigeria. The samples were transported immediately in an ice box to the laboratory for microbiological analysis.

Isolation and identification of LAB from *Nunu* (fermented milk)

One milliliter of fermented milk samples was homogenized with 9 mL of sterile maximum recovery diluent (MRD, Oxoid) in a test tube and serially diluted in the same diluent. Precisely 0.1 mL of relatively diluted sample was spread–plated on De Man, Rogosa, and Sharpe agar (MRSA) according to the procedure reported by **Plessas *et al*. (2017)** with some modification. Under anaerobic conditions, plates were incubated at 30°C for 48 h. Colonies obtained were purified by sub-culturing successively on MRS agar and then subjected to morphological, biochemical, and microscopic characteristics. Gram-positive isolates having catalase negative were confirmed as LAB and preserved on MRSA slants in the refrigerator at 4°C.

Screening of LAB isolates for EPS production

Screening isolates for the production of EPS was conducted following the method reported by **Derdak *et al*. (2022)**. Strains of LAB were pre-cultivated on MRS agar and streaked onto LTV agar: 1% (w/v) meat extract,0.5% (w/v) tryptone, 0.65% (w/v) NaCl, 0.8% (w/v) KNO_3, 0.8% (w/v) sucrose, 0.1% (v/v) Tween 80 (Merck), 1.7% (w/v) agar, pH 7.1 ± 0.2 **(Sawadogo-Lingani *et al*., 2007)** and incubated anaerobically at 30°C for 48 h. Following the inoculation loop method, colonies were tested for slime formation to verify their stickiness. Isolates whose length of the slime exceeds 1.5 mm were regarded as positive slime producers **(Knoshaug *et al*., 2000)**. MRS-sucrose broth without peptone and glucose, was used to confirm the positive results **(Omafuvbe and Enyioha, 2011)**. The MRS-sucrose broth was used in culturing the isolates and incubated anaerobically at 30°C for 24 h. Exactly 1.5 mL of 24 h old culture of the test organism was centrifuged at 5000×g for 10 minutes (4°C) and 1 mL of the supernatant was pipetted into a glass tube, with the addition of the same volume of ethanol. Confirming the presence of EPS, an opaque link was formed at the interface of the cell supernatant and ethanol. The intensity of the opaque link was used to describe the positive isolates. Lactic acid bacteria isolate that produced a higher quantity of EPS was chosen for further analysis.

Optimization of culture parameters for EPS production

The effects of pH, temperature, incubation time, and different carbon sources as reported by **Zehir Şentürk *et al*. (2020)** were used to determine optimal conditions and other factors for enhanced EPS production. For optimization studies, LAB isolate was grown anaerobically on an MRSA plate at 30°C for 24 h, then a loopful of an overnight culture was inoculated into 10 mL MRS broth and incubated anaerobically for 24h at 30°C. Ten millilitres of overnight culture were diluted further in fresh MRS broth to give an absorbance of 0.08 in a spectrophotometer set at 540 nm wavelengths. This gave a cell suspension of approximately 10^6CFU/mL which was used for EPS production.

EPS production

The selected LAB isolate was propagated in an EPS Selection Medium (ESM) following the procedure previously reported by **Abid *et al*. (2018)** and referred to as modified EPS Selection Medium (mESM). The modified medium contained 0.5% yeast extract, 10% skim milk, 0.5% peptone, and 5% starch. The isolate was transferred from MRS agar slants into 10mL MRS broths and incubated for 24h at 30ºC under anaerobic conditions. The culture was standardized to give a cell suspension of approximately 10^6 CFU/mL as described in the section above. One millilitre from the standardized culture was transferred into 100mL conical flasks which contained 10 mL of mESM broth and incubated anaerobically for 24h at 30ºC. Ten millilitres of the inocula were then added to 90 mL of mESM brothinside a 200 mL conical flask, and incubated for 24 h at 30ºC.

Extraction of EPS

The extraction process of the EPS out of the culture medium after 24 h was achieved following the method described by **Taj *et al*. (2022)**. Grown cultures were heated in boiling water to inactivate enzymes for 15 mins, and later cooled down to room temperature. The cells were removed through centrifugation of the sample for 20 mins at 10,000 rpm. After which, the coagulate proteins and supernatant were recovered.

Precipitation of EPS

EPS were precipitated with three volumes of cold ethanol (96%) from the supernatant as illustrated by **Xiao *et al*. (2020)**, shaken vigorously and stored overnight at 4ºC. The EPS precipitates were removed after centrifugation at 8000 rpm (4°C, 20 mins).

Purification of EPS

In purifying the EPS fraction, the EPS precipitates were first re-dissolved in distilled water and centrifuged for 20 mins at 10,000 rpm. The recovered supernatant was then precipitated in cold ethanol (1:3) in order to re-obtain the precipitate **(Amao *et al*., 2019)**. This method was repeated twice to ensure proper purification of the EPS fraction. For the removal of impurities, EPS precipitates obtained were dissolved in distilled H_2O and dialyzed against the same solution for 24 h at 4ºC.

Lyophilization of EPS fraction

The dialyzed EPS fraction was taken to the Central Science Laboratory of the Obafemi Awolowo University, Ile-Ife, Nigeria for lyophilization. The EPS yield was calculated by weighing the dried weight of the precipitates on the weighing balance and expressed as mg/L **(Adebayo-Tayo and Fashogbon, 2020)**, and stored for future use.

Chemical characterization of the EPS

The EPS was analyzed for its chemical characteristics like total carbohydrate content (TCHO) and monosaccharide composition **(Aslam *et al*., 2016)**. The total carbohydrate contained in the EPS was analyzed following the phenol-sulfuric acid approach reported by **Zhang *et al*. (2020)**. One millilitre of a cold solution of 5 % (w/v) phenol with 5 mL concentrated sulphuric acid was added to 1 mL of the EPS sample while keeping the test tubes in a block of ice. The absorbance of the mixture was read at 490 nm after 20 mins of room temperature incubation using the spectrophotometer. Glucose was employed as the standard in the range of 0-100 µg concentration from 1 mg/mL stock solution. Absorbance at 490 nm was plotted against glucose concentration on a standard graph.

In determining its monosaccharide composition, polysaccharides hydrolysis was performed using the hot ethanol extraction procedure described by **Chow and Landhausser (2004)**. The derivative products were used in determining the monomer composition by spectrophotometry analysis with four standard sugars (glucose, fructose, galactose, and mannose).

Statistical analysis

The data obtained were presented as means ± standard deviation (SD). The descriptive analysis of the quantitative data was done using the Microsoft Excel Package (2019) and the production of graphs. Experiments were carried out in triplicate.

RESULTS

Colonial and morphological characteristics of isolates

In totality, three strains of lactic acid bacteria (LAB) were isolated from the fermented milk (*Nunu*) sample. The morphological characteristics of the lactic acid bacteria isolated from fermented milk samples are shown in Table 1. The biochemical and physiological characteristics of LAB isolates are shown in Table 2. Depending on morphological, biochemical, and physiological characteristics, all isolates were identified and confirmed with the Advanced Bacterial Identification software **(Sorescu & Stoica, 2021)**. The LAB species were found to belong to the genus *Lactobacillus*.

Table 1 Morphological Characteristics of Lactic Acid Bacteria Isolated from Fermented Milk Samples

Isolates Code	Characteristics					
	Shape	Colour	Opacity	Elevation	Gram's reaction	Source
Nu1	Circular	Cream	Translucent	Flat	+ve rods	Nunu
Nu3	Circular	Cream	Opaque	Flat	+ve rods	Nunu
Nu5	Circular	Cream	Translucent	Low convex	+ve rods	Nunu

Key: +ve = positive

Table 2 Biochemical and Physiological Characteristics of Lactic Acid Bacteria Isolated from Fermented Milk Samples

														Sugar fermentation																	Probable Identity
Isolate code	**Cell Morphology**	**Catalase test**	**Production of gas from MRS broth**	**Arginine hydrolysis**	**Methyl red test**	**Methyl red test**	**NO^{3-} reduction**	**Growth at 15°C**	**Growth at 45°C**	**Growth at pH 3.9**	**Growth at pH 9.6**	**Growth at 4.5% NaCl**	**Growth at 6.5% NaCl**	**Xylose**	**Galactose**	**Sorbitol**	**Mannitol**	**Maltose**	**Melobiose**	**Arabinose**	**Trehalose**	**Salicin**	**Lactose**	**Raffinose**	**Cellobiose**	**Sucrose**	**Rhamnose**	**Mannose**	**Ribose**	**Fructose**	
Nu1	R	-	-	-	-	-	-	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus* sp.
Nu3	R	-	+	+	-	-	-	-	+	+	-	+	+	-	+	-	-	+	+	-	+	-	+	+	+	+	+	+	+	+	*Lactobacillus* sp.
Nu5	R	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+	-	-	+	+	-	+	+	+	*Lactobacillus* sp.

Key: + = Positive reaction, - = Negative reaction, R = Rod**EPS production by**

LAB isolates

The ability of the isolated lactic acid bacteria to produce EPSs is shown in Table 3. Visual observation of slime production was very difficult. All the LAB isolates were good EPS producers. However, the isolate identified as *Lactobacillus* sp. (*Nu*3) showed the highest degree of EPS production, while *Lactobacillus* sp. (*Nu*1) and *Lactobacillus* sp. (*Nu*5) showed a moderate level and slight production of EPSs, respectively.

Table 3 EPS Production of Lactic Acid Bacteria Isolated from Fermented Milk Samples

LAB Isolates	**Slime formation**	**Confir**
Lactobacillus sp.(Nu1)	-	+
Lactobacillus sp.(Nu3)	-	+ +
Lactobacillus sp.(Nu5)	-	+ +

Key: + = Positive, - = Negative, ++ = Slightly opaque = Slight production of EPS, +++ = Opaque = Moderate production of EPS, ++++ = Very opaque = High production of EPS

Optimization of culture parameters

Effect of pH on EPS production

EPS production was found maximum at pH 7.0 having 1.87 g/100 mL of production medium. An initial increase in the EPS yield was recorded with an increase in pH from pH 3 to 7, followed by a gradual decrease at pH 8.0. However, at pH 9 and beyond no EPS could be recovered (Figure 1).

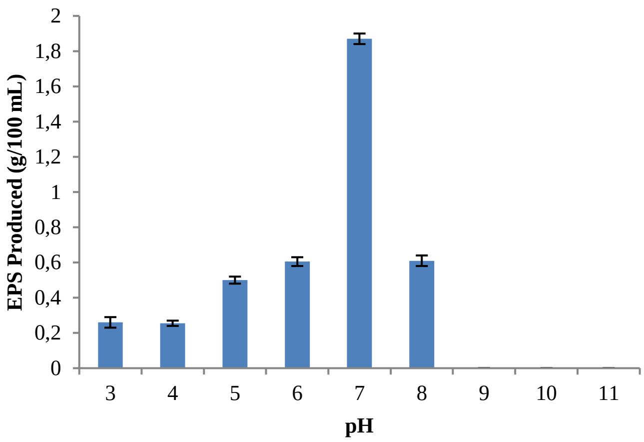

Figure 1 Effects of pH on EPS production by *Lactobacillus* sp. (Nu3) isolated from fermented milk sample.

Effect of temperature on EPS production

In considering the temperature effects for EPS production, maximum production was achieved at 30ºC with 1.15 g/100 mL of dried EPS. At 40ºC, a sharp decrease was observed with EPS yield of 0.74 g/100 mL which finally dropped to 0.62 g/100 mL at 50ºC (Figure 2).

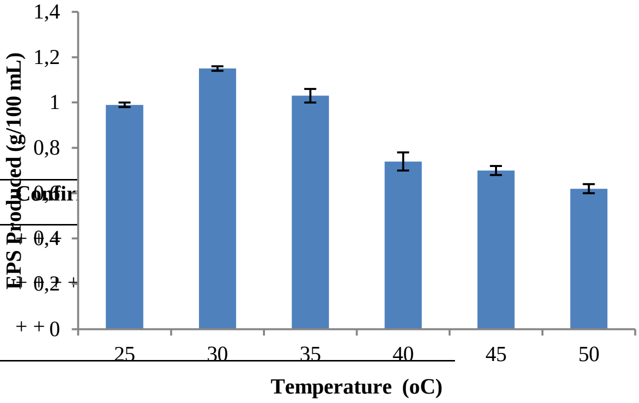

Figure 2 Effects of temperature on EPS production by *Lactobacillus* sp. (Nu3) isolated from fermented milk sample.

Effect of incubation time on EPS production

The result for the incubation period showed that maximum EPS production was accomplished within 48 h of incubation with 1.02 g/100 mL of dried EPS. However, a gradual decrease was obtained from 72 to 166 h as shown in Figure 3.

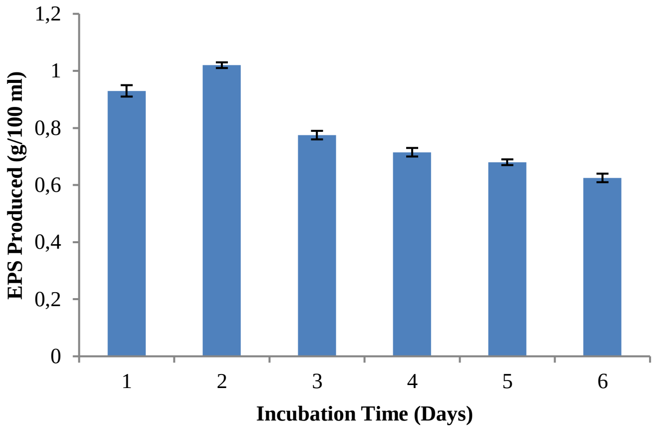

Figure 3 Effects of incubation time on EPS production by *Lactobacillus* sp. (Nu3) isolated from fermented milk sample.

Effect of different carbon sources on EPS production

The effects of different carbon sources (glucose, galactose, fructose, maltose, sucrose, lactose, pectin, starch, and cellulose) used for EPS production are illustrated in Figure 4. The highest exopolysaccharide production was achieved when the culturing medium (mESM) was enriched with starch as the sole carbon source with 4.86 g/100 mL of dried EPS. On the other hand, cellulose gave the least yield of 0.12 g/100 mL (Figure 4).

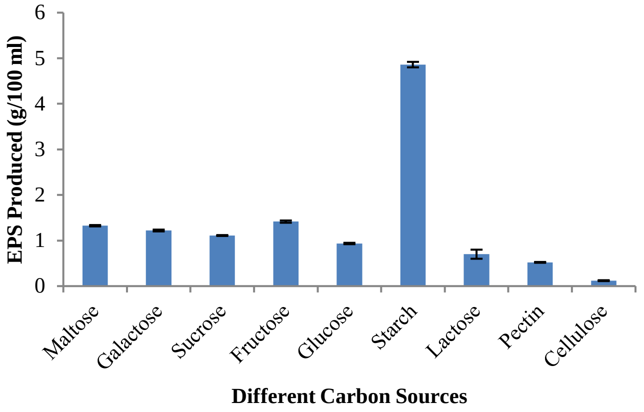

Figure 4 Effects of different carbon sources on EPS production by *Lactobacillus* sp. (Nu3) isolated from fermented milk sample.

Purification of EPS

The purified EPS precipitate produced a whitish coloration and was entirely soluble in water, showing a clear EPS solution with ropy characteristics. Lyophilization of the EPS fraction gave a dry weight of 5.31g/100 mL.

Chemical characterization of EPS

Total carbohydrate content

The total carbohydrate content in the exopolysaccharides was found to be 73.64 g/mL from the glucose standard curve with an OD value of 490 nm.

Monosaccharides composition of EPS

The results showed that glucose (13.92 g/mL) and galactose (9.31 g/mL) are the two primary sugars in the high-molecular-weight portion of the EPS. Other sugar components included fructose (1.53 g/mL)and mannose (1.94 g/mL) as shown in Figure 5.

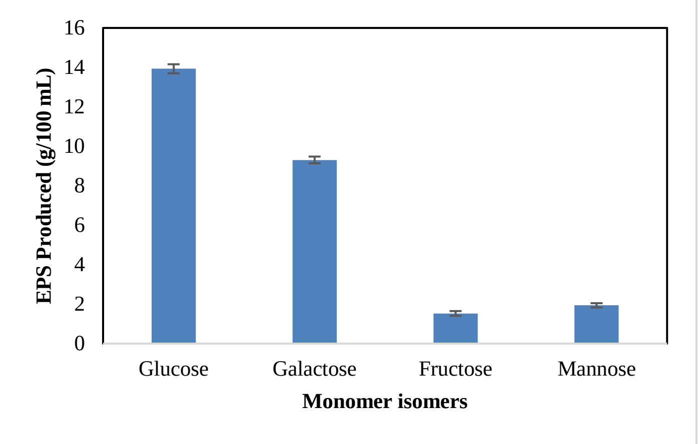

Figure 5 Composition of EPS determined by Spectronic 21D Spectrophotometer

DISCUSSION

Based on the phenotypic characteristics, LAB isolated from fermented milk samples were identified as *Lactobacillus* species. These isolates belonged to the lactic group that has been implicated in fermented dairy products (cheese and milk) **(Nagalakshmi *et al.*, 2013)**. *Lactiplantibacillus plantarum* was isolated from Algerian traditional dairy products **(Bachtarzi *et al.*, 2019)**, in camel milk **(Ayyash *et al.*, 2020)**, in human breast milk **(Nambiar *et al.*, 2018)**, and from some bottled yoghurt commercially sold in Nigeria **(Omafuvbe and Enyioha, 2011)**.
In the quest for EPS-producing LAB strains, all the different lactic acid bacteria isolates from fermented milk were tested for EPS-producing activity. All three lactic acid bacteria screened in the present study have the potential to produce exopolysaccharides. However, this was expected, as many LAB strains have been shown potential to produce technologically important substances such as exopolysaccharides **(Adesulu-Dahunsi *et al.*, 2018; Lynch *et al.*, 2018)**.
In this study, the isolate identified as *Lactobacillus* sp. (Nu3) appeared to be the most promising in terms of EPS production in comparison to other isolates. Therefore, isolate *Lactobacillus* sp. (Nu3) was selected for bulk production. Many species of *Lactobacillus* have been examined for EPS production and those usually reported were *L. rhamnosus*, *L. plantarum*, *Lactococcus lactis*, and *L. helveticus* **(Wang *et al.*, 2014)**. Previously L. *plantarum* YM11 was reported to produce EPS of 0.09 g/L at 56 h (SDM medium) **(Wang *et al.*, 2015)**.
Previous work has established that EPS production by LAB can be enhanced by altering the medium composition and growth requirements **(Chaisuwan *et al.*, 2020)**, such as carbon source and nitrogen source **(Imran *et al.*, 2016; Zhang *et al.*, 2019)**, and environmental conditions, that is, initial pH **(Oleksy-Sobczak *et al.*, 2020)**, incubation time **(Krishnamurthy *et al.*, 2020)**, temperature, agitation speed, and aeration rate **(Su *et al.*, 2007)**. For certain microbial strains that are EPS-producers, such as those from *Aspergillus* **(Costa *et al.*, 2019)**, *Leuconostoc* **(Du *et al.*, 2017)**, *Lactobacillus* **(Imran *et al.*, 2016; Oleksy-Sobczak *et al.*, 2020)** and *Streptomyces* **(Vinothini *et al.*, 2019)**, enhancing their growth conditions could upsurge their EPS synthesis.
The culture conditions in this study revealed that production of EPS by *Lactobacillus* sp. (Nu3) significantly reduced towards the late stationary phase of growth (beyond 48 h) as the maximum growth was observed from 24 to 48 h. This was apparently due to the activity of glycohydrolases likely produced in the production medium that catalyzed the breakdown of polysaccharides, thereby resulting in lower EPS yields **(Moghannem *et al.*, 2018)**, or under a physiological alteration of the cell environment **(Bancalari *et al*., 2019)**.
Other LAB strains that show exopolysaccharide degradation with prolonged incubation have also been reported **(Zhang *et al*., 2011)**, however, less degradation of polysaccharides was accomplished when the strains were grown at lower temperatures and pH than those required for optimal growth **(Prete *et al*., 2021; Zhang *et al.*, 2011)**. Conversely, for some EPS-producing LAB strains, for example, *Streptococcus thermophilus* ST111, degradation of EPS yield was not recorded during the fermentation; hence maximal EPS production was obtained at the conclusion of the fermentation process **(Zhang *et al*., 2011)**.
In the synthesis of polysaccharides, temperature plays a significant role. The temperature range of 25°C to 35°C was favourable for proper growth and polysaccharide synthesis **(Zhang *et al.*, 2011)**. The impact of temperature on exopolysaccharide production is related to the growth of the organism. The maximum EPS-producing organism activity was studied for the temperature range from 25ºC to 50ºC. It was observed that EPS synthesis by *Lactobacillus* sp. (Nu3) was found to be maximal at 30°C. Temperature above 30°C resulted in a decline growth of the strain, thus a decrease in the production rate of EPS was rapid with an increase in temperature. This finding agreed with the investigation of **Pawar *et al.*, (2013)** who reported that 30°C was optimum for EPS production for the Alpha Proteobacterium group and also **Singh and Das (2011)** reported that 30ºC temperature was suitable for EPS production. Contradictory findings have been found in the literature examining how temperature affects LAB exopolysaccharide synthesis. According to some studies, more EPS is synthesized at temperatures that are within the maximum growth range **(Peng *et al.*, 2020)**, whereas other studies reported that a higher amount of EPS is biosynthesized at temperatures quite below the optimum range **(Bengoa *et al.*, 2018)**. Yet, some authors claimed that the production of EPS was not significantly impacted by temperature **(Deepak *et al.*, 2016)**. These discrepancies exist for several reasons, including EPS measurement methods, growth media, measurement conditions and timing, an absence of a pH regulator, and different ways of expressing EPS synthesis (milligrams of EPS per litre, milligrams of EPS, milligrams of EPS per CFU) **(Tiwari *et al*., 2015)**.
The pH affects the activity of glucosyltransferases and hence serves as a key factor in the biosynthesis of EPS **(Paulo *et al*., 2012)**. In the report of **Chai *et al*. (2022)**, the pH of media is one of the controlling parameters during fermentation. Depending on the specific bacterial species, the optimum pH for exopolysaccharide synthesis is typically close to neutrality **(Staudt *et al.*, 2012)**. In this study, at pH 7, an optimum amount of EPS was synthesized by *Lactobacillus* sp.(Nu3), and low levels of exopolysaccharide were recovered from the fermented medium below and above this pH, while basic conditions (pH 9, 10, and 11) were found to extremely inhibit the organism growth. This result is consistent with the report of **Zhang *et al*. (2011)** who reported that more EPS was synthesized by *L. fermentum* F6 strain when grown at optimal pH 6.5. It also correlates with the study of **Imran *et al*. (2016)** who established that neutral pH is most appropriate for enhancing the EPS production by *L. plantarum*.
Although, the optimal pH for the productionof EPS varied amongst LAB strains, it was frequently discovered to be close to pH 6.0 **(Gayathiri *et al.*, 2017)**. Maximum EPS production was obtained with *Bifidobacterium animalis* subsp. *lactis* BB12 and *Lactobacillus acidophilus* LA5 with rising pH, due to the fact that higher EPSs were recovered around the pH 5.8 and reduced with an increase in pH value **(Amiri et al., 2019)**.
Carbohydrates are the primary building block of the cytoskeleton and a significant nutritional requirement for cellular growth and advancement **(Alizadeh *et al.*, 2023)**. The impacts of different carbon sources on the exopolysaccharide production were investigated. Here, varying carbon sources such as fructose, galactose, sucrose, maltose, glucose, lactose, pectin, starch, and cellulose were varied in the composition medium. Of all the carbohydrates considered, starch followed by fructose, was most effective as a carbon source utilized by *Lactobacillus* sp.(Nu3) for the optimal growth and biosynthesis of EPS. This was most likely caused by the strain's more effective use of starch as an energy source or precursor for the synthesis of EPS as opposed to other sugars. **Kumar *et al.***

(2014) reported starch as the prime substrate based on a number of factors, including specific cell growth rate, degree of enzyme activity, specific enzyme formation, etc. This was in contrast to the report of **Zhang *et al*. (2019)**, who found maltose as more favourable for the production of exopolysaccharides by *L. sanfranciscensis* Ls-1001 than other carbon sources. According to **Adesulu-Dahunsi *et al*. (2018)**, EPS production by *L. plantarum* OF101 in sucrose-modified medium yielded the highest amount of 2.18 g/L.

The findings of this optimization routine suggest that to maximize exopolysaccharide production by *Lactobacillus* sp. (Nu3), optimization of environmental factors such as medium composition, initial pH of the growth medium, temperature, and fermentation periods should be highly considered. An increase in EPS production by *Lactobacillus* sp. (Nu3) was obtained, with a significant EPS yield of 5.31 g/100 mL when the strain was grown at 30°C for 48 hours and at initial pH 7.0 in the mESM supplemented with 10% (w/v) skim milk, 0.5% (w/v) yeast extract, 0.5% (w/v) peptone and 5% (w/v) starch. The growth conditions and medium composition are responsible for the LAB strain's high EPS output **(Sanalibaba and Cakmak, 2016)**. Previous studies have demonstrated the effects of nutritional and growth requirements on EPS synthesis yield by LAB isolate **(Cirrincione *et al*., 2018; Helal *et al*., 2015; Nguyen *et al*., 2020)**. In the current study, the selected LAB strain showed a higher yield compared to other LAB strains in EPS production yield **(Nemati and Mozafarpour, 2024; Pramudito *et al*., 2024; Tarannum *et al*., 2023)**. According to these findings, the *Lactobacillus* strain (Nu3) can be used as a novel source of EPS for a range of industrial applications.

Centrifugation was used to extract the EPS produced; removing the bacterial cells, after which it was precipitated in ethanol. This method ensured the total recovery of EPS from the growth medium. The EPS fraction was purified by subjecting the EPS to two ethanol precipitation steps and was further dialyzed to remove other impurities for lyophilization. The total carbohydrate content in the exopolysaccharides produced from this strain of *Lactobacillus* sp. (Nu3) was 73.64% after the purification. The result of **Cerning *et al*. (1994)** is similar to this finding, reporting almost the quantity of EPS from LAB. It is however below the carbohydrate composition of EPS biosynthesized by *Lactobacillus plantarum* YW11 documented to be 92.35% **(Wang *et al*., 2015)**.

The EPS was found to be a heteropolysaccharide (HePS). According to **Korcz and Varga (2021)**, most EPS produced by strains of lactic acid bacteria are HePS. After the complete hydrolysis of the EPS, the dominant neutral sugars were glucose and galactose. Other sugars present in the sample include fructose and mannose. The occurrence of various functional groups disclosed a typical hetero-polymeric nature of the products. **Widyaningrum and Meindrawan, (2020)** claimed that the sugar content and monomer arrangement of EPS determines its forms and structures, which in turn influence its physical characteristics. The result of monomer composition was consistent with those linked with EPSs from *Lactobacillus* strains as reported **(Ai *et al*., 2016)**. **Amiri *et al*. (2019)** revealed that microbial EPSs from *Lactobacillus acidophilus* LA5 are constituents of glucose, galactose, mannose, glucuronic acid, xylose, and fructose. Also, the monomer analysis of the EPS of *Lactobacillus plantarum* KF5 confirmed the presence of galactose, mannose, and glucose **(Wang *et al*., 2010)**. These monomer constituents provide the EPS its unique structural characteristics, such as branching patterns, which enhance its thickening, gel-forming, and emulsifying capabilities, making it appropriate for use in the biotechnology, pharmaceutical, food, and cosmetics industries **(Prete *et al*., 2021)**.

To date, numerous analytical techniques have been developed for determining carbohydrate concentration in the EPS **(Zhang *et al*., 2020)**. However, caution must be taken in selecting a suitable approach, as they tend to influence the quantity of EPS generated. The phenol-sulfuric acid method, for instance, is a dependable and widely used technique; yet, some studies have identified issues with its accuracy, reproducibility, and repeatability **(Gao *et al*., 2022)**. Hence, discrepancies in the amount of EPS between those observed in our study and those reported by other authors using the same LAB strain could be anticipated.

5.0 CONCLUSION

This research demonstrated the diversity of LAB in fermented milk samples with good EPS-producing activity. Screening of the lactic acid bacteria to confirm their exopolysaccharide-producing activity enabled the selection of the most appropriate LAB strains with the highest exopolysaccharide production for starter culture fermentation. The selected starter culture *Lactobacillus* sp. (Nu3) synthesized a substantialamount of EPS when under optimal growth conditions. Findings revealed that the LAB strain (Nu3) produced a high level of EPS compared to other LAB strains. Conclusively, *Lactobacillus* sp. (Nu3) was discovered to be a unique EPS producer, and its EPS material could be adopted for biotechnological use.Nevertheless, further investigations are required to examine the structural characteristics of these microbial EPS and evaluate their significant effect on human health through in-vivo studies.

Competing interests: The authors declare that they have no competing or financial interests.

Author contributions:
Naheem Adekilekun Tijani: Conceptualization, Methodology, Resources, Data curation, Writing – original draft, Writing – review &editing, Software. **Kaltume Umar Hambali:** Writing – review & editing. **Saheed Adekunle Akinola:** Writing – review & editing. **Abdullateef Opeyemi Afolabi:** Writing – review &editing. **Kehinde Olusayo Awojobi:** Supervision, Methodology, Formal analysis, Validation, Writing – review & editing.

Data availability: All relevant data are contained in the article.

REFERENCES

Abid, Y., Casillo, A., Gharsallah, H., Joulak, I., Lanzetta, R., Corsaro, M. M., Attia, H., & Azabou, S. (2018). Production and structural characterization of exopolysaccharides from newly isolated probiotic lactic acid bacteria. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2017.10.155

Adebayo-Tayo, B., & Fashogbon, R. (2020). In vitro antioxidant, antibacterial, in vivo immunomodulatory, antitumor and hematological potential of exopolysaccharide produced by wild type and mutant Lactobacillus delbureckii subsp. bulgaricus. *Heliyon*. https://doi.org/10.1016/j.heliyon.2020.e03268

Adesulu-Dahunsi, A. T., Sanni, A. I., & Jeyaram, K. (2017). Rapid differentiation among Lactobacillus, Pediococcus and Weissella species from some Nigerian indigenous fermented foods. *LWT*. https://doi.org/10.1016/j.lwt.2016.11.007

Adesulu-Dahunsi, A. T., Sanni, A. I., & Jeyaram, K. (2018). Production, characterization and In vitro antioxidant activities of exopolysaccharide from Weissella cibaria GA44. *LWT*. https://doi.org/10.1016/j.lwt.2017.09.013

Ai, L., Guo, Q., Ding, H., Guo, B., Chen, W., & Cui, S. W. (2016). Structure characterization of exopolysaccharides from Lactobacillus casei LC2W from skim milk. *Food Hydrocolloids*. https://doi.org/10.1016/j.foodhyd.2015.10.023

Alizadeh, J., Kavoosi, M., Singh, N., Lorzadeh, S., Ravandi, A., Kidane, B., Ahmed, N., Mraiche, F., Mowat, M. R., & Ghavami, S. (2023). Regulation of Autophagy via Carbohydrate and Lipid Metabolism in Cancer. In *Cancers*. https://doi.org/10.3390/cancers15082195

AMAO, J. A., Omojasola, P. F., & Barooah, M. (2019). Isolation and characterization of some exopolysaccharide producing bacteria from cassava peel heaps. *Scientific African*. https://doi.org/10.1016/j.sciaf.2019.e00093

Amiri, S., Rezaei Mokarram, R., Sowti Khiabani, M., Rezazadeh Bari, M., & Alizadeh Khaledabad, M. (2019). Exopolysaccharides production by Lactobacillus acidophilus LA5 and Bifidobacterium animalis subsp. lactis BB12: Optimization of fermentation variables and characterization of structure and bioactivities. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2018.11.084

Aslam, S. N., Michel, C., Niemi, A., & Underwood, G. J. C. (2016). Patterns and drivers of carbohydrate budgets in ice algal assemblages from first year Arctic sea ice. *Limnology and Oceanography*. https://doi.org/10.1002/lno.10260

Ayyash, M., Abu-Jdayil, B., Itsaranuwat, P., Galiwango, E., Tamiello-Rosa, C., Abdullah, H., Esposito, G., Hunashal, Y., Obaid, R. S., & Hamed, F. (2020). Characterization, bioactivities, and rheological properties of exopolysaccharide produced by novel probiotic Lactobacillus plantarum C70 isolated from camel milk. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2019.09.171

Bachtarzi, N., Kharroub, K., & Ruas-Madiedo, P. (2019). Exopolysaccharide-producing lactic acid bacteria isolated from traditional Algerian dairy products and their application for skim-milk fermentations. *LWT*. https://doi.org/10.1016/j.lwt.2019.03.005

Bajpai, V. K., Rather, I. A., Majumder, R., Shukla, S., Aeron, A., Kim, K., Kang, S. C., Dubey, R. C., Maheshwari, D. K., Lim, J., & Park, Y. H. (2016). Exopolysaccharide and lactic acid bacteria: Perception, functionality and prospects. In *Bangladesh Journal of Pharmacology*. https://doi.org/10.3329/bjp.v11i1.23819

Bancalari, E., D'Incecco, P., Savo Sardaro, M. L., Neviani, E., Pellegrino, L., & Gatti, M. (2019). Impedance microbiology to speed up the screening of lactic acid bacteria exopolysaccharide production. *International Journal of Food Microbiology*. https://doi.org/10.1016/j.ijfoodmicro.2019.108268

Bengoa, A. A., Llamas, M. G., Iraporda, C., Dueñas, M. T., Abraham, A. G., & Garrote, G. L. (2018). Impact of growth temperature on exopolysaccharide production and probiotic properties of Lactobacillus paracasei strains isolated from kefir grains. *Food Microbiology*. https://doi.org/10.1016/j.fm.2017.08.012

Bukar, *, & Salami-Suleiman. (2019). Nutritional and Sensory Evaluation of Soy-Yoghurt Produced with Lactobacillus plantarum Isolated from Locally Fermented Milk (Nono). *Nigerian Journal of Microbiology*, *33*(1), 4633–4644. http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Cerning, J., Renard, C. M. G. C., Thibault, J. F., Bouillanne, C., Landon, M., Desmazeaud, M., & Topisirovic, L. (1994). Carbon source requirements for exopolysaccharide production by Lactobacillus casei CG11 and partial structure analysis of the polymer. *Applied and Environmental Microbiology*. https://doi.org/10.1128/aem.60.11.3914-3919.1994

Chai, W. Y., Teo, K. T. K., Tan, M. K., & Tham, H. J. (2022). Fermentation Process Control and Optimization. In *Chemical Engineering and Technology*. https://doi.org/10.1002/ceat.202200029

Chaisuwan, W., Jantanasakulwong, K., Wangtueai, S., Phimolsiripol, Y., Chaiyaso, T., Techapun, C., Phongthai, S., You, S. G., Regenstein, J. M., &

Seesuriyachan, P. (2020). Microbial exopolysaccharides for immune enhancement: Fermentation, modifications and bioactivities. In *Food Bioscience*. https://doi.org/10.1016/j.fbio.2020.100564
Chen, M., Ye, X., Shen, D., & Ma, C. (2019). Modulatory effects of gut microbiota on constipation: The commercial beverage Yakult Shapes Stool Consistency. In *Journal of Neurogastroenterology and Motility*. https://doi.org/10.5056/jnm19048
Chow, P. S., & Landhäusser, S. M. (2004). A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology*. https://doi.org/10.1093/treephys/24.10.1129
Cirrincione, S., Breuer, Y., Mangiapane, E., Mazzoli, R., & Pessione, E. (2018). 'Ropy' phenotype, exopolysaccharides and metabolism: Study on food isolated potential probiotics LAB. *Microbiological Research*. https://doi.org/10.1016/j.micres.2018.07.004
Costa, C. R. L. d. M., Menolli, R. A., Osaku, E. F., Tramontina, R., de Melo, R. H., do Amaral, A. E., Duarte, P. A. D., de Carvalho, M. M., Smiderle, F. R., Silva, J. L. d. C., & Mello, R. G. (2019). Exopolysaccharides from Aspergillus terreus: Production, chemical elucidation and immunoactivity. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2019.08.039
Deepak, V., Ram Kumar Pandian, S., Sivasubramaniam, S. D., Nellaiah, H., & Sundar, K. (2016). Optimization of anticancer exopolysaccharide production from probiotic Lactobacillus acidophilus by response surface methodology. *Preparative Biochemistry and Biotechnology*. https://doi.org/10.1080/10826068.2015.1031386
Derdak, R., Sakoui, S., Pop, O. L., Cristian Vodnar, D., Addoum, B., Elmakssoudi, A., Errachidi, F., Suharoschi, R., Soukri, A., & El Khalfi, B. (2022). Screening, optimization and characterization of exopolysaccharides produced by novel strains isolated from Moroccan raw donkey milk. *Food Chemistry: X*. https://doi.org/10.1016/j.fochx.2022.100305
Dertli, E., Colquhoun, I. J., Côté, G. L., Le Gall, G., & Narbad, A. (2018). Structural analysis of the α-D-glucan produced by the sourdough isolate Lactobacillus brevis E25. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2017.09.017
Dilna, S. V., Surya, H., Aswathy, R. G., Varsha, K. K., Sakthikumar, D. N., Pandey, A., & Nampoothiri, K. M. (2015). Characterization of an exopolysaccharide with potential health-benefit properties from a probiotic Lactobacillus plantarum RJF4. *LWT*. https://doi.org/10.1016/j.lwt.2015.07.040
Du, R., Xing, H., Yang, Y., Jiang, H., Zhou, Z., & Han, Y. (2017). Optimization, purification and structural characterization of a dextran produced by L. mesenteroides isolated from Chinese sauerkraut. *Carbohydrate Polymers*. https://doi.org/10.1016/j.carbpol.2017.06.084
Gao, L., Wang, Y., Zhang, F., Li, S., Zhao, J., Zhang, Q., Ye, J., Ma, Y., Wang, Z., & Chen, W. (2022). A standardized method for the quantification of polysaccharides: An example of polysaccharides from Tremella fuciformis. *LWT*. https://doi.org/10.1016/j.lwt.2022.113860
García-Burgos, M., Moreno-Fernández, J., Alférez, M. J. M., Díaz-Castro, J., & López-Aliaga, I. (2020). New perspectives in fermented dairy products and their health relevance. In *Journal of Functional Foods*. https://doi.org/10.1016/j.jff.2020.104059
Gayathiri, E., Bharathi, B., Velu, S., Siva, N., Natarajan S, Prabavathi, S., & Selvadhas, S. (2017). Isolation, Identification and Optimization of Exopolysaccharide Producing Lactic Acid Bacteria from Raw Dairy Samples. *International Journal of Pharma And Chemical Research*.
Ghareeb, A., Fouda, A., Kishk, R. M., & El Kazzaz, W. M. (2024). Unlocking the therapeutic potential of bioactive exopolysaccharide produced by marine actinobacterium Streptomyces vinaceusdrappus AMG31: A novel approach to drug development. *International Journal of Biological Macromolecules*, *276*(P2), 133861. https://doi.org/10.1016/j.ijbiomac.2024.133861
Granato, D., Branco, G. F., Cruz, A. G., Faria, J. de A. F., & Shah, N. P. (2010). Probiotic dairy products as functional foods. *Comprehensive Reviews in Food Science and Food Safety*. https://doi.org/10.1111/j.1541-4337.2010.00120.x
Han, X., Yang, Z., Jing, X., Yu, P., Zhang, Y., Yi, H., & Zhang, L. (2016). Improvement of the Texture of Yogurt by Use of Exopolysaccharide Producing Lactic Acid Bacteria. *BioMed Research International*. https://doi.org/10.1155/2016/7945675
Helal, M., Hussein, M.-D., Osman, M., Shalaby, A. S., & Ghaly, M. (2015). Production and prebiotic activity of exopolysaccharides derived from some probiotics. *Egyptian Pharmaceutical Journal*. https://doi.org/10.4103/1687-4315.154687
Imran, M. Y. M., Reehana, N., Jayaraj, K. A., Ahamed, A. A. P., Dhanasekaran, D., Thajuddin, N., Alharbi, N. S., & Muralitharan, G. (2016). Statistical optimization of exopolysaccharide production by Lactobacillus plantarum NTMI05 and NTMI20. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2016.09.007
Kaur, N., & Dey, P. (2023). Bacterial exopolysaccharides as emerging bioactive macromolecules: from fundamentals to applications. In *Research in Microbiology*. https://doi.org/10.1016/j.resmic.2022.104024
Knoshaug, E. P., Ahlgren, J. A., & Trempy, J. E. (2000). Growth associated exopolysaccharide expression in Lactococcus lactis subspecies cremoris Ropy352. *Journal of Dairy Science*. https://doi.org/10.3168/jds.S0022-0302(00)74923-X
Korcz, E., & Varga, L. (2021). Exopolysaccharides from lactic acid bacteria: Techno-functional application in the food industry. In *Trends in Food Science and Technology*. https://doi.org/10.1016/j.tifs.2021.02.014
Krishnamurthy, M., Jayaraman Uthaya, C., Thangavel, M., Annadurai, V., Rajendran, R., & Gurusamy, A. (2020). Optimization, compositional analysis, and characterization of exopolysaccharides produced by multi-metal resistant Bacillus cereus KMS3-1. *Carbohydrate Polymers*. https://doi.org/10.1016/j.carbpol.2019.115369
Kumar, Y., Kumar Singh, P., Singh, A. K., Masih, H., Kiran Peter, J., Benjamin, J. C., & Rath, S. (2014). Production Optimization of Alpha Amylase from Bacillus Altitudinis. *International Journal of Scientific Engineering and Technology Research*, *3*(4), 564–573. https://doi.org/10.13140/RG.2.2.14726.42569
Lynch, K. M., Coffey, A., & Arendt, E. K. (2018). Exopolysaccharide producing lactic acid bacteria: Their techno-functional role and potential application in gluten-free bread products. *Food Research International*. https://doi.org/10.1016/j.foodres.2017.03.012
Malang, S. K., Maina, N. H., Schwab, C., Tenkanen, M., & Lacroix, C. (2015). Characterization of exopolysaccharide and ropy capsular polysaccharide formation by Weissella. *Food Microbiology*. https://doi.org/10.1016/j.fm.2014.08.022
Moghannem, S. A. M., Farag, M. M. S., Shehab, A. M., & Azab, M. S. (2018). Exopolysaccharide production from Bacillus velezensis KY471306 using statistical experimental design. *Brazilian Journal of Microbiology*. https://doi.org/10.1016/j.bjm.2017.05.012
Nagalakshmi, P. K., Sumathi, R., Kanimozhi, K., & Sivakumar, T. (2013). Isolation of bacteriocin nisin producing Lactococcus lactis from dairy products. *J. Acad. Indus. Res*.
Nambiar, R. B., Sellamuthu, P. S., Perumal, A. B., Sadiku, E. R., Phiri, G., & Jayaramudu, J. (2018). Characterization of an exopolysaccharide produced by Lactobacillus plantarum HM47 isolated from human breast milk. *Process Biochemistry*. https://doi.org/10.1016/j.procbio.2018.07.018
Nemati, V., & Mozafarpour, R. (2024). Exopolysaccharides isolated from fermented milk-associated lactic acid bacteria and applied to produce functional value-added probiotic yogurt. *Lwt*, *199*(April), 116116. https://doi.org/10.1016/j.lwt.2024.116116
Nguyen, P. T., Nguyen, T. T., Bui, D. C., Hong, P. T., Hoang, Q. K., & Nguyen, H. T. (2020). Exopolysaccharide production by lactic acid bacteria: The manipulation of environmental stresses for industrial applications. *AIMS Microbiology*. https://doi.org/10.3934/MICROBIOL.2020027
Oleksy-Sobczak, M., Klewicka, E., & Piekarska-Radzik, L. (2020). Exopolysaccharides production by Lactobacillus rhamnosus strains – Optimization of synthesis and extraction conditions. *LWT*. https://doi.org/10.1016/j.lwt.2020.109055
Omafuvbe, B. O., & Enyioha, L. C. (2011). Phenotypic identification and technological properties of lactic acid bacteria isolated from selected commercial Nigerian bottled yoghurt. *Frican Journal of Food Science*.
Paulo, E. M., Vasconcelos, M. P., Oliveira, I. S., Affe, H. M. de J., Nascimento, R., Melo, I. S. de, Roque, M. R. de A., & Assis, S. A. de. (2012). An alternative method for screening lactic acid bacteria for the production of exopolysaccharides with rapid confirmation. *Food Science and Technology*. https://doi.org/10.1590/s0101-20612012005000094
Pawar, S. T., Bhosale, A. A., Gawade, T. B., & Nale, T. R. (2013). Isolation, screening and optimization of exopolysaccharide producing bacterium from saline soil. *Journal of Microbiology and Biotechnology Research Scholars Research Library J. Microbiol. Biotech. Res*.
Peerzada Gh, J., Sinclair, B. J., Perinbarajan, G. K., Dutta, R., Shekhawat, R., Saikia, N., Chidambaram, R., & Mossa, A. T. (2023). An overview on smart and active edible coatings: safety and regulations. In *European Food Research and Technology*. https://doi.org/10.1007/s00217-023-04273-2
Peng, K., Koubaa, M., Bals, O., & Vorobiev, E. (2020). Recent insights in the impact of emerging technologies on lactic acid bacteria: A review. In *Food Research International*. https://doi.org/10.1016/j.foodres.2020.109544
Plessas, S., Nouska, C., Karapetsas, A., Kazakos, S., Alexopoulos, A., Mantzourani, I., Chondrou, P., Fournomiti, M., Galanis, A., & Bezirtzoglou, E. (2017). Isolation, characterization and evaluation of the probiotic potential of a novel Lactobacillus strain isolated from Feta-type cheese. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2017.01.052
Pramudito, T. E., Desai, K., Voigt, C., Smid, E. J., & Schols, H. A. (2024). Dextran and levan exopolysaccharides from tempeh-associated lactic acid bacteria with bioactivity against enterotoxigenic Escherichia coli (ETEC). *Carbohydrate Polymers*. https://doi.org/10.1016/j.carbpol.2023.121700
Prete, R., Alam, M. K., Perpetuini, G., Perla, C., Pittia, P., & Corsetti, A. (2021). Lactic acid bacteria exopolysaccharides producers: A sustainable tool for functional foods. In *Foods*. https://doi.org/10.3390/foods10071653
Sanalibaba, P., & Cakmak, G. A. (2016). Exopolysaccharides Production by Lactic Acid Bacteria. *Applied Microbiology: Open Access*. https://doi.org/10.4172/2471-9315.1000115
Sawadogo-Lingani, H., Lei, V., Diawara, B., Nielsen, D. S., Møller, P. L., Traoré, A. S., & Jakobsen, M. (2007). The biodiversity of predominant lactic acid bacteria in dolo and pito wort for the production of sorghum beer. *Journal of Applied Microbiology*. https://doi.org/10.1111/j.1365-2672.2007.03306.x
Singh, S., & Das, S. (2011). Screening, production, optimization and

characterization of cyanobacterial polysaccharide. *World Journal of Microbiology and Biotechnology*. https://doi.org/10.1007/s11274-011-0657-y

Sørensen, H. M., Rochfort, K. D., Maye, S., MacLeod, G., Brabazon, D., Loscher, C., & Freeland, B. (2022). Exopolysaccharides of Lactic Acid Bacteria: Production, Purification and Health Benefits towards Functional Food. In *Nutrients*. https://doi.org/10.3390/nu14142938

SORESCU, I., & STOICA, C. (2021). Online Advanced Bacterial Identification Software, an Original Tool for Phenotypic Bacterial Identification. *Romanian Biotechnological Letters*. https://doi.org/10.25083/rbl/26.6/3047-3053

Staudt, A. K., Wolfe, L. G., & Shrout, J. D. (2012). Variations in exopolysaccharide production by Rhizobium tropici. *Archives of Microbiology*. https://doi.org/10.1007/s00203-011-0742-5

Su, C., Chi, Z., & Lu, W. (2007). Optimization of medium and cultivation conditions for enhanced exopolysaccharide yield by marine Cyanothece sp. 113. *Chinese Journal of Oceanology and Limnology*. https://doi.org/10.1007/s00343-007-0411-3

Taj, R., Masud, T., Sohail, A., Sammi, S., Naz, R., Sharma Khanal, B. K., & Nawaz, M. A. (2022). In vitro screening of EPS-producing Streptococcus thermophilus strains for their probiotic potential from Dahi. *Food Science and Nutrition*. https://doi.org/10.1002/fsn3.2843

Tarannum, N., Hossain, T. J., Ali, F., Das, T., Dhar, K., & Nafiz, I. H. (2023). Antioxidant, antimicrobial and emulsification properties of exopolysaccharides from lactic acid bacteria of bovine milk: Insights from biochemical and genomic analysis. *LWT*. https://doi.org/10.1016/j.lwt.2023.115263

Tiwari, O. N., Khangembam, R., Shamjetshabam, M., Sharma, A. S., Oinam, G., & Brand, J. J. (2015). Characterization and Optimization of Bioflocculant Exopolysaccharide Production by Cyanobacteria Nostoc sp. BTA97 and Anabaena sp. BTA990 in Culture Conditions. *Applied Biochemistry and Biotechnology*. https://doi.org/10.1007/s12010-015-1691-2

Vinothini, G., Latha, S., Arulmozhi, M., & Dhanasekaran, D. (2019). Statistical optimization, physio-chemical and bio-functional attributes of a novel exopolysaccharide from probiotic Streptomyces griseorubens GD5. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2019.05.011

Wang, J., Zhao, X., Tian, Z., Yang, Y., & Yang, Z. (2015). Characterization of an exopolysaccharide produced by Lactobacillus plantarum YW11 isolated from Tibet Kefir. *Carbohydrate Polymers*. https://doi.org/10.1016/j.carbpol.2015.03.003

Wang, K., Li, W., Rui, X., Chen, X., Jiang, M., & Dong, M. (2014). Structural characterization and bioactivity of released exopolysaccharides from Lactobacillus plantarum 70810. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2014.02.056

Wang, Y., Li, C., Liu, P., Ahmed, Z., Xiao, P., & Bai, X. (2010). Physical characterization of exopolysaccharide produced by Lactobacillus plantarum KF5 isolated from Tibet Kefir. *Carbohydrate Polymers*. https://doi.org/10.1016/j.carbpol.2010.06.013

Widyaningrum, D., & Meindrawan, B. (2020). The application of microbial extracellular polymeric substances in food industry. *IOP Conference Series: Earth and Environmental Science*. https://doi.org/10.1088/1755-1315/426/1/012181

Xiao, L., Han, S., Zhou, J., Xu, Q., Dong, M., Fan, X., Rui, X., Chen, X., Zhang, Q., & Li, W. (2020). Preparation, characterization and antioxidant activities of derivatives of exopolysaccharide from Lactobacillus helveticus MB2-1. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2019.09.192

Yadav, M. K., Song, J. H., Vasquez, R., Lee, J. S., Kim, I. H., & Kang, D. K. (2024). Methods for Detection, Extraction, Purification, and Characterization of Exopolysaccharides of Lactic Acid Bacteria—A Systematic Review. *Foods*, *13*(22). https://doi.org/10.3390/foods13223687

You, X., Yang, L., Zhao, X., Ma, K., Chen, X., Zhang, C., Wang, G., Dong, M., Rui, X., Zhang, Q., & Li, W. (2020). Isolation, purification, characterization and immunostimulatory activity of an exopolysaccharide produced by Lactobacillus pentosus LZ-R-17 isolated from Tibetan kefir. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2020.05.027

Zannini, E., Waters, D. M., Coffey, A., & Arendt, E. K. (2016). Production, properties, and industrial food application of lactic acid bacteria-derived exopolysaccharides. In *Applied Microbiology and Biotechnology*. https://doi.org/10.1007/s00253-015-7172-2

Zehir Şentürk, D., Dertli, E., Erten, H., & Şimşek, Ö. (2020). Structural and technological characterization of ropy exopolysaccharides produced by Lactobacillus plantarum strains isolated from Tarhana. *Food Science and Biotechnology*. https://doi.org/10.1007/s10068-019-00641-5

Zhang, G., Zhang, W., Sun, L., Sadiq, F. A., Yang, Y., Gao, J., & Sang, Y. (2019). Preparation screening, production optimization and characterization of exopolysaccharides produced by Lactobacillus sanfranciscensis Ls-1001 isolated from Chinese traditional sourdough. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2019.08.077

Zhang, T., Zhang, C., Li, S., Zhang, Y., & Yang, Z. (2011). Growth and exopolysaccharide production by Streptococcus thermophilus ST1 in skim milk. *Brazilian Journal of Microbiology*. https://doi.org/10.1590/S1517-83822011000400033

Zhang, W. H., Wu, J., Weng, L., Zhang, H., Zhang, J., & Wu, A. (2020). An improved phenol-sulfuric acid method for the determination of carbohydrates in the presence of persulfate. *Carbohydrate Polymers*. https://doi.org/10.1016/j.carbpol.2019.115332

Zhang, Y., Li, S., Zhang, C., Luo, Y., Zhang, H., & Yang, Z. (2011). Growth and exopolysaccharide production by Lactobacillus fermentum F6 in skim milk. *African Journal of Biotechnology*.