Isolation and Characterization of Exopolysaccharide-Producing *Lactobacillus Paracasei* Strain and It's Impact on Yoghurt Properties

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ABSTRACT: In the last decade, microbial polysaccharides have received increasing attention, and it has been demonstrated that many microbial strains can produce polysaccharides with different compositions and functionality. The isolated and characterized *Lactobacillus paracasei* has been studied for its ability to produce exopolysaccharides (EPS). In addition, the effect of carbon sources on EPS production was investigated. The effect of *Lactobacillus paracasei* on the viscosity and sensory properties of yoghurt made from whole milk and skim milk was studied. *Lactobacillus paracasei* had excellent biomass (4.4 g/L) with good biomass separation (0.11). Using sucrose 100 g/L as a carbon source for 3 days gave the best EPS content (0.7 g/L). Infrared spectra showed that the EPS produced by *Lactobacillus paracasei* was a hetero-polysaccharide. Fortification with *Lactobacillus paracasei* in the production of yoghurt from skim milk resulted in a relatively similar viscosity (5155.20 ± 4.53 mPa.S) to that of whole milk yoghurt (5264.30 ± 2.40 mPa.S), reflecting the improvement in the sensory characteristics of yoghurt from skim milk, especially mouthfeel. Consequently, fortification with *Lactobacillus paracasei* is recommended in low-fat yoghurt.

Keywords: *Exopolysaccharides; Yoghurt; Low-fat yoghurt; FT-IR; Viscosity; Sensory characteristics.*

INTRODUCTION

Lactic acid bacteria (LAB) are Gram-positive, catalase-negative, oxidase-negative, and non-sporulating microaerophilic bacteria that mainly ferment carbohydrates to lactic acid. The LAB include both cocci (e.g. *Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Tetragenococcus, Streptococcus, Enterococcus*) and rods (*Lactobacillus, Carnobacterium, Bifidobacterium*) **(Mokoena, 2017)**. LAB are generally recognized as safe (GRAS); therefore, they can be successfully used in the food industry. LAB generally contribute to flavour and aroma development and delay spoilage **(Lorenzo *et al.*, 2018)**. LAB produce different nutraceuticals such as teichoic acids, peptidoglycan and polysaccharides that could be beneficial for both human health and the food industry **(Xiao *et al.*, 2023)**.

Exopolysaccharides are water-soluble gums with novel and unique physical properties **(Prajapati *et al.*, 2013)**. They can be classified into two categories depending on their location: released exopolysaccharides (released into the surrounding environment as slime) and cell-bound exopolysaccharides (adhered to the bacterial cell as a capsule) **(Xiao *et al.*, 2023)**. In many medical applications, such as scaffolds or matrices in tissue engineering, drug delivery, and wound treatment, some of these bacterial exopolysaccharides are used because of their inherent biocompatibility and apparent non-toxicity. This makes them more desirable than polysaccharides derived from plants and microalgae **(Nwodo *et al.*, 2012)**.

Yoghurt is one of the most consumed dairy products worldwide **(Bachtarzi *et al.*, 2019; Gomaa *et al.*, 2022)**; using low-fat dairy products is considered a health-promoting trend by consumers **(Mathur and Singh, 2005)**. However, skim milk affects the sensory attributes of the products, particularly the mouthfeel **(Gomaa *et al.*, 2018; Hamza and Gomaa, 2020)**. Using food thickening and stabilizing agents could enhance the textural properties of the products **(Bachtarzi *et al.*, 2019)**. Due to the high demand for natural food additives, finding a natural EPS producer has become crucial.

The aim of the present study was the isolation and characterization of the *L. paracasei* strain, investigation of its produced EPS properties and its potential role in the improvement of viscosity and sensory characteristics of yoghurt prepared from skimmed and whole milk.

2.MATERIALS AND METHODS

2.1.Bacterial strains and materials

Salmonella typhimurium was kindly provided by the American Type Culture Collection (ATCC), Virginia, USA. *Escherichia coli* was kindly provided by the Institute of Graduate Studies and Research (IGSR), Alexandria, Egypt.

Whole milk (fat 3.02%, protein 3.08%, lactose 4.75%, minerals 0.60%, freezing point – 0.457 °C, and density 1.0333 gm/mL); skim milk (fat 0.22%, protein 3.29%, lactose 4.96%, minerals 0.82%, freezing point – 0.51 °C, and density 1.0386 gm/mL); yoghurt, naturally produced without using commercial starters, were collected from Alexandria Governorate, Egypt, and kept in a refrigerator at 4 °C until use.

De Man, Rogosa, and Sharpe (MRS) broth/ agar for *Lactobacilli* counts, tryptone bile x-glucuronide medium (TBX) agar for *E. coli* count, *Salmonella Shigella* agar (SS agar) for *Salmonella* count (Oxoid, Paisley, UK), and Columbia agar with 5% sheep blood (Merck, Darmstadt, Germany) for hemolytic activity assessment. Ampicillin trihydrate (HiMedia, Maharashtra, India), tetracycline hydrochloride and chloramphenicol (Bioanalyse, Ankara, Turkey). Trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), mutanolysin, lysozyme, isoamyl alcohol, and nigrosin stain (Merck, Darmstadt, Germany). Chloroform, ethanol, glycerol, sucrose, sodium chloride (NaCl), isopropanol, and sodium hydroxide (NaOH) were obtained from Aljumhuria, Alexandria, Egypt.

2.2 .Isolation of *Lactobacillus paracasei*

Lactobacillus paracasei was isolated from fermented milk products using MRS media. After preparing serial dilutions in peptone (0.1%), the dilutions were incubated on MRS agar media at 37 °C/ 48 h anaerobically to isolate *Lactobacillus paracasei* strains **(Savadogo *et al.*, 2004)**. Colonies were then tested for Gram reaction test and catalase production according to the methods described by Harrigan & McCance **(1976)**. The Gram-positive and catalase-negative colonies were then subcultured in MRS broth to maintain their purity and stored in glycerol (50%) at −80 °C **(Al-Nabulsi *et al.*, 2022)**. Subsequently, bacterial growth from the slant culture was reactivated in 10 mL broth medium at 37 °C/ 24 h anaerobically under static conditions for further analysis.

2.3. Identification of isolated *Lactobacilli*

2.3.1.Genomic DNA Preparation of Isolates

To extract total DNA, 1.5 mL of the culture was centrifuged in the middle of the logarithmic phase (OD_{650} of 0.5 - 1) at 3000xg for 10 min at 4 °C to collect the cells (pellet). Cells were then frozen at -20 °C for 1 h, thawed, and washed in 1 mL TES buffer (6.7 % sucrose, 50 mM Tris-HCl, 1 mM EDTA, pH 8.0). Afterwards, the cells were re-suspended in 300 µl STET buffer (8 % sucrose, 5 % Triton X-100, 50 mM Tris-HCl, 50 mM EDTA, pH 8.0). After adding 75 µL lysis buffer (Triethylsilane (TES) containing 1330 U/mL mutanolysin and 40 mg/mL lysozyme), the suspension was incubated at 37 °C/ 1h. Later, 40 µL Sodium dodecyl sulfate (SDS) (20%) in Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0, 37 °C) was vortexed with the cells for 60 s. The cells were then incubated at 37 °C/ 10 min, followed by 65 °C/ 10 min. Subsequently, 100 µL TE buffer was added, and the lysate was extracted with 1 volume of phenol/chloroform/isoamyl alcohol (49:49:1 v/v/v). The mixture was centrifuged with an Eppendorf phase lock gel (18000 xg, 5 min); the aqueous phase was mixed gently with 70 µL 1M NaCl, 1 mL isopropanol; the mixture was then placed on ice for 15 min. The precipitated DNA was collected by centrifugation at 20000 xg at 4°C/ 30 min, and the pellet was washed in ice-cold 70 % ethanol. Finally, the DNA was dried and resuspended in 100 µL TE **(Savadogo *et al.*, 2004)**.

2.3.2. 16S rDNA sequencing

The 16S rDNA of the bacterial isolates was amplified by polymerase chain reaction (PCR) using forward primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). PCR mixtures were prepared according to the manufacturer's protocol (Zymo Research, Irvine, CA, USA). Amplification was then performed at 94 °C/ 2 min (denaturation; 1 cycle), 94 °C/ 20 s (35 cycles), 53 °C/ 20 s (annealing; 1 cycle), and 70 °C/ 1.5 min (extension; 1 cycle). Genomic DNA was analyzed by electrophoresis in 1 % (w/v) agarose gels in 1X Tris-acetate-EDTA buffer. DNA sequencing of PCR products was carried out by Macrogen, Seoul, South Korea, and the results were aligned with the US National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool algorithm (BLAST). A neighbour-joining phylogenetic tree of *Lactobacillus paracasei* was constructed using Molecular Evolutionary Genetics Analysis (MEGA) software.

2.4.Probiotic characterization studies

2.4.1.Antimicrobial activity of isolated *Lactobacillus paracasei*

Antimicrobial activity was determined by agar well diffusion assay against *Escherichia coli* K12 and *Salmonella typhimurium* (indicators). Plate count agar medium (PCA) at a temperature of 45 °C was inoculated with 0.2% with the broth cultures grown separately overnight. The prepared medium was poured into Petri dishes and allowed to solidify. Two wells with a 4 mm diameter in each plate were filled with either; 100 µL of *L. paracasei* supernatant (pH 6 supernatant

neutralized with 1N NaOH obtained by centrifugation and membrane filtration (0.2 µm; OE 66, Whatman®, Dassel, Germany)), or *L. paracasei* cultured in broth medium for 18 h under standard conditions or sterile broth medium (negative control). Subsequently, the plates were incubated at 4 °C/ 4 h, followed by 18-24 h under standard conditions for each indicator strain. Later, the diameter of the inhibition zones was measured; the inhibition zones were scored **(Gomaa, 2005)**.

2.4.2.Antibiotics sensitivity

Bacterial susceptibility to antibiotics was determined by the disk diffusion assay described by Ayad *et al.* **(2004)** using a 3 mm paper disc containing ampicillin trihydrate 10 mcg/disc, tetracycline hydrochloride 30 mcg/disc, and chloramphenicol 30 mcg/disc. The discs were placed on agar plates inoculated with 0.5% *L. paracasei* grown overnight in broth culture. The plates were then incubated at 37 °C/ 2 days and screened for clear zones as an indicator of sensitivity to antibiotics.

2.4.3.Assessment of hemolytic activity

Briefly, 5 µL of *L. paracasei* broth culture was inoculated onto blood agar plates; plates were incubated at 37ºC/ 18 h anaerobically under standard conditions. Subsequently, the plates were screened for hemolytic activity: β-hemolytic activity completely lyses the red blood cells surrounding the colony; α-hemolysis partially lyses the haemoglobin and turns it green; γ-hemolysis (or non-hemolytic) means that there is no hemolytic activity **(Morán *et al.*, 2002)**.

2.5.Production and technological characteristics

2.5.1.Yield of biomass

L. paracasei were subcultured twice under standard conditions; the broth medium was inoculated with 10% of the culture. Bacterial growth was measured spectrophotometrically (Thermo Scientific, EVOLUTION 300 UV-VIS, Paisley, UK) at 650 nm (OD_{650}) every two hours (in triplicate) for 24 h. The difference between the initial OD_{650} (0-time) and the OD_{650} at the time the sample was collected (ΔOD650) was used to indicate the extent of growth. Afterwards, the cells were harvested from 20 mL of the medium by centrifugation at 6988 xg for 15 min at 4 °C. The pellet was then washed twice with potassium phosphate buffer (50 mM, pH 6) and dried at 105 °C for 4 h to a constant weight to determine the cell dry weight (CDW) in mg L^{-1} as described by Gerhardt **(1981)**. The remaining media were used to study biomass separation **(Gerhardt *et al.*, 1981)**.

2.5.2.Separation of biomass

The remaining supernatant after harvesting the cells in section 2.6.1 was used to express biomass separation by measuring its OD at 650 nm (OD_{650}). The readings at OD_{650} reflect the quality of separation: 0 represents excellent separation, 0 - 0.1 represents a good separation of biomass, 0.2 - 0.3 is fair biomass, and greater than 0.3 represents poor biomass separation **(Gerhardt *et al.*, 1981)**.

2.5.3.Acidification activity

Acidification activity was determined by the change in pH (ΔpH) over time as Ayad et al. (2004) described. The broth MRS (50 mL) was inoculated with 5% of *L. paracasei* culture in the early stationary growth phase and incubated at an appropriate temperature. The pH was measured every hour for 6 h using a pH meter (Adwa, AD1030, Szeged, Hungary). The acidification rate was calculated using equation 1; cultures were classified as fast, medium, or slow acidifying when a ΔpH of 0.4 U was achieved after 3 h, 3-5 h, and > 5 h, respectively.

$$\Delta pH = pH\ at\ time(x) - pH\ at\ zero\ time \quad (1)$$

While the overall acidification activity was determined using equation 2; values < 2 units reflect poor acidification activity **(Gerhardt *et al.*, 1981)**.

$$\Delta pH = pH\ at\ zero\ time - pH\ at\ the\ end\ of\ growth \quad (2)$$

2.5.4.Exopolysaccharides production

L. paracasei cultures were incubated at 37ºC/ 24 h on MRS agar medium. They were then superficially screened for EPS production based on their texture on the medium using the inoculation loop method **(Jeong *et al.*, 2017)**. The colonies formed were pulled up with a metal loop and the strains were considered positive EPS producers if the length of the slime exceeded 1.5 mm. Subsequently, the positive strains were tested for capsule production using a phase contrast microscope (ZEISS microscope, Germany) according to the method of Prescott et al. **(1996)**. After mixing the bacterial culture with the nigrosine dye was spread in a thin film on a slide and air-dried. The bacteria appear as lighter-coloured bodies amidst a blue-black background because the dye particles cannot penetrate the bacterial cell or its capsule.

2.6.Exopolysaccharides extraction

EPS was extracted according to Savadogo et al. **(2004)**. *L. paracasei* was cultured in MRS broth at 37 °C/ 12h. Then, 1% of the incubated inoculum was added to 200 mL of MRS broth and incubated anaerobically for 37 °C/ 72 h under static conditions to produce exopolysaccharides. After centrifuging the mixture at 22337 xg/ 15 min at 4

°C, the biomass was removed. Afterwards, samples were treated with trichloroacetic acid (10% w/v) for 30 min with agitation and centrifuged at 22337 xg/ 15 min to remove proteins. A double volume of chilled ethanol was added to the supernatant and stored overnight at 4 °C. The mixture was centrifuged at 22337 xg/ 20 min at 4 °C; the collected precipitate was dissolved in demineralized water and precipitated again with twice the volume of chilled ethanol. The mixture was further centrifuged at 22337 xg/ 20 min at 4 °C to recover the crude EPS. The crude EPS was lyophilized using a freeze dryer (FDF 0350, Humanlab Inc., Bucheon-si, Gyeonggi-do, Korea).

2.7. Characterization of exopolysaccharide by FT-IR Spectroscopy

To prepare the pellets, we pressed the freeze-dried EPS (1 mg) with 100 mg KBr **(Sharma *et al.*, 2023).** Fourier Transform-Infrared (FT-IR) Spectra of pure EPS were recorded from 450 to 4600 cm^{-1} with a resolution of 4.0 cm^{-1} and 25 scans by subtracting both background and atmospheric water at resolution using a Shimadzu FT-IR spectrophotometer (Spectrum BX FTIR).

2.8. Application of EPS-producing *Lactobacillus paracasei* in yoghurt production

Skim and whole milk were pasteurized at 80 °C/ 5 min; the milk was immediately cooled to 42 ºC before adding the starter cultures (Table 1) and transferred to 15 cups of 100 g. The prepared mixtures were incubated at 42 ºC/ 3 h. The yoghurt was then cooled to 4 °C to stop the fermentation process.

Table (1): Types of produced yoghurt

Milk Type	Trial code	Starter culture
Skim milk	S0	Yoghurt standard starter culture*
	C0S	*Lactobacillus paracasei*
	C0M	*Lactobacillus paracasei* and yoghurt starter*
Full cream milk	S3	Yoghurt standard starter culture*
	C3S	*Lactobacillus paracasei*
	C3M	*Lactobacillus paracasei* and yoghurt starter*

*Commercial yoghurt standard starter culture (*Streptococcus thermophilus* TLDB1 and *Lactobacillus delbrueckii* subsp. *bulgaricus* TST22).

2.9.Physical properties of yoghurt

2.9.1.Determination of pH and viscosity

The pH was measured at 28 °C with a pH meter (Adwa, AD1030, Szeged, Hungary). Viscosity was measured using a viscometer (Rogo-Sampaic, STS-2011 R, Wissous, France) at a speed of 60 to 200 rpm with spindles R2 and R5. Viscosity was measured at 26 °C and expressed in mPa.S.

2.9.2.Sensory evaluation

Seven panellists experienced in the evaluation of fermented dairy products were selected to evaluate the sensory attributes of yoghurt samples. A predetermined list of 22 sensory attributes was used to describe the sensory characteristics of yoghurt **(Tamime and Robinson, 2007)**. The introduced sensory attributes allowed the differentiation of samples in terms of odour, appearance, texture, flavour and mouthfeel. Samples (coded with 3 letters) were stored at 7 °C/ 24 h before sensory assessment. Subsequently, the samples were served in 100 g plastic cups. The panellists were asked to rate the yoghurt using a 5-point scale (1= little; 5= too much). The procedure for evaluating the sensory attributes of the yoghurt was divided into 4 main stages: Odor was assessed by removing the lid of the cup and rating the intensity of the volatile odour substances; appearance by visual observation; textural properties by breaking the yoghurt gel and agitating the product; Finally, the flavour and mouthfeel of the yoghurt were assessed by swallowing 10 g (a teaspoon portion) of the sample. Later, the overall acceptability was evaluated at the end of the sensory evaluation of each sample **(Soukoulis *et al.*, 2007)**.

2.10.Statistical analysis

The data were analyzed with IBM SPSS 25 (Armonk, New York, United States) using an independent-sample t-test and one-way ANOVA followed by Duncan's test at a 95% confidence level ($p < 0.05$). Data were expressed as mean ± standard deviation (SD).

3.RESULTS AND DISCUSSION

3.1.Identification of *L. paracasei* by 16S rRNA sequencing

The 16S rRNA sequence was aligned with other 16S rRNA gene sequences in GenBank using the NCBI Basic Local Alignment Search Tools BLAST n program. Multiple sequence alignment was performed using the Clustal X Version program **(Larkin *et al.*, 2007)**, and the resulting alignment was edited using SeaView **(Galtier *et al.*, 1996)**. The phylogenetic tree was constructed based on sequence distances using the neighbour-joining algorithm (NJ) with the Tamura-Nei substitution model. Phylogenetic analyses were performed using the Genius program. The stability or accuracy of the inferred topology was assessed

using a bootstrap analysis with 1,000 replicates. Sequence identities were determined based on the highest percentage (at least 99%) of total nucleotide match with sequences from GenBank. Blast results of PCR amplicons from isolate (C) showed 100 % identity with *Lactobacillus paracasei* (Figure 1) and the results were aligned with the US National Center for Biotechnology Information (NCBI: Accession: OQ144640.1 GI: 2418755615)

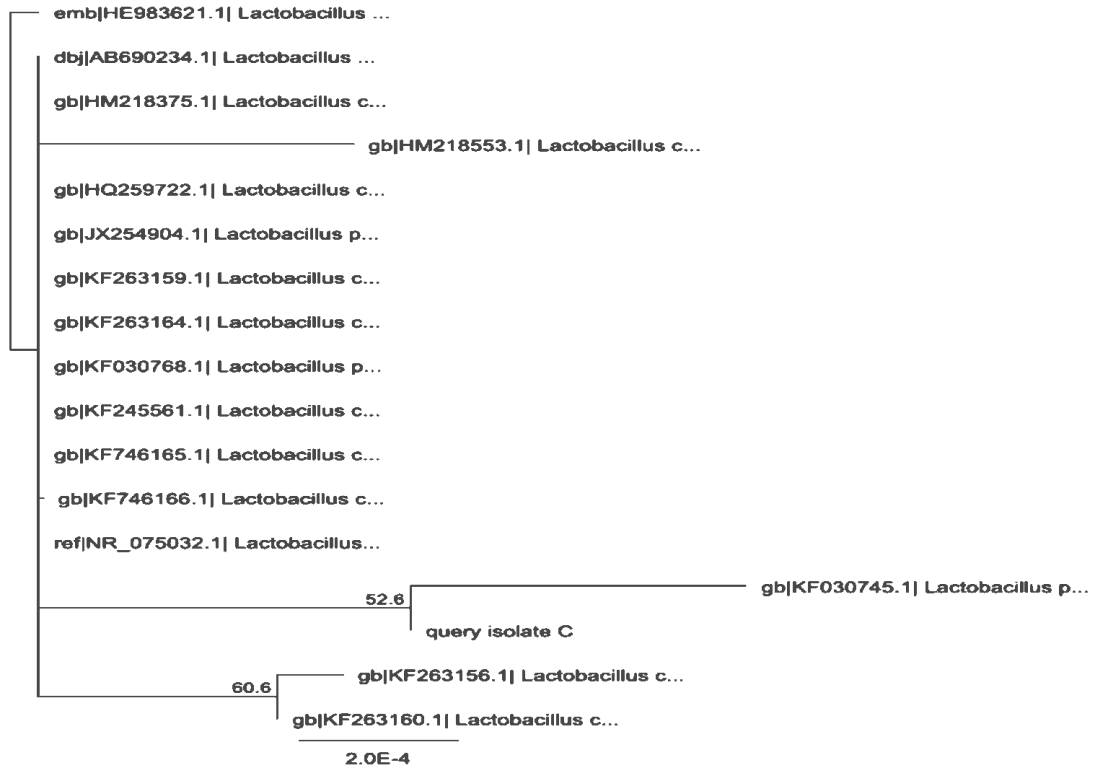

Figure 1: Dendrogram of 16S rRNA of isolate (C) compared with *Lactobacillus paracasei* by the Geneious program shown as a phylogenetic tree. Branch lengths are proportional to genetic distance, and numbers at the branch points indicate bootstrap values. The tree was generated using the neighbour-joining method. The dataset was subjected to 1,000 bootstrap replicates. The reference sequences were obtained from the GenBank database.

3.2.Probiotic characterization

3.2.1.Antimicrobial activity of *Lactobacillus paracasei*

Antimicrobial activity is of critical importance in the selection of probiotic cultures as natural inhibitors of harmful bacteria. The use of strains that inhibit human pathogens may provide a safe alternative for food preservation and therapeutic formulas **(Georgieva *et al.*, 2015)**. The supernatant obtained from *L. paracasei* was tested against potential human pathogens such as *Salmonella typhimurium* and *Escherichia coli* (Table 2). According to John *et al.* **(2017)**, the extract shows an intermediate inhibitory effect when bacterial growth is inhibited by 11-15 mm but an effective inhibitory effect above 16 mm. In this regard, *L. paracasei* was more efficient against *Salmonella typhimurium* than against *Escherichia coli*: it efficiently inhibited the growth of *Salmonella typhimurium* (25 mm), while it intermediately inhibited *E. coli* growth (15 mm). However, *L. paracasei* showed more robust antimicrobial activity against *Salmonella typhimurium* than that reported by Lando et al. **(2023)**, where *L. fermentum* LBF 233, *fermentum* LBF *433*, and *L. casei* LBC 237 inhibited bacterial growth by 8.33, 10.67, and 9.00 mm, respectively.

Lactobacillus isolates helped to promote bacterial interference by producing inhibitory substances such as organic acids, bacteriocin-like compounds, hydrogen peroxide, and bacteriocins **(Georgieva *et al.*, 2015)**. Here, the effect of organic acids was eliminated by neutralizing the supernatant. Besides, the strain could not produce hydrogen peroxide (data not shown), consequently, the antimicrobial effect of all strains is probably due to the production of bacteriocins. Our result is in contradiction with Georgieva *et al.* **(2015)**, reporting that bacteriocins inhibited the Gram-positive strains, while hydrogen peroxide and organic acids inhibited the Gram-negative strains.

3.2.2. Antibiotic sensitivity test

Antibiotics are critical for fighting bacterial infections. However, bacteria are adaptable and can develop antibiotic resistance **(Mathur and Singh, 2005)**, a dangerous high-level problem that complicates the treatment process for common bacterial infections **(Andersson *et al.*, 2020; WHO, 2020)**. Antibiotic resistance could transfer to the bacterial gut and worsen the situation by

developing new antibiotic-resistant pathogens. In this regard, the absence of transferable resistance to therapeutic antibiotics is crucial for the selection of a functional strain **(Georgieva *et al.*, 2015)**; only non-resistant strains are safe for human consumption **(Lu *et al.*, 2021)**.

In the present study, *L. paracasei* was tested against gram-positive spectrum antibiotics that inhibit cell wall syntheses, such as ampicillin, and protein synthesis, including chloramphenicol and tetracycline. In general, antibiotic-sensitive strains show weak activity with a larger transparent circle around the drug paper **(Lu *et al.*, 2021)**. *L. paracasei* revealed the highest sensitivity to tetracycline (20 mm), while it showed intermediate sensitivity to chloramphenicol (15 mm). However, the inhibition zone around the ampicillin disk (10 mm) indicates the resistance of the strain to ampicillin **(Sarker *et al.*, 2014)**.

3.2.3.Assessment of hemolytic activity

Some bacterial strains can produce exoenzymes called hemolysins that lyse red blood cells to varying degrees: Complete lysis of red blood cells (β-hemolytic activity; *Streptococcus haemolyticus*); partial lysis of haemoglobin to a greenish colour (α-hemolytic activity; *Streptococcus viridans*); no lysis of blood (γ-hemolysis or non-hemolytic). The introduction of bacteria with hemolytic activity into the gastrointestinal tract is dangerous; therefore, it is important to test the hemolytic activity of bacterial strains **(Morán *et al.*, 2002; Ismael *et al.*, 2023)**. *L. paracasei* did not lyse red blood cells, reflecting its safety as a functional strain in food production.

Table 2: Antimicrobial activity and Antibiotic sensitivity of *Lactobacillus* paracasei

Antimicrobial effect (mm)	
Salmonella typhimurium	25.00 ± 1.00^{a}
E. coli	15.00 ± 1.00^{b}
Antibiotic sensitivity (mm)	
Ampicillin (10 mcg/disc)	10 ± 0.00^{c}
Chloramphenicol (30 mcg/disc)	15 ± 1.00^{b}
Tetracycline (30 mcg/disc)	20 ± 1.00^{a}

Data are means of triplicate ± SD

The means of each character with different superscripts are statistically different ($p < 0.05$)

3.3.Assessment of production and technological characteristics *Lactobacillus paracasei*

3.3.1Growth parameters

The OD of growing *L. paracasei* was measured at 650 nm during incubation at 37 ºC/ 26h. As can be seen in (Figure 2), no lag phase was observed, indicating that growth was maintained throughout the cultivation period. It is evident that as the cultivation period increases, the growth and biomass of *Lactobacillus paracasei* increase. Therefore, *Lactobacillus paracasei* can be used in technological applications; its biomass can be cultivated for 7-9 h.

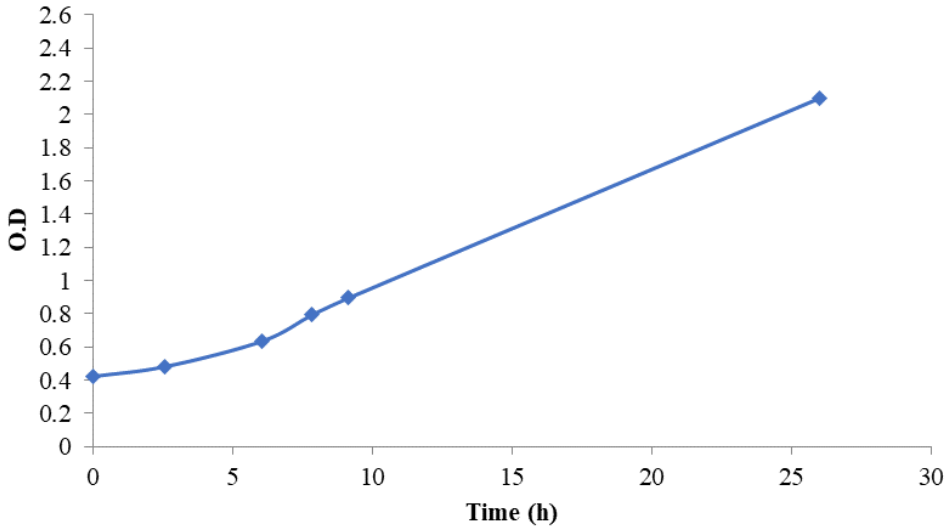

Figure 2: Growth curve of *Lactobacillus paracasei* cultured on broth MRS at 37 °C/ 26 h.

3.3.2.Yield of biomass

Bacterial strains can be classified into different categories based on their culture dry weight (CDW) at the end of the fermentation process: Strains with excellent biomass (greater than 3 gm L^{-1}); good biomass (3-1 gm L^{-1}); fair biomass (1-0.6 gm L^{-1}); poor biomass (less than 0.6 gm L^{-1}) **(Costilow, 1981)**. *Lactobacillus paracasei* has an excellent biomass yield of 4.4 g/L.

3.3.3.Assessment of biomass separation

Biomass separation at the end of the fermentation process (OD_{650}) ranged from 0-0.1, 0.2-0.3, and > 0.3 is good, fair, and poor biomass separation, respectively **(Gerhardt *et al.*, 1981)**. The assessment indicated a good biomass separation of *L. paracasei* (0.11).

3.3.4.Acidification activity of *Lactobacillus paracasei*

3.3.4.1.Acidification Activity

Measurement of the pH of media inoculated with *L. paracasei* for 26 h showed a decline in pH from 5.6 (at zero time) to 4.7 (after 26 h) due to the production of lactic acid (Figure 3). *L. paracasei* revealed a slow acidification pattern as it reached ΔpH ≥ 0.4 in >5 h **(Gomaa, 2010)**.

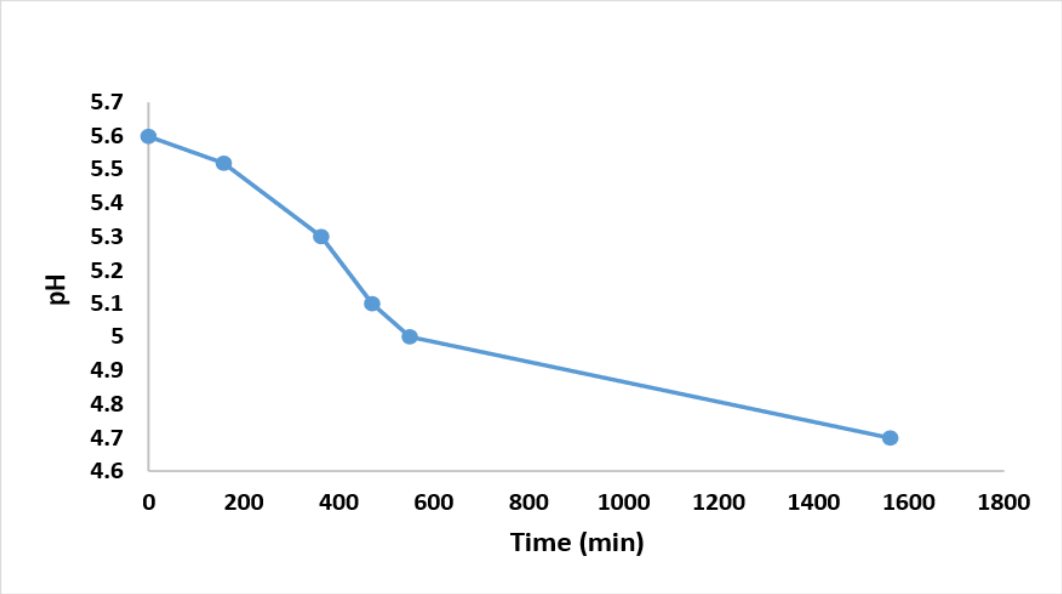

Figure 3: Acidity development of MRS broth inoculated with 1% *L. paracasei* and incubated at 37 °C/ 24 h.

3.3.4.2. Change in pH through 24 hours

The overall acidification activity was determined by calculating ΔpH (pH at zero-time - pH at end of growth). The Δ pH of *Lactobacillus paracasei* was 0.9 after 24 h, reflecting its poor acidification activity, as it achieved a final pH value of < 2 units **(Gomaa, 2010)**.

3.3.4.3.Exopolysaccharides production

Exopolysaccharide production trails were performed with two different carbon sources, sucrose (100 g/L) and glucose (20 g/L), at two different incubation periods (2 and 3 days). The amount of EPS was determined by measuring the final dry weight **(Savadogo *et al.*, 2004)**. The incubation period and carbon source significantly affect EPS production. The final EPS dry weight of *L. paracasei* cultured with glucose 20 g/L for 2 and 3 days was 0.1 ± 0.03 and 0.2 ± 0.02 g/L, respectively. In contrast, the final EPS dry weight of *L. paracasei* cultured with sucrose 100 g/L for 2 and 3 days was 0.24 ± 0.03 and 0.7 ± 0.01 g/L, respectively. Accordingly, we observed an increase in the amount of exopolysaccharides when the incubation time was prolonged. Besides, sucrose 100 g/L was the best carbon source for *L. paracasei*; it significantly increased EPS production by almost 3.5-fold compared with glucose 20 g/L (Figure 4). We obtained higher EPS values than those reported by Shankar et al. **(2021)** and Bengoa *et al.* **(2020)**, 258 mg/100 mL and 130-160 mg/L, respectively. Similarly, Fuso et al. **(2023)** obtained a close amount of EPS when they used sucrose as a carbon source for *L. paracasei 2333*. However, glucose was better than sucrose, resulting in nearly 1.5 g/L EPS **(Fuso *et al.*, 2023)**.

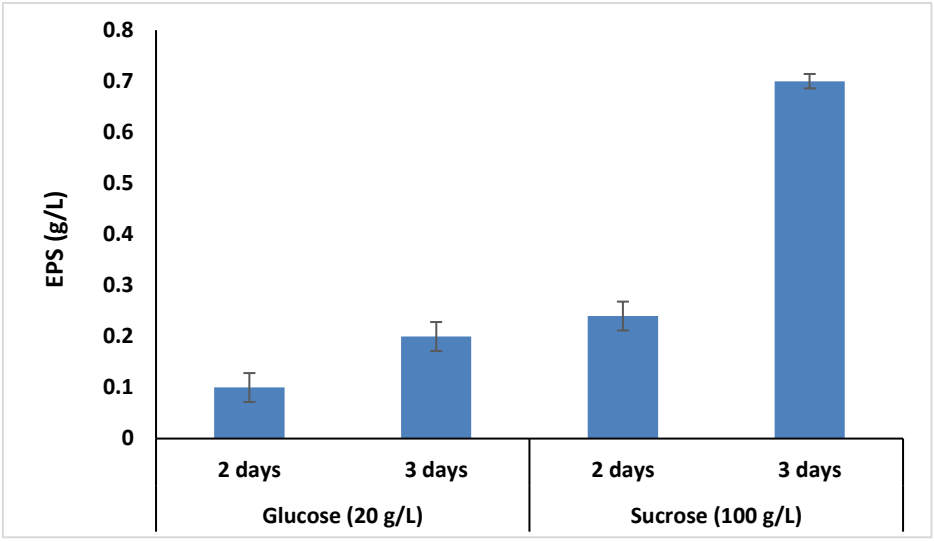

Figure 4: Exopolysaccharides production in g/L by *Lactobacillus paracasie* using glucose 20g/L and sucrose 100 g/L as a carbon source for 2 and 3 days.

3.5.FT-IR spectra Analysis of *Lactobacillus paracasei*

The IR spectra of the EPS of *L. paracasei* show different stretching vibrations (Figure 5). In the functional region, a stretching vibration of an OH group was observed at 3453cm^{-1}, characterizing the presence of a polysaccharide **(Xiao *et al.*, 2023)**. In addition, the stretching of an NH_2 group with a hydrocarbon was signalled at 1653 cm^{-1}, reflecting the presence of glucosamine in the purified EPS **(Krishnaveni and Ragunathan, 2015)**. In the fingerprint region, a weak stretching was detected at 1230 cm^{-1}, indicating the possible presence of sulfated groups **(Wang *et al.*, 2014)**. Moreover, the band at 1064 cm^{-1} corresponds to fructose **(Černá *et al.*, 2003)**. Accordingly, the sugar composition of the purified exopolysaccharide extracted from *Lactobacillus paracasei* contains fructose and glucosamine which means that this exopolysaccharide is a heteropolysaccharide.

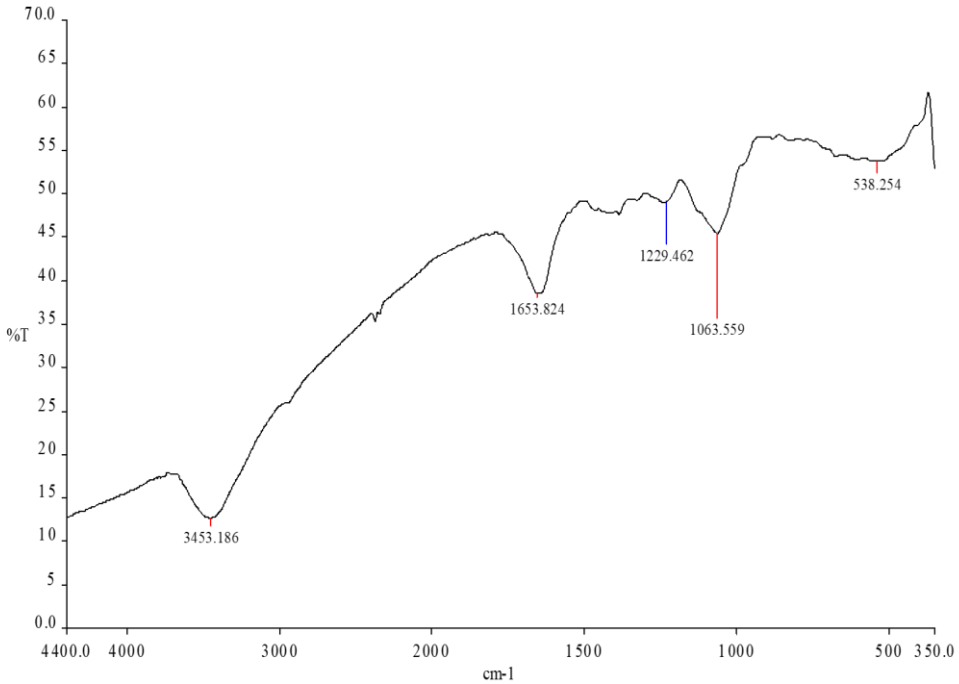

Figure 5: Fourier- transformed infrared spectra of purified exopolysaccharide produced by *Lactobacillus paracasei*

3.6.Application of EPS producer *Lactobacillus paracasei* in yoghurt

3.6.1.Physical properties of yoghurt types

The final pH values of all yoghurt trails (S0, C0S, and COM) prepared with skim milk were relatively close ($p > 0.05$), ranging from 4.00 to 4.04. In contrast, the yoghurt trials with whole milk (S3, C3S and C3M) had different pH values than the yoghurt made with skim milk ($p < 0.05$); the pH of C3S was the lowest (3.86), while C3M had the highest pH (4.20). The obtained result denotes that pH is affected by the strain and fat content of milk. Viscosity was also affected by strain and fat content: yoghurt with the standard starter culture and *L. paracasei* had the highest viscosity regardless of fat content ($p < 0.05$). Using the standard starter resulted in a higher viscosity in the whole fat yoghurt (4433.00 mPa.S) than that of the skimmed yoghurt (1193.00 mPa.S). Generally, yoghurt made from whole milk has a viscosity of 2340 – 2800 mPa.S **(Hanif *et al.*, 2012)**. The obtained result reveals that fat content significantly affects the viscosity of yoghurt. On the other hand, the viscosity of yoghurt decreased almost 25-fold when only *L. paracasei* was used, reaching 105.60 ± 0.85 and 113.70 ± 1.84 for skimmed and full-fat yoghurt. Interestingly, yoghurt with the standard starter culture and *L. paracasei* from skim and whole milk had viscosity values of 5155.20 ± 4.53 and 5264.30 ± 2.40 mPa.S, respectively. The obtained result indicates that adding *L. paracasei* to the starter culture improved the yoghurt curd compared to the exclusive use of the yoghurt starter culture, which can be attributed to the production of EPS.

Table 3: Physical properties of yoghurt types

Trail	pH	Viscosity (mPa.S)
S0	4.00 ± 0.03 ^{c}	1193.00 ± 4.24 ^{d}
C0S	4.04 ± 0.01 ^{bc}	105.60 ± 0.85 ^{c}
C0M	4.01 ± 0.01 ^{c}	5155.20 ± 4.53 ^{b}
S3	4.10 ± 0.04 ^{b}	4433.00 ± 4.24 ^{c}
C3S	3.86 ± 0.06 ^{d}	113.70 ± 1.84 ^{c}
C3M	4.20 ± 0.03 ^{a}	5264.30 ± 2.40 ^{a}

- Data are presented as means ± SD
- Means in the same column with different superscripts are statistically different ($p < 0.05$)
- S0, Skimmed milk with yoghurt standard starter culture; C0S, Skimmed milk with *Lactobacillus paracasei*; C0M, Skimmed milk with *Lactobacillus paracasei* and yoghurt starter; S3, Full cream with yoghurt standard starter culture; C3S, Full cream with *Lactobacillus paracasei*; C3M, Full cream with *Lactobacillus paracasei* and yoghurt starter.

3.6.2.Sensory evaluation of yoghurt types

The standard trail of full-fat yoghurt with yoghurt starter culture shows a strong yoghurt odour, thick appearance, strong creamy flavour, and strong light mouthfeel. In contrast, for the skim milk yoghurt with a standard starter culture, the panellists noted a strong sour milk odour, soft appearance, strong sour milk flavour, and a very light mouthfeel (Figure 6). The use of *Lactobacillus paracasei* showed a very strong cheesy odour, good thick appearance, strong acid flavour and thick mouthfeel in skim milk yoghurt, while a good cheese odour, very strong thick appearance, very strong acid flavour and strong thick mouthfeel were noted in full-fat yoghurt. Consistent with the physical characteristics (Table 3), the trials made from skim and full-fat milk using *Lactobacillus paracasei* with yoghurt starter show a very strong yoghurt odour, thick appearance, good creamy flavour, and thick mouthfeel. As noticed, adding *Lactobacillus paracasei* to the standard starter culture resulted in a fat mouthfeel, denoting that *Lactobacillus paracasei* was able to produce compounds that affect fat mouthfeel.

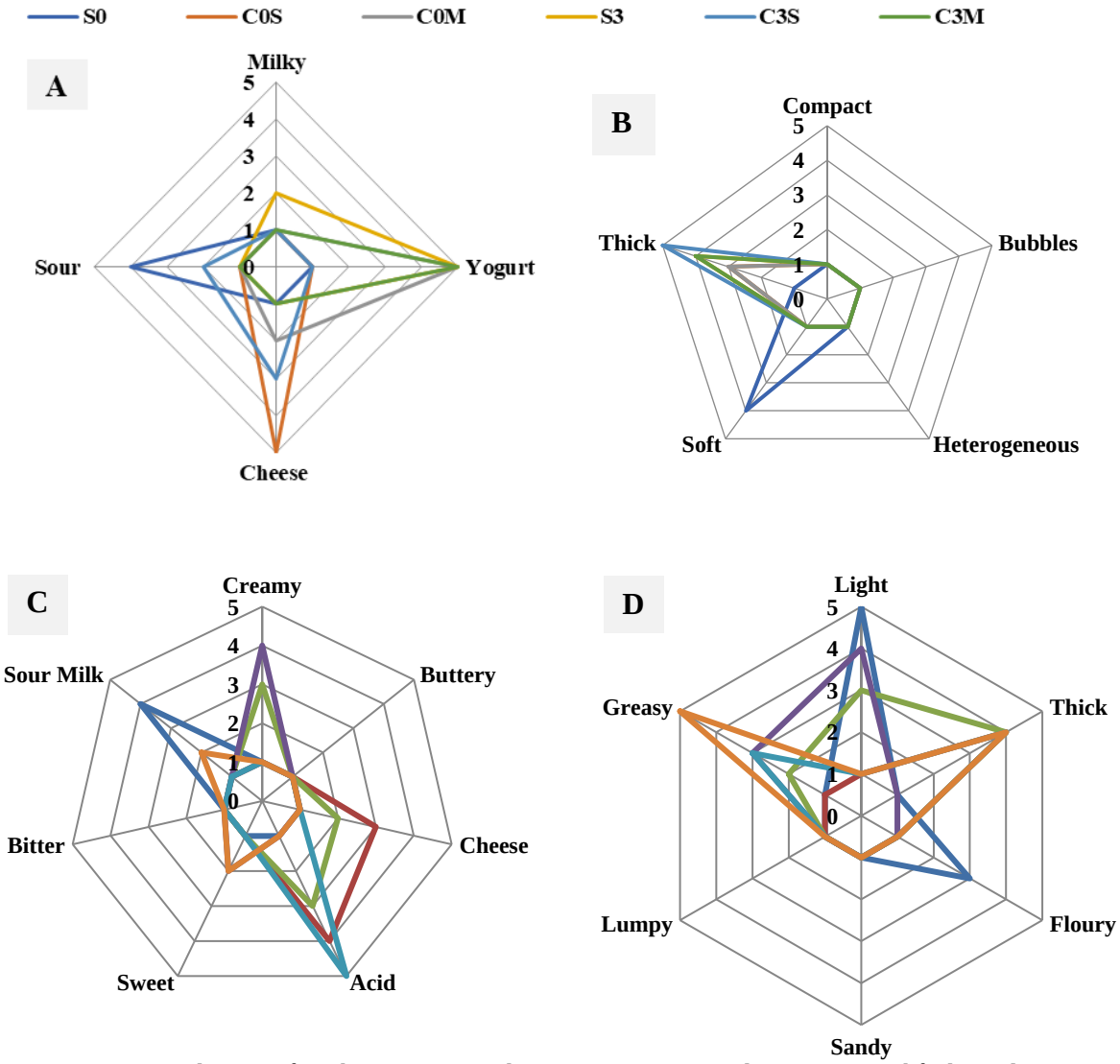

Figure 6: Sensory evaluation of yoghurt types: A Odor, B Appearance, C Flavour, D Mouthfeel. Trials: S0 Skimmed milk with yoghurt standard starter culture; C0S Skimmed milk with *Lactobacillus paracasei*; C0M Skimmed milk with *Lactobacillus paracasei* and yoghurt starter; S3 Full cream milk with yoghurt standard starter culture; C3S Full cream milk with *Lactobacillus paracasei*; C3M Full cream milk with *Lactobacillus paracasei* and yoghurt starter

4.CONCLUSION

The study showed that the fortification with exopolysaccharide-producing bacteria in foods improved the mouthfeel, appearance, and textural properties of yoghurt. Exopolysaccharide-producing bacteria act as a fat substitute in yoghurt made from skim milk, giving these products the same fatty mouthfeel as products made from whole milk. It is recommended to add *Lactobacillus paracasei* to low-fat yoghurt, as these bacteria act as a fat substitute.

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الملخص العربي

عزل وتوصيف سلالة *Lactobacillus paracasei* المنتجة لعديد السكريات الخارجية وتأثيرها على خصائص الزبادي

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في العقد الماضي، حظيت عديد السكريات الخارجية الميكروبية باهتمام متزايد، وقد ثبت أن العديد من السلالات الميكروبية يمكن أن تنتج تلك المركبات بتركيبات ووظائف مختلفة. تمت دراسة *Lactobacillus paracasei* المعزولة والمعرفة لقدرتها على إنتاج عديد السكريات الخارجية(EPS) . بالإضافة إلى ذلك، تم دراسة تأثير مصادر الكربون على إنتاجEPS . تمت دراسة تأثير *Lactobacillus paracasei* على اللزوجة والخصائص الحسية للزبادي المصنوع من الحليب كامل الدسم والحليب الخالي من الدسم. كان لدى *Lactobacillus paracasei* *قدرة انتاج* كتلة حيوية ممتازة (4.4 جم / لتر) مع فصل جيد للكتلة الحيوية (0.11). أعطى استخدام السكروز 100 جم/لتر كمصدر كربوني لمدة 3 أيام أفضل محتوى EPS(0.7 جم / لتر). أظهرت أطياف الأشعة تحت الحمراء أن EPS الذي تنتجه *Lactobacillus paracasei* كان ضمن نطاق عديد السكاريد غير المتجانسة. أدى التدعيم باستخدام *Lactobacillus paracasei* في إنتاج الزبادي من الحليب الخالي من الدسم إلى لزوجة مماثلة نسبيًا (4.53 mPa.S ± 5155.20) للزبادي كامل الدسم ± 5264.30) (2.40 mPa.S، مما يعكس التحسن في الخصائص الحسية للزبادي من الحليب الخالي من الدسم ، وخاصةً الإحساس بالفم. وبالتالي ، يوصى بتدعيم الزبادي قليل الدسم ببكتريا *Lactobacillus paracasei* .

الكلمات الرئيسية: عديد السكريات الخارجية. زبادي قليل الدسم .FTIR لزوجة. الخصائص الحسية.