Agro-industrial Residues: Low-cost Biomass for the Production of Bioactive Extracellular Polysaccharides by *Lactobacillus plantarum* in Solid State Fermentation

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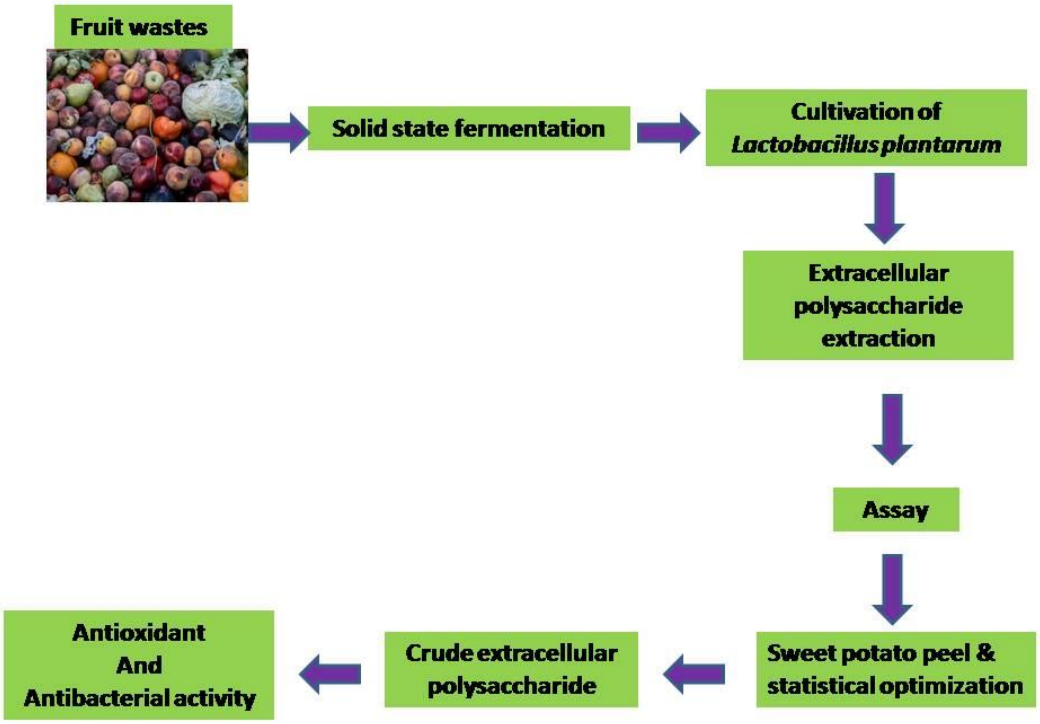
GRAPHICAL ABSTRACT



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Agro-residues, such as apple peel, banana peel, orange peel, tapioca peel, and sweet potato peel powders, were screened for the biosynthesis of bioactive extracellular polysaccharide (EPS) in solid-state fermentation. *Lactobacillus plantarum* utilized sweet potato peel and improved EPS production compared with other wastes, so it was used for optimization studies. Compared with the unoptimized medium, the response surface methodology improved EPS production twofold during solid-state fermentation. In the central composite design, the experimental model was significant ($p<0.01$), and the selected variables (moisture, sucrose and yeast extract) significantly influenced EPS production. The maximum yield was 59.1 mg/g with 64.5% moisture content, 5.74% sucrose, and 1.39% yeast extract. In addition, the extracted EPS exhibited strong antioxidant activity, and it scavenged ABTS, DPPH, and hydroxyl radicals in a concentration-dependent manner. The antibacterial activity of EPS was greatest against *Staphylococcus aureus*, and the corresponding zone of inhibition was18±1 mm, followed by *Pseudomonas aeruginosa*(14±1 mm).The results showed that sweet potato peels can be used as a cheap substrate for EPS production in solid-state fermentation.

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Keywords: Agro-wastes; Sweet potato; Valorization; Solid-state fermentation; Extracellular polysaccharide

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INTRODUCTION

Bacterial exopolysaccharides (EPSs) are extracellular carbohydrate-based biopolymers secreted by several microbes such as fungi, microalgae, bacteria, and yeast (Rana *et al*. 2020). Bacterial EPSs are produced through various pathways. They are either in free form into the medium of cell growth (slime EPSs) or in bound form around the cell surface (capsular EPSs). Once EPSs are released in the medium, EPSs bind with other macromolecules such as lipids, proteins, and DNA to form an extracellular matrix. The

term EPS is used to illustrate polysaccharides that represent about 40 to 95% of the extracellular polymeric substances produced by bacteria into the surrounding ecosystem (Xu *et al*. 2019). Bacterial EPSs are produced by various bacteria and have wide applications in various industries, including the cosmetics, textile, pharmaceutical, medical, food and petrochemical industries (Bueno *et al*. 2013; Zhao *et al*. 2018). Only a few bacterial EPSs, such as dextran, xanthan, curdlan, and gellan, have been commercialized, and a few bacterial EPSs have failed to enter the commercial market because of their high production cost (Freitas *et al*. 2011; Mouro *et al.* 2024). Therefore, identifying new bacteria with the high EPS yield is necessary. Low-cost substrates such as molasses, cheese whey, glycerol, and saline byproducts contain significant quantities of carbohydrates that are widely used for the production of EPS by microorganisms (Niknezhad *et al*. 2015, 2016; Imran *et al*. 2016; He *et al*. 2018; Rath *et al*. 2022).

Extracellular polysaccharides are secondary metabolites synthesized by various bacteria, such as *Bacillus* and *Lactobacillus*; they are produced in the extracellular space (Bachtarzi *et al*. 2020; Cao *et al*. 2020). Postbiotics are a product of microorganisms or their components that have health benefits. Compared with other reported postbiotics, polysaccharides have several advantages, including cost-effectiveness, simple extraction procedures, low-cost media, and the fact that the structure of EPS depends on the type of microorganism and the composition of the medium (Daba *et al*. 2021). EPS-producing strains in different culture media can produce EPS with different structures. These findings provide a quantitative basis for screening EPS as a means to select those having several advantages. Compared with xanthan gum, the supplementation of EPS with low-fat contents may increase the stability and rheological properties of the final product (Yalmanci *et al*. 2023) and improve emulsification activity (Sran *et al*. 2019; Nguyen *et al*. 2024). Certain extracellular polysaccharides are used also as antioxidants in the food industry (Zhang *et al*. 2013). Considering their physical and chemical structures, these polysaccharides can be applied for suspending, thickening, and gelling applications. In addition to these prebiotic properties, these polysaccharides present additional features, such as antimicrobial, antioxidant, blood cholesterol-lowering, and anticancer activities (Upadhyaya *et al*. 2025).

Sweet potato (*Ipomoea batatas* Lam.) is cultivated in various countries, and potato processing industries generate potato residues and wastewater. China is one of the largest producers of sweet potato, producing more than 52 million tonnes in 2019 (FAO STAT 2019). Approximately 4.5 to 5tonnes of potato residue are generated for every tonne of the final starch product. Moreover, <10% of potato residues are utilized for the preparation of animal feed, and not all of the remaining amounts are utilized. Unutilized residues cause environmental pollution. Sweet potato residues consist of protein (5.5%), dietary fibre (21.4%), and starch (52.0%). The dried residues include cellulose (31.2%), lignin (16.8%), pectin (15.6%), and hemicelluloses (11.4%) (Mei *et al*. 2010). These nutrient-rich agro-residues are valuable for the production of lipopeptides and poly-γ-glutamic acid (Wang *et al*. 2008), hydrogen (Yokoi *et al*. 2001), emulsifiers (Xie *et al*. 2020), and cellulose nanocrystals (Lu *et al*. 2013). Mathematical modelling of the optimization process is a useful strategy for understanding EPS bioprocess conditions and determining the most significant variables. Response surface methodology (RSM) is a prominent statistical and mathematical technique that is used to optimize the bioprocess conditions of variables for the required response and analyse the significance of the selected variables. This RSM can be performed even in the presence of multiple interactions, which potentially affect the yield of the final product (Vijayaraghavan and Vincent 2016). Response surface

methodology has been used for optimizing bioprocess conditions and analysing the stoichiometric relationships among variables, namely, substrate utilization, cell growth, and product formation. Central composite design is one of the optimization methods widely used in bioprocess development. This approach provides more information on the interactive effects of the variables for bioprocess design and optimization (Bekar and Kamiloglu 2024).

The yield of extracellular polysaccharides can be improved by optimizing culture conditions (Biji *et al*. 2016; El-Sheikh *et al*. 2020). In this study, the crude extracellular polysaccharide obtained from the optimized medium using sweet potato peel was investigated for its antibacterial and antioxidant activities. Response surface methodology was used to improve the culture conditions of *Lactobacillus plantarum* in solid-state fermentation *via* agro-residues.

EXPERIMENTAL

Microorganisms and Culture Conditions

Lactobacillus plantarum (MTCC9510) was obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), India. It was cultivated in De Mann, Rogosa Sharpe (MRS) agar medium with the following composition (g/L): yeast extract, 5; beef extract, 10; peptone, 10; Tween 80 (polysorbate 80), 1; glucose, 20; magnesium sulfate, 0.10; sodium acetate, 5; di-potassium phosphate, 2; and agar–agar, 10. The pH of the medium was tested and adjusted to 6.2 ± 0.2. The culture was incubated for 24 h at 37 °C. The colonies were isolated and used for the preparation of the inoculum.

Chemicals

Yeast extract, beef extract, peptone, polysorbate 80, glucose, magnesium sulfate, sodium acetate, di-potassium phosphate, and agar–agar were purchased from Himedia, India. The other chemicals were either analytical grade or ACS grade. The solvents were purchased from Merck, Germany, with >99% purity.

Agro-residues

Apple peel, banana peel, orange peel, tapioca peel, and sweet potato peel powders were collected from a local market in Kanniyakumari, India. It was dried for five days under sunlight and individually ground into powder *via* a mechanical grinder. The fine powder was sieved, and a particle size between 0.8 and 1.2 mm was used as the substrate.

Solid-state Fermentation (SSF)

Agro-industrial residues (5 g) were mixed with 3 mL of 0.1 M sodium phosphate buffer (pH 6.5)to maintain 60% initial moisture content. The mixture was sterilized at 15 psi for 30 min and cooled. It was inoculated with 5% inoculum (1×10^7 colony forming units (CFU)/mL) and incubated for four days at 37 °C. Uninoculated medium was used as a control. All experiments were performed in triplicate, and the mean value was used for data processing (Marraiki *et al*. 2020).

Extraction of Extracellular Polysaccharide and Assay

The fermented solid medium was mixed with 25 mL of double distilled water and vortexed for 20 min, after which the extracellular matrix was removed from the surface of the agroresidues. The sample was filtered and centrifuged at 5000×g for 10 min. It was stored at 4 °C for further studies(Liu *et al*. 2022).

Extrapolysaccharide Assay

The supernatant was mixed with three volumes of ethanol (95%) and stirred vigorously. The mixture was incubated for 24 h at 4 °C, after which the extracellular polysaccharide was precipitated. The EPS precipitate was dialyzed against double distilled water for 24 h at 4 °C (three changes). The dialyzed sample was lyophilized, and the EPS powder was used for the assay. The amount of EPS was determined *via* the phenol–sulfate acid method with glucose as a standard (Dubois *et al*. 1956). Briefly, 0.1 mL of the sample (10 mg/mL) was mixed with 1 mL of phenol (5%) and 2.5 mL of 98% sulfuric acid. The mixture was incubated for 20 min at room temperature (28±1 °C), and the absorbance was read at 490 nm. Glucose was used as the standard (10 to 100 μg).

Screening of the Process Variable by a Two-level Full Factorial Design

A two-level full factorial design was used to screen the significant media components from the five selected variables (2^5 level).This is an economical factorial design that analyses the significance level at two different levels (low and high). This model shows the interactions among the analysed factors that influence EPS production. A total of 32 experimental runs were performed to analyse the effects of five selected variables. Five variables, sweet potato, ammonium sulfate, yeast extract, sucrose, and glucose, were used as variables. Table 1 shows the factors and levels for EPS production, and fermentation experiments were performed for 96 h at 37 °C under static conditions (Al Farraj *et al*. 2020).

Central Composite Design and Response Surface Methodology

A central composite design was used to determine the optimum concentrations of the variables (Kalaiyarasi *et al*. 2020). The other components were maintained at moderate levels. Each variable in the experimental design was analysed at five different levels. The experimental design included 20 runs, and solid-state fermentation was performed as described earlier in separate experiments. The amount of EPS produced was calculated after the EPS assay, and the result was expressed as the response (Y). An analysis of variance (ANOVA) was used to analyse the significance level of the designed model. Three-dimensional (3-D) response surface graphs were plotted *via* Design Expert software (version 8.0), and the interactive effects between variables were analysed.

Antioxidant Activity

DPPH-free radical scavenging activity

DPPH radical-scavenging activity was determined as described previously (Cheng *et al*. 2019). Briefly, various concentrations (0.25, 0.5, 0.75, 1.0, and 1.25 mg/mL) of EPS were allowed to react with 0.2 mm DPPH solution (2 mL), which was prepared in methanol. The mixture was further incubated for 30 min in the dark. The absorbance of each sample was read at 517 nm. DPPH was not included in the blank vials. For the control, EPS was not added, and ascorbic acid was used as the positive control. The DPPH scavenging activity of each sample was analysed *via* the following formula,

$$\text{Scavenging activity (\%)} = \frac{A_0-(A_1-A)}{A_0} \times 100\% \quad (1)$$

where A is the absorbance of the blank sample, A_0 is the absorbance of the control sample, and A_1 is the absorbance of the test.

ABTS radical-scavenging assay

The ABTS radical scavenging assay was performed as described previously by Wang *et al*. (2017). Briefly, an ABTS solution was prepared at a 7 mM concentration, and 2.4 mm potassium persulfate was prepared separately. To 1 mL of ABTS solution, 1 mL of potassium persulfate solution was added, and the mixture was incubated for 16 h in the dark. EPS (0.2 mL) was then added at various concentrations (0.25, 0.5, 0.75, 1.0, and 1.25 mg/mL), mixed with 1.8 mL of the ABTS solution and incubated in the dark for 10 min. The absorbance of the sample was read at 734 nm. To the blank, ABTS was not added, whereas to the control, EPS was not incorporated. Ascorbic acid was prepared at various concentrations, and the ABTS radical scavenging activity was determined using Eq. 1.

Hydroxyl radical-scavenging assay

In this method, EPS (0.5 mL) was prepared at various concentrations (0.25, 0.5, 0.75, 1.0, and 1.25 mg/mL) and mixed with 1.8 mmol salicylic acid (2 mL), .8 mmolFeSO4 (0.25 mL), and 6 mmol H_2O_2 (1 mL). The mixture was incubated at 37 °C for 30 min, and the supernatant was collected after centrifugation at 5000 rpm for 10 min. The absorbance of the sample was read at 510 nm. Salicylic acid was not added to the blank group, and EPS was not added to the control group (Wang *et al*. 2017). The hydroxyl radical-scavenging activity of the EPS was determined by use of Eq. 1.

Antibacterial Activity of EPS Isolated from *Lactobacillus plantarum* (MTCC9510)

The antibacterial activity of the EPS was tested against four indicator bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus cereus*. The antibacterial test was carried out *via* the agar disc diffusion assay method according to the manufacturer's instructions. Briefly, Mueller–Hinton agar plates (Himedia, Mumbai, India) containing each indicator organism (0.1 mL) (10^7 CFU/mL) were spread on plates. A disc (6 mm diameter) containing 5 mg EPS was subsequently loaded, placed on the plates, and allowed to diffuse for 2 h at 4 °C. The mixture was incubated for 24 h, after which the plates were tested. The diameter of the clear zone was observed, and chloramphenicol was used as the positive control (Al-Ansari *et al*. 2021).

RESULTS AND DISCUSSION

Agro-residues: A Low-cost Substrate for Extracellular Polysaccharide Production

Substrates such as apple peels, banana peels, orange peels, tapioca peels, and sweet potato peel powder were utilized for the production of extracellular polysaccharides in solid-state fermentation (Fig. 1). The selected substrates are agro-residues or leftovers chosen on the basis of their ability to produce EPS in microbial fermentation. These starch-rich substrates, especially tapioca peel and sweet potato, are suitable for the production of

EPS by *L. plantarum* to promote bacterial growth and EPS production. EPS production was 20.5±4.1 mg/g in the apple peel substrate and reached a maximum in the sweet potato substrate (34.5±9.3 mg/g), followed by the orange peel substrate (34.5±3.3 mg/g). Apple pomace has previously been considered a substrate for the production of SSF products because of its increased amount of crude fibre, pectin, and various minerals, including Fe, Mg, K, and Mn (Shalini and Gupta 2010). *Xanthomonas* strains produce xanthan gum *via* solid-state fermentation using agroresidues, including pineapple peel, sugarcane peel, and sugarcane bagasse (Itunu *et al.* 2025). *Lactobacillus confuses* are cultivated *via* solid-state fermentation and submerged fermentation, and EPS production has been reported (Seesuriyachan *et al*. 2010). An EPS-producing *Bacillus licheniformis* UD061 strain was cultivated in SSF using agro-residues, and maize cobs were reported as an ideal substrate for EPS production (Fang *et al*. 2013).

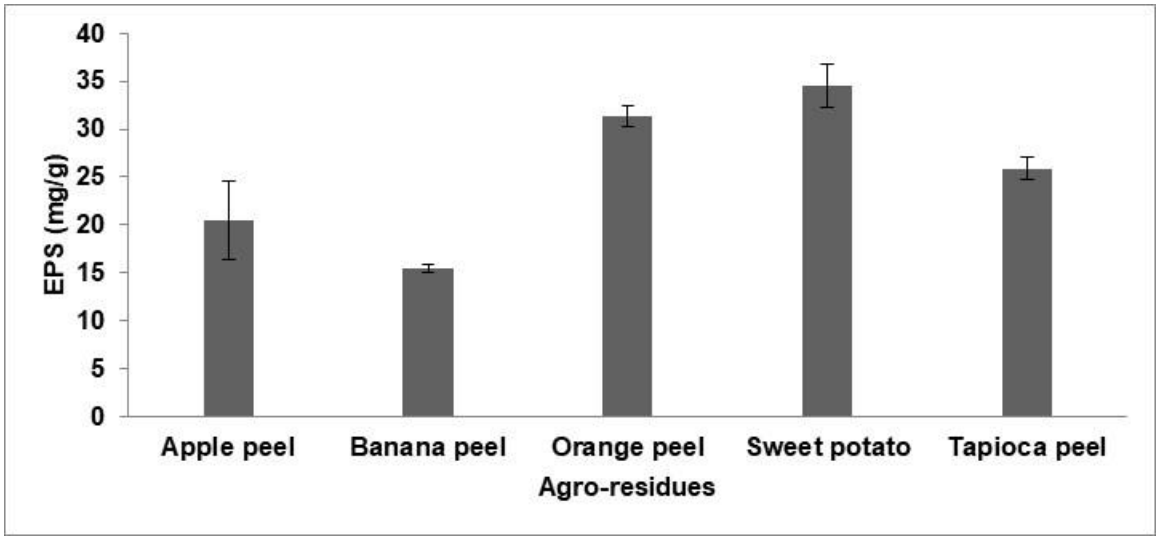

Fig. 1. Production of extracellular polysaccharide by *Lactobacillus plantarum* in solid-state fermentation using agro-residues

Screening of Variables *via* a Two-level Full Factorial Design

The screening experiments were performed to evaluate the effects of five factors: moisture content of the medium, pH of the medium, sucrose, yeast extract, and ammonium sulfate. A two-level full factorial design was used, and the results of the full factorial design are described in Table 1.

The biosynthesis of EPSs, described as mg/g, varied significantly from 1.3 to 37.8 mg/g. A decreased level of EPS production was observed at the lowest moisture, sucrose, yeast extract, and ammonium sulfate contents. As shown in Table 2, in addition to pH, other variables, such as moisture content, sucrose, yeast extract, and ammonium sulfate, significantly improved EPS production. The main effect of these selected factors was positive, and pH had an insignificant effect on the production of EPS. The effects of moisture content, sucrose, yeast extract, and ammonium sulfate on EPS production were analysed *via* multiple regression. The results of the F test for variations between the responses of two-level full factorial experiments revealed that the variation was significant ($p<0.001$).

The optimal combination of physical and nutritional factors varies on the basis of the bacteria being cultivated for the production of EPS and the type of fermentation. Gu*et al*. (2017) used a fractional factorial design to screen the variables, and KCl and $MgSO_4$ significantly improved EPS production. The optimum nutrient compositions were 0.50% peptone, 0.50% KCl, 5.80% $MgSO_4$, 0.30% yeast extract, 1.00% sodium citrate, and 0.50% casein hydrolysate. In EPS production, pH, incubation time, temperature, and nitrogen and carbon sources were significantly influenced, whereas salts or vitamins were considered the least influential factors. The supplemented nitrogen sources influenced EPS production. Inorganic nitrogen sources such as ammonium sulfate, ammonium chloride, potassium nitrate, sodium nitrate, diammonium oxalate, and urea are commonly screened for EPS production. In this study, only organic sources were used for the production of EPS in SSF.

Table 1. Range of Five Selected Variables and Results for a Two-level Full Factorial Design

Run	pH (A)	Moisture (B) %	Sucrose (C)%	Y. extract (D) %	A. sulfate (E) %	EPS (mg/g)
1	5.5	40	0.5	0.1	1	2.03
2	7.5	80	5	0.1	0.1	8.05
3	5.5	40	0.5	1	0.1	15.43
4	5.5	80	5	0.1	1	8.3
5	7.5	80	5	1	0.1	37.8
6	7.5	40	5	1	1	15.4
7	5.5	80	5	1	0.1	13.9
8	5.5	80	0.5	1	0.1	10.3
9	7.5	80	5	1	1	35.3
10	5.5	40	5	1	1	34.2
11	7.5	80	0.5	1	1	21.1
12	7.5	40	5	0.1	0.1	10.1
13	5.5	40	0.5	1	1	25.4
14	5.5	40	5	1	0.1	12.15
15	7.5	80	5	0.1	1	13.3
16	5.5	40	5	0.1	1	8.11
17	5.5	80	0.5	1	1	12.12
18	5.5	40	0.5	0.1	0.1	2.07
19	7.5	80	0.5	0.1	0.1	14.19
20	5.5	80	5	0.1	0.1	8.7
21	5.5	80	0.5	0.1	1	11.9
22	7.5	40	0.5	1	0.1	8.1
23	7.5	40	5	0.1	1	7.9
24	7.5	40	0.5	0.1	1	2.9
25	7.5	40	0.5	1	1	12.3
26	7.5	40	0.5	0.1	0.1	1.3
27	7.5	40	5	1	0.1	10.2
28	5.5	80	0.5	0.1	0.1	18.3
29	5.5	40	5	0.1	0.1	10.1
30	7.5	80	0.5	1	0.1	4.05
31	5.5	80	5	1	1	28.3
32	7.5	80	0.5	0.1	1	12.3

Table 2. Analysis of Variance for the Influence of Variables on EPS Production in a Two-Level Full Factorial Design

Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob> F
Model	2748.932	21	130.9015	43.89198	< 0.0001
B-Moisture	201.1015	1	201.1015	67.43041	< 0.0001
C-Sucrose	242.11	1	242.11	81.18078	< 0.0001
D-Yeast extract	765.3828	1	765.3828	256.637	< 0.0001
E-Ammonium sulfate	136.6205	1	136.6205	45.80957	< 0.0001
AB	178.4161	1	178.4161	59.82386	< 0.0001
AC	39.605	1	39.605	13.27977	0.0045
BD	13.57205	1	13.57205	4.550781	0.0587
CD	148.2642	1	148.2642	49.71378	< 0.0001
DE	191.3946	1	191.3946	64.17564	< 0.0001
ABC	42.45811	1	42.45811	14.23643	0.0036
ABD	171.4026	1	171.4026	57.47221	< 0.0001
ABE	27.5282	1	27.5282	9.230353	0.0125
ACD	12.62531	1	12.62531	4.233335	0.0667
ACE	60.2802	1	60.2802	20.21227	0.0012
ADE	40.23045	1	40.23045	13.48949	0.0043
BCD	333.2071	1	333.2071	111.7261	< 0.0001
ABCD	12.65045	1	12.65045	4.241764	0.0664
ABCE	10.19261	1	10.19261	3.417637	0.0942
ACDE	56.44531	1	56.44531	18.92642	0.0014
BCDE	47.38511	1	47.38511	15.88848	0.0026
ABCDE	18.06005	1	18.06005	6.055631	0.0336
Residual	29.82356	10	2.982356		
Cor Total	2778.756	31			

Central Composite Design and Response Surface Methodology

Microbial EPS have various functions, including removal of pollutants from wastewater, bioflocculation, sedimentation, and activated sludge dewatering. However, several factors affect the commercialization of EPS because the production cost is relatively high (Patel and Prajapat 2013). The optimization method using a statistical approach reduced the number of experimental runs and helped to analyse the interactive effects between the variables (Hao *et al*. 2019). Extracellular polysaccharide production is affected by several environmental factors, including the pH and moisture content of the substrate in the SSF. In this study, variations in pH and moisture content influenced EPS production. In the present study, response surface methodology with a central composite design was used to analyse the optimal levels of three factors (moisture, sucrose, and yeast extract). The central composite design and the results are described in Table 3. EPS production ranged between 0.33 mg/g and 58.04 mg/g in the central composite design experiment. Analysis of variance was performed *via* the Design Expert software package.

As described in Table 4, the designed model was significant ($p<0.05$), and the nonsignificance of the lack of fit revealed that the determined model had a high quality of fit and was valid in the screened experimental range. The obtained p values of the quadratic terms of the variables revealed very good dependence of the results on the selected factors. The moisture content of the medium ($p<0.05$) and the supplemented yeast extract ($p<0.05$) significantly improved EPS production. The model F value of 6.72 implies that the designed model was significant. In this case, A, B, C, A^2, and B^2 are significant model terms.

Table 3. Central Composite Design of Three Variables and Five Different Levels and the Experimental Results

Run	Moisture (%)	Sucrose (%)	Yeast extract (%)	EPS (mg/g)
1	40	1	1.5	2.5
2	40	1	0.5	2.65
3	62.5	5.5	1	34.2
4	40	10	1.5	49.4
5	62.5	-2.07	1	0.33
6	62.5	5.5	0.159	12.84
7	85	10	0.5	45.9
8	62.5	5.5	1	44
9	62.5	5.5	1.84	58.04
10	24.7	5.5	1	0.48
11	62.5	13.1	1	50.7
12	85	1	1.5	31
13	62.5	5.5	1	54.91
14	62.5	5.5	1	47
15	62.5	5.5	1	48.3
16	85	10	1.5	31.8
17	85	1	0.5	12.01
18	62.5	5.5	1	50.3
19	40	10	0.5	13.2
20	100.3	5.5	1	25

Table 4. Analysis of Variance for Extracellular Polysaccharide Production in the Central Composite Design

Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob> F
Model	6769.262	9	752.1403	6.716946	0.0032
A-Moisture	649.7236	1	649.7236	5.80232	0.0368
B-Sucrose	2290.176	1	2290.176	20.45228	0.0011
C-Yeast extract	1001.618	1	1001.618	8.944891	0.0136
AB	64.7522	1	64.7522	0.578266	0.4645
AC	121.3682	1	121.3682	1.083872	0.3223
BC	1.32845	1	1.32845	0.011864	0.9154
A^2	2024.119	1	2024.119	18.07628	0.0017
B^2	775.2898	1	775.2898	6.923682	0.0251
C^2	210.9182	1	210.9182	1.883593	0.1999
Residual	1119.765	10	111.9765		
Lack of Fit	873.5812	5	174.7162	3.548488	0.0954
Pure Error	246.1841	5	49.23682		
Cor Total	7889.028	19			

The "lack-of-fit F value" of 3.55 implies that there was a 9.54% chance that a "lack-of-fit F value,"which could be attributed to noise. Central composite design is a suitable method for optimizing medium components in solid-state fermentation. It has been used for the production of enzymes, EPS and other biomolecules in solid-state fermentation. The optimum culture conditions for each organism are unique; hence, the optomization of any organism is warranted to improve yield. Saeed *et al*. (2023) used mango peel as an effective medium for the production of EPS, and process parameters such as incubation time, temperature, inoculum, pH, and agitation speed were optimized. A central composite design was used to optimize the variables, and the maximum production of EPS (0.717 g/L

mango peel hydrolysate) was obtained after incubation for 64 h at 35 °C and pH 7.5 and inoculation with 2% inoculum. In *Rhodotorula mucilaginosa*, EPS production was improved by optimizing culture conditions *via* a central composite design. A total of three factors were optimized (pH, sucrose, and ammonium sulfate), and nutrient factors (sucrose and ammonium sulfate) improved the EPS yield (Okoro *et al*. 2021).

The relationships between the selected variables (moisture, sucrose, and yeast extract) and the experimental levels of the selected factors are illustrated in 3D response surface graphs (Fig. 2). The response surface plots revealed interactions between each pair of factors (moisture and sucrose, moisture and yeast extract, and yeast extract and sucrose). The optimum level was analysed by observing the response surface plots and solving the regression equation. The maximum EPS production was achieved when the moisture, sucrose, and yeast extract contents were 64.5%, 5.74%, and 1.39%, respectively. EPS production was 59.1 mg/g, which was highly consistent with the predicted response (58.4 mg/g). Xanthan gum was produced from *Xanthomonas* spp., and the initial process parameters were optimized *via* a 2^3 full factorial central composite design. The supplemented sucrose, magnesium sulfate and dipotassium hydrogen phosphate significantly influenced xanthan gum production. The strain *Bacillus subtilis* ES used sucrose (4%), 0.002% Na_2SO4, and 0.1% $NaNO_3$ for xanthan gum production (Bashandy and Abd-Alla 2024). One of the main advantages of applying the RSM is that it can determine the effects of experimental variables more accurately than conventional methods, which involve the possible consequences of the selected variables. The interactions between the selected factors can be explained in 3D graphs, revealing the interactive effect between two variables and the middle level. Carbon and nitrogen sources play critical roles because these nutrients are directly involved in metabolic processes and cell proliferation (Lakra *et al*. 2024). The yield of EPS improved with increasing sucrose concentration, possibly because sucrose provided the required carbon source for *Lactobacillus plantarum*, resulting in a high yield of EPS. When the level of yeast extract and sucrose was above the optimum level, the yield of EPS decreased because an adequate supply of carbon could cause the production of lipids, which affects EPS production (Chen*et al*. 2024).

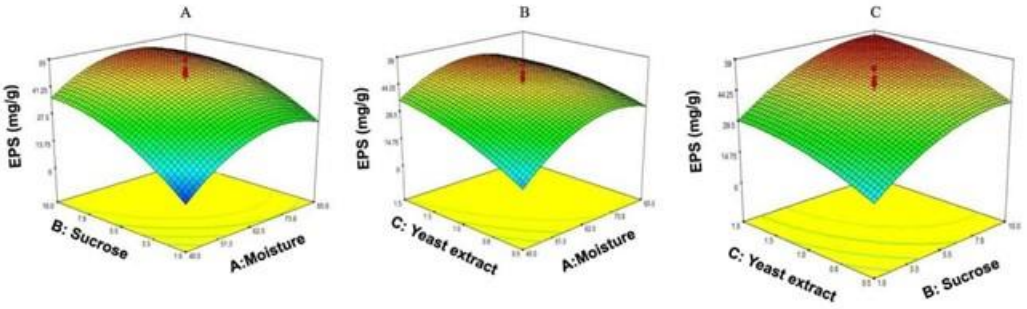

Fig. 2. 3D-response surface plot showing the effects of sucrose and moisture (A), yeast extract and moisture (B), and yeast extract and sucrose (C)

Antioxidant Activity

The DPPH radical-scavenging activity of EPS is illustrated in Fig. 3A. At concentrations ranging from 0.25 to 1.0 mg/mL, the radical scavenging activity increased significantly.

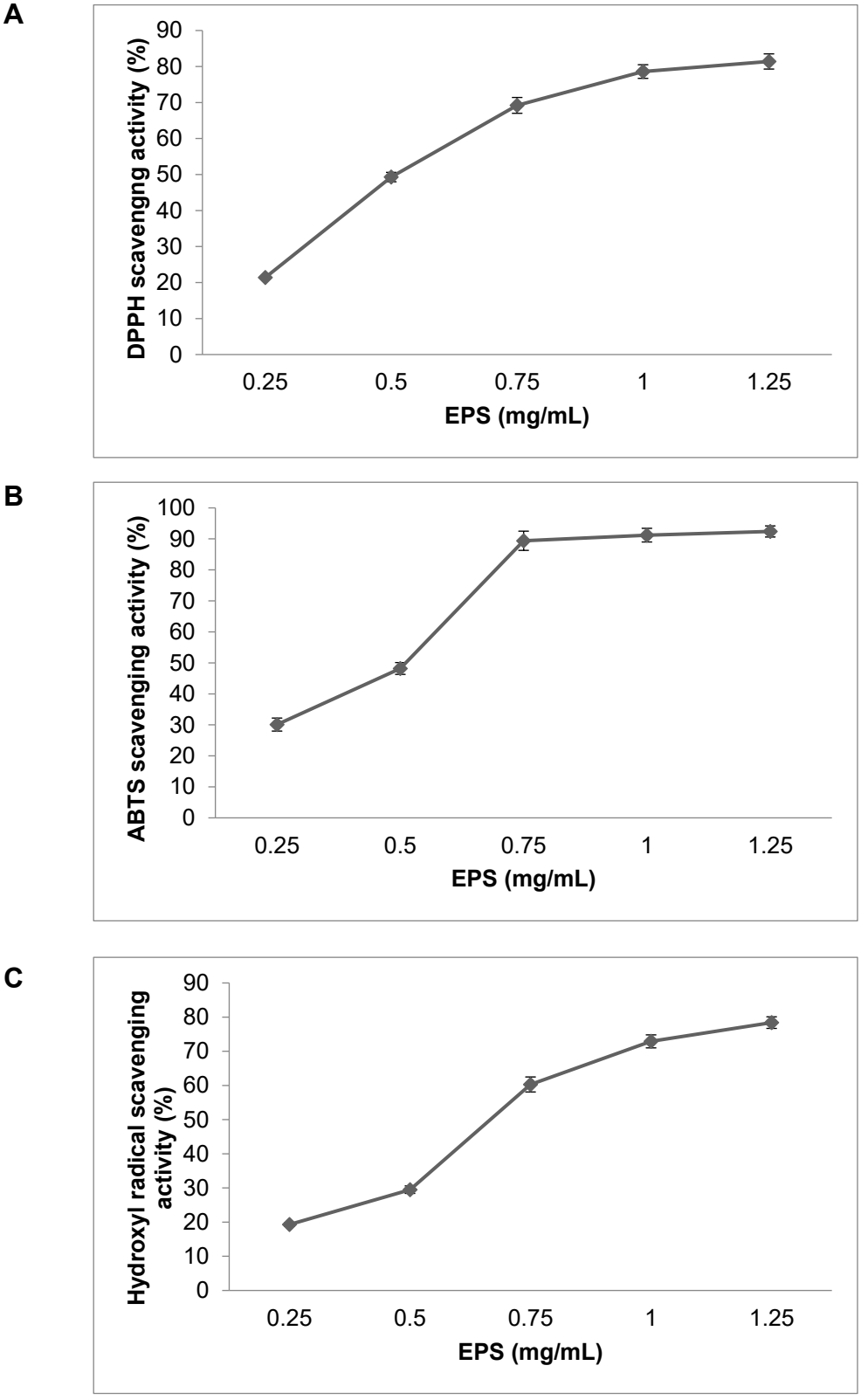

Fig. 3. Antioxidant activity of EPS isolated from *Lactobacillus plantarum* and cultivated in solid-state fermentation using sweet potato peels. (A) DPPH activity, (B) ABTS activity, and (C) hydroxyl radical scavenging activity

At the 0.25 mg/mLEPS concentration, the scavenging activity was 21.4±0.3 mg/mL, while the scavenging activity of EPS increased to 81.4±2.1 mg/mL at the 1 mg/mL EPS concentration. The ABTS scavenging activity of EPS ranged from 30.1±2.1% at 0.25 mg/mL, and it increased at a concentration of 1.25 mg/mL. No significant variation was observed between the 1.0 mg/mL EPS and the 1.25 mg/mL EPS (Fig. 3B). The hydroxyl radical-scavenging ability of EPS is illustrated in Fig. 3C. The hydroxyl radical scavenging activity of EPS was 19.3±0.5% at 0.25 mg/mL, and the scavenging ability of EPS increased slowly at higher concentrations (78.4±1.7%). As was discussed earlier, the bioactivity of EPS varies with culture conditions. In this study, the antioxidant activity was determined *via* three different methods to determine the antioxidant properties. Reactive oxygen species (ROS) have several functions in various cellular processes, including cell signalling, apoptosis, ion transport, and genetic regulation (Harman 1993).Although ROS participate in several cellular processes, an excess of RPS can lead to several harmful effects on the body. These include promoting the development of cancer, damaging DNA, and accelerating cellular degeneration, which can lead to the development of various diseases, including lung injury, inflammation, and cancer (Bjørn and Hasselbalch 2015; Espinosa *et al*. 2016). For this reason, it is important to search for novel antioxidants from biological sources. In this study, the antioxidant activity of the crude EPS isolated from *Lactobacillus plantarum* was tested *via* three different methods. The scavenging activity was dose-dependent, and the maximum activity was observed at higher concentrations. In *L. plantarum* KX041 EPS, a >80% scavenging efficacy was observed at 8 mg/mL EPS (Wang *et al*. 2017). In *Bacillus velezensis* SN-1, approximately 60% activity was observed at an EPS concentration of 8 mg/mL (Cao *et al*. 2020). The free-radical scavenging assay method is a useful and state-of-the-art preliminary evaluation method. *In vivo* and *in vitro* experimental results may not be similar (Ahmad *et al*. 2020).

Antibacterial Activity of EPS

The antibacterial activity of EPS was tested against four indicator bacteria, and the results are shown in Fig. 4. The extracted EPS showed antibacterial activity against both gram-positive bacteria and gram-negative bacteria. The antibacterial activity of EPS was greatest against *S. aureus*, and the corresponding zone of inhibition was the 18±1 mm zone of inhibition, followed by *P. aeruginosa* (14±1 mm zone of inhibition). Moreover, the antibacterial activity was significantly lower against *E. coli* and *B. cereus*. Similarly, EPS isolated from *L. plantarum* exhibited varying antibacterial effects against *S. aureus* and *E. coli* (Ayyash *et al*. 2020). The antibacterial activity of EPS has been reported previously; however, the mechanism of action has not been clearly established (Abdalla *et al*. 2021; Yang *et al*. 2023). The antibacterial property of EPSs is attributed mainly to their activity on the peptidoglycan layer of the bacterial envelope, which is a major component of the cell structure (Sivasankar *et al*. 2018). The antibacterial activity of lactic acid bacterial EPS is due mainly to the presence of available functional groups, such as hydroxyl, phosphate and carbonyl groups (Rajoka *et al*. 2020). The pathogenic bacteria have several receptors on their cell surfaces. The cell surface of pathogenic bacteria plays an important role in cell-to-cell-to-host communications (Dertli *et al*. 2015). The negatively charged EPS produced by *Lactococcus* sp. showed higher inhibitory action against Gram-positive than Gram-negative pathogens (Zhou *et al*. 2019). In the present study, EPS extracted from *L. plantarum* showed maximum activity against Gram positive *S. aureus* strain. Moreover, EPSs exhibit antagonistic activity against both gram-positive and gram-negative bacteria (Salachna *et al*. 2018).

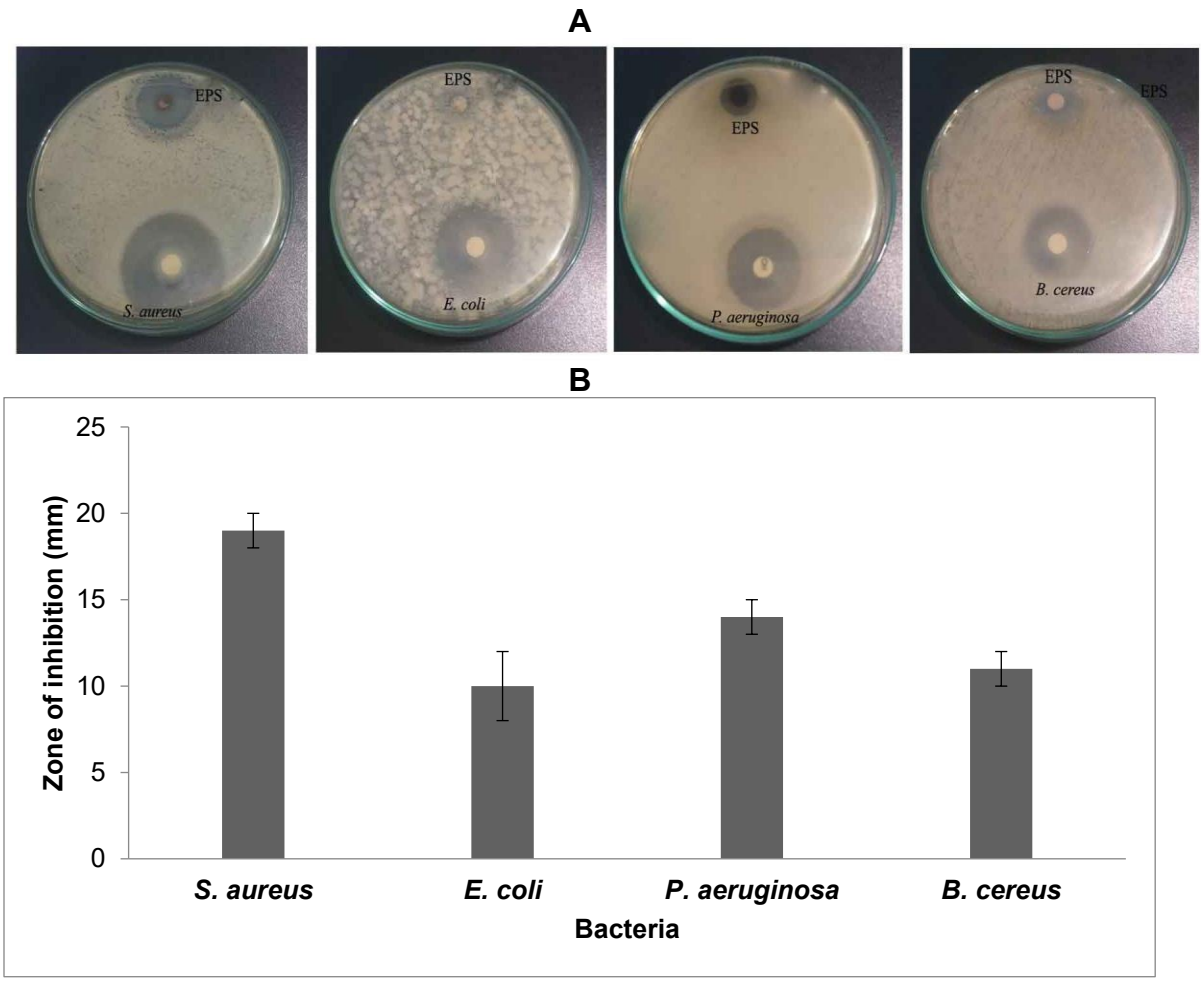

Fig. 4. Antibacterial activity of extracellular polysaccharides against bacterial pathogens. The bacterial strains were cultured on Mueller–Hinton agar medium, EPS inhibited bacterial growth (A), and the inhibitory effect was expressed as the zone of inhibition (mm) (B)

Several research works reported that EPS produced by LAB is associated with several health benefits, including anticancer, antioxidant, and anti-biofilm effects. Moreover, the biological activity of different EPS is affected by their chemical structure. EPS extracted from LAB can inhibit or reduce biofilm production by pathogenic microorganisms, thereby preventing infectious diseases (Oerlemans *et al.* 2021). LAB-synthesized EPSs are useful and are found in several applications in therapeutic medicine, and in adjuvant therapy for the treatment of cancer due to their potential to form hydrogels. The limitation for the industrial application of EPS is the wide variation in the yield, and functional properties of EPS of EPS-producing bacteria. Therefore, it is very important to develop new ideas to improve the production of EPS for its future industrial applications. The monosaccharide composition, molecular weight, and functional groups of EPSs influence biological and technological activities (Mahmoud *et al*. 2021). Most of the research work performed in recent years has focused on evaluating structural characters and functional properties. Moreover, the mechanism of EPS's antibacterial properties is highly complex and still not clearly understood. A better understanding of the underlying molecular mechanism of EPS could lead to the development of EPSs with known functional properties as therapeutic agents or functional foods.

Levilactobacillus brevis was used to produce both homopolysaccharides and heteropolysaccharides, and these have several functional properties. They were purified from the culture supernatant of LAB previously (Notararigo *et al*. 2013). Moreover, the composition of carbon/nitrogen level in the medium affected the final composition and functional properties of EPS (Le *et al.* 2017). Recently, Wang *et al*. (2024) purified heteropolysaccharide from *Levilactobacillus brevis* AM7 strain using oat flour, hemp protein, and oat bran concentrate medium. The recent focus was mainly towards the development of healthy and safe functional food additives for modern consumers using homo and heteropolysaccharides (Liu *et al*. 2025).

CONCLUSIONS

1. Agro-industrial residues were exploited for the production of extracellular polymeric substances by *Lactobacillus plantarum*. Compared with other substrates, sweet potato peel induced EPS production.
2. The response surface methodology improved bioactive extracellular polysaccharides (EPS) production in solid-state fermentation twofold, and the central composite design was ideal for determining the interactive effects among variables.
3. The crude EPS exhibited antioxidant activity and scavenged ABTS, DPPH, and hydroxyl radicals. The antagonistic property of EPS was evident against gram-positive and gram-negative bacteria.

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REFERENCES CITED

Abdalla, A. K., Ayyash, M. M., Olaimat, A. N., Osaili, T. M., Al-Nabulsi, A. A., Shah, N. P., and Holley, R. (2021). "Exopolysaccharides as antimicrobial agents: Mechanism and spectrum of activity," *Frontiers in Microbiology* 12, article 664395. DOI:10.3389/fmicb.2021.664395

Ahmad, A., Wali, A. F., Rehman, M. U., Khan, A., Raish, M., Kazi, M., Alnemer, O., and G. M. Rao, P. (2020). "Therapeutic potential of *Rhododendron arboreum* polysaccharides in an animal model of lipopolysaccharide-inflicted oxidative stress and systemic inflammation," *Molecules* 25(24), article 6045. DOI: 10.3390/molecules25246045

Al-Ansari, M. M., Andeejani, A. M., Alnahmi, E., AlMalki, R. H., Masood, A., Vijayaraghavan, P., Rahman, A. A., and Choi, K. C. (2021). "Insecticidal, antimicrobial and antioxidant activities of essential oil from *Lavandula latifolia* L. and its deterrent effects on *Euphoria leucographa*," *Industrial Crops and Products* 170, article 113740. DOI:10.1016/j.indcrop.2021.113740

Al Farraj, D. A., Kumar, T. S. J., Vijayaraghavan, P., Elshikh, M. S., Alkufeidy, R. M., Alkubaisi, N. A., and Alshammari, M. K. (2020). "Enhanced production, purification and biochemical characterization of therapeutic potential fibrinolytic enzyme from a new *Bacillus flexus* from marine environment," *Journal of King Saud University-Science* 32(7), 3174-3180. DOI:10.1016/j.jksus.2020.09.004

Ayyash, M., Abu-Jdayil, B., Itsaranuwat, P., Galiwango, E., Tamiello-Rosa, C., Abdullah, H., Esposito, G., Hunashal, Y., Obaid, R.S., and 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* 144, 938-946. DOI:10.1016/j.ijbiomac.2019.09.171

Bachtarzi, N., Speciale, I., Kharroub, K., De Castro, C., Ruiz, L., and Ruas-Madiedo, P. (2020). "Selection of exopolysaccharide-producing *Lactobacillus plantarum* (*Lactiplantibacillus plantarum*) isolated from Algerian fermented foods for the manufacture of skim-milk fermented products," *Microorganisms* 8(8), article 1101. DOI: 10.3390/microorganisms8081101

Bashandy, S. R., Abd-Alla, M. H., Mohammed, E. A., and Hassan, E. A. (2024). "Optimizing exopolysaccharide production by *Bacillus subtilis* using spoiled fig and grape," *Current Microbiology* 81(12), article ID 408. DOI: 10.1007/s00284-024-03913-4

Bekar, F., and Kamiloglu, A. (2024). "Effect of different carbon sources on exopolysaccharide production of *Lactiplantibacillus pentosus* KS27 and optimization of production conditions using Box-Behnken designing," *Biomass Conversion and Biorefinery* 14(20), 26139-26148. DOI: 10.1007/s13399-023-04545-7

Biji, G. D., Arun, A., Muthulakshmi, E., Vijayaraghavan, P., Arasu, M. V., and Al-Dhabi, N. A. (2016). "Bio-prospecting of cuttle fish waste and cow dung for the production of fibrinolytic enzyme from *Bacillus cereus* IND5 in solid state fermentation," *3 Biotech* 6, 1-13. DOI:10.1007/s13205-016-0553-0

Bjørn, M. E., and Hasselbalch, H. C. (2015). "The role of reactive oxygen species in myelofibrosis and related neoplasms," *Mediators of Inflammation* 2015(1), article 648090. DOI: 10.1155/2015/648090

Bueno, V. B., Bentini, R., Catalani, L. H., and Petri, D. F. S. (2013). "Synthesis and swelling behavior of xanthan-based hydrogels," *Carbohydrate Polymers* 92(2), 1091-1099. DOI: 10.1016/j.carbpol.2012.10.062

Cao, C., Liu, Y., Li, Y., Zhang, Y., Zhao, Y., Wu, R., and Wu, J. (2020). "Structural characterization and antioxidant potential of a novel exopolysaccharide produced by *Bacillus velezensis* SN-1 from spontaneously fermented Da-Jiang," *Glycoconjugate Journal* 37, 307-317. DOI: 10.1007/s10719-020-09923-1

Chen, J., Lu, Y., Liu, L., Bai, R., Zhang, S., Hao, Y., Xu, F., Wei, B., and Zhao, H. (2024). "Characteristic analysis and fermentation optimization of a novel *Aureobasidiumpullulans* RM1603 with high pullulan yield," *Journal of Bioscience and Bioengineering* 137(5), 335-343. DOI: 10.1016/j.jbiosc.2023.12.018

Cheng, X., Huang, L., and Li, K. T. (2019). "Antioxidant activity changes of exopolysaccharides with different carbon sources from *Lactobacillus plantarum* LPC-1 and its metabolomic analysis," *World Journal of Microbiology and Biotechnology* 35(5), article 68. DOI: 10.1007/s11274-019-2645-6

Daba, G. M., Elnahas, M. O., and Elkhateeb, W. A. (2021). "Contributions of exopolysaccharides from lactic acid bacteria as biotechnological tools in food,

pharmaceutical, and medical applications," *International Journal of Biological Macromolecules* 173, 79-89. DOI: 10.1016/j.ijbiomac.2021.01.110

Dertli, E., Mayer, M. J., and Narbad, A. (2015). "Impact of the exopolysaccharide layer on biofilms, adhesion and resistance to stress in *Lactobacillus johnsonii* FI9785," *BMC Microbiology* 15, 1-9. DOI: 10.1186/s12866-015-0347-2

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., and Smith, F. (1956). "Colorimetric method for determination of sugars and related substances," *Analytical Chemistry* 28(3), 350-356. DOI: 10.1021/ac60111a017

El-Sheikh, M. A., Rajaselvam, J., Abdel-Salam, E. M., Vijayaraghavan, P., Alatar, A. A., and Biji, G. D. (2020). "*Paecilomyces* sp. ZB is a cell factory for the production of gibberellic acid using a cheap substrate in solid state fermentation," *Saudi Journal of Biological Sciences* 27(9), 2431-2438. DOI:10.1016/j.sjbs.2020.06.040

Espinosa, A., Henríquez-Olguín, C., and Jaimovich, E. (2016). "Reactive oxygen species and calcium signals in skeletal muscle: A crosstalk involved in both normal signaling and disease," *Cell Calcium* 60(3), 172-179. DOI: 10.1016/j.ceca.2016.02.010

Fang, Y., Ahmed, S., Liu, S., Wang, S., Lu, M., and Jiao, Y. (2013). "Optimization of antioxidant exopolysaccharides production by *Bacillus licheniformis* in solid state fermentation," *Carbohydrate Polymers* 98(2), 1377-1382. DOI: 10.1016/j.carbpol.2013.07.076

FAO STAT (2019). "Statistical Database," (www.fao.org), accessed 10 February 2024.

Freitas, F., Alves, V. D., and Reis, M. A. (2011). "Advances in bacterial exopolysaccharides: From production to biotechnological applications," *Trends in Biotechnology* 29(8), 388-398. DOI: 10.1016/j.tibtech.2011.03.008

Gu, D., Jiao, Y., Wu, J., Liu, Z., and Chen, Q. (2017). "Optimization of EPS production and characterization by a halophilic bacterium, *Kocuria rosea* ZJUQH from Chaka Salt Lake with response surface methodology," *Molecules* 22(5), article 814. DOI: 10.3390/molecules22050814

Hao, L., Liu, W., Liu, K., Shan, K., Wang, C., Xi, C., Liu, J., Fan, Q., Zhang, X., Lu, X., and Cui, B. (2019). "Isolation, optimization of fermentation conditions, and characterization of an exopolysaccharide from *Pseudoalteromonas agarivorans* Hao 2018," *Marine Drugs* 17(12), article 703. DOI: 10.3390/md17120703

Harman, D. (1993). "Free radical involvement in aging: Pathophysiology and therapeutic implications," *Drugs and Aging* 3, 60-80. DOI: 10.2165/00002512-199303010-00006

He, X., He, F., Hang, J., Li, H., Chen, Y., Wei, P., Chen, K., Li, Y., and OuYang, P. (2018). "Enhanced production of exopolysaccharides using industrial grade starch as sole carbon source," *Bioprocess and Biosystems Engineering* 41, 811-817. DOI: 10.1007/s00449-018-1915-2

Imran, M. Y. M., Reehana, N., Jayaraj, K. A., Ahamed, A. A. P., Dhanasekaran, D., Thajuddin, N., Alharbi, N. S., and Muralitharan, G. (2016). "Statistical optimization of exopolysaccharide production by *Lactobacillus plantarum* NTMI05 and NTMI20," *International Journal of Biological Macromolecules* 93, 731-745. DOI: 10.1016/j.ijbiomac.2016.09.007

Itunu, A. O., Titilope, O., Onyemali, C. P., Ehiwuogu-Onyibe, J., Aina, A. D., Thonda, O. A., Lawal, A. K., and Tutuwa, J. A. (2025). "Microbial production of xanthan gum using various agro wastes and molecular characterization of xanthan gum producing isolates," *International Journal of Scientific Research and Technology* 2(12), 1-1. DOI: 10.5281/zenodo.14586979

Kalaiyarasi, M., Ahmad, P., and Vijayaraghavan, P. (2020). "Enhanced production antibiotics using green gram husk medium by *Streptomyces* sp. SD1 using response surface methodology," *Journal of King Saud University-Science* 32(3), 2134-2141. DOI: 10.1016/j.jksus.2020.02.014

Lakra, U., Lincoln Singh Munda, V., Nigam, V. K., and Sharma, S. R. (2024). "Optimisation, characterization, and biological evaluation of novel exopolysaccharide from *Bacillus licheniformis* (BITSL006)," *Natural Product Research* 38(21), 3783-3792. DOI: 10.1080/14786419.2023.2265041

Le, C., and Stuckey, D. C. (2017). "Influence of feed composition on the monomeric structure of free bacterial extracellular polysaccharides in anaerobic digestion," *Environmental Science and Technology* 51(12), 7009-7017. DOI: 10.1021/acs.est.7b00925

Liu, L., Xu, J., Na, R., Du, R., Ping, W., Ge, J., and Zhao, D. (2022). "Purification, characterization and partial biological activities of exopolysaccharide produced by *Saccharomyces cerevisiae* Y3," *International Journal of Biological Macromolecules* 206, 777-787. DOI: 10.1016/j.ijbiomac.2022.03.083

Liu, W., Wei, Y., Xiang, R., Dong, B., and Yang, X. (2025). "Lactic acid bacteria exopolysaccharides unveiling multifaceted insights from structure to application in foods and health promotion," *Foods* 14(5), article 823. DOI: 10.3390/foods14050823

Lu, H., Gui, Y., Zheng, L., and Liu, X. (2013). "Morphological, crystalline, thermal and physicochemical properties of cellulose nanocrystals obtained from sweet potato residue," *Food Research International* 50(1), 121-128. DOI: 10.1016/j.foodres.2012.10.013

Mahmoud, Y. A. G., El-Naggar, M. E., Abdel-Megeed, A., and El-Newehy, M. (2021). "Recent advancements in microbial polysaccharides: Synthesis and applications," *Polymers* 13(23), article ID 4136. DOI: 10.3390/polym13234136

Marraiki, N., Vijayaraghavan, P., Elgorban, A. M., Dhas, D. D., Al-Rashed, S., and Yassin, M. T. (2020). "Low cost feedstock for the production of endoglucanase in solid state fermentation by *Trichoderma hamatum* NGL1 using response surface methodology and saccharification efficacy," *Journal of King Saud University-Science* 32(2), 1718-1724. DOI:10.1016/j.jksus.2020.01.008

Mei, X., Mu, T. H., and Han, J. J. (2010). "Composition and physicochemical properties of dietary fiber extracted from residues of 10 varieties of sweet potato by a sieving method," *Journal of Agricultural and Food Chemistry* 58(12), 7305-7310. DOI: 10.1021/jf101021s

Mouro, C., Gomes, A. P., and Gouveia, I. C. (2024). "Microbial exopolysaccharides: Structure, diversity, applications, and future frontiers in sustainable functional materials," *Polysaccharides* 5(3), 241-287. DOI: 10.3390/polysaccharides5030018

Nguyen, H. T., Pham, T. T., Nguyen, P. T., Le-Buanec, H., Rabetafika, H. N., and Razafindralambo, H. L. (2024). "Advances in microbial exopolysaccharides: Present and future applications," *Biomolecules*14(9), article ID 1162. DOI: 10.3390/biom14091162

Niknezhad, S. V., Asadollahi, M. A., Zamani, A., and Biria, D. (2016). "Production of xanthan gum by free and immobilized cells of *Xanthomonas campestris* and *Xanthomonas pelargonii*," *International Journal of Biological Macromolecules* 82, 751-756. DOI: 10.1016/j.ijbiomac.2015.10.065

Niknezhad, S. V., Asadollahi, M. A., Zamani, A., Biria, D., and Doostmohammadi, M. (2015). "Optimization of xanthan gum production using cheese whey and response

surface methodology," *Food Science and Biotechnology* 24, 453-460. DOI: 10.1007/s10068-015-0060-9

Notararigo, S., Nácher-Vázquez, M., Ibarburu, I., Werning, M. L., de Palencia, P. F., Dueñas, M. T., ... and Prieto, A. (2013). "Comparative analysis of production and purification of homo-and hetero-polysaccharides produced by lactic acid bacteria," *Carbohydrate Polymers* 93(1), 57-64. DOI: 10.1016/j.carbpol.2012.05.016

Oerlemans, M. M., Akkerman, R., Ferrari, M., Walvoort, M. T., and de Vos, P. (2021). "Benefits of bacteria-derived exopolysaccharides on gastrointestinal microbiota, immunity and health," *Journal of Functional Foods* 76, article ID 104289. DOI: 10.1016/j.jff.2020.104289

Okoro, O. V., Gholipour, A. R., Sedighi, F., Shavandi, A., and Hamidi, M. (2021). "Optimization of exopolysaccharide (EPS) production by *Rhodotorula mucilaginosa* sp. GUMS16," *Chemical Engineering* 5(3), article ID 39. DOI: 10.3390/chemengineering5030039

Patel, A., and Prajapati, J. B. (2013). "Food and health applications of exopolysaccharides produced by lactic acid bacteria," *Advances in Dairy Research* 1(2), 1-7. DOI: 10.4172/2329-888X.1000107

Rajoka, M. S. R., Wu, Y., Mehwish, H. M., Bansal, M., and Zhao, L. (2020). "*Lactobacillus* exopolysaccharides: New perspectives on engineering strategies, physiochemical functions, and immunomodulatory effects on host health," *Trends in Food Science and Technology* 103, 36-48. DOI:10.1016/j.tifs.2020.06.003

Rath, T., Rühmann, B., Schmid, J., and Sieber, V. (2022). "Systematic optimization of exopolysaccharide production by *Gluconacetobacter* sp. and use of (crude) glycerol as carbon source," *Carbohydrate Polymers* 276, article 118769. DOI: 10.1016/j.carbpol.2021.118769

Saeed, S., Ahmed, S., Naz, A., Arooj, F., and Mehmood, T. (2023). "Valorization of using agro-wastes for levan through submerged fermentation and statistical optimization of the process variables applying response surface methodology (RSM) design," *Microorganisms*11(6), article 1559. DOI: 10.3390/microorganisms11061559

Salachna, P., Mizielińska, M., and Soból, M. (2018). "Exopolysaccharide gellan gum and derived oligo-gellan enhance growth and antimicrobial activity in *Eucomis* plants," *Polymers* 10(3), article 242. DOI:10.3390/polym10030242

Seesuriyachan, P., Techapun, C., Shinkawa, H., and Sasaki, K. (2010). "Solid state fermentation for extracellular polysaccharide production by *Lactobacillus confusus* with coconut water and sugar cane juice as renewable wastes," *Bioscience, Biotechnology, and Biochemistry* 74(2), 423-426. DOI: 10.1271/bbb.90663

Shalini, R., and Gupta, D. K. (2010). "Utilization of pomace from apple processing industries: A review," *Journal of Food Science and Technology* 47, 365-371.DOI: 10.1007/s13197-010-0061-x

Sivasankar, P., Seedevi, P., Poongodi, S., Sivakumar, M., Murugan, T., Sivakumar, L., Sivakumar, K., and Balasubramanian, T. (2018). "Characterization, antimicrobial and antioxidant property of exopolysaccharide mediated silver nanoparticles synthesized by *Streptomyces violaceus* MM72," *Carbohydrate Polymers* 181, 752-759. DOI: 10.1016/j.carbpol.2017.11.082

Sran, K. S., Sundharam, S. S., Krishnamurthi, S., and Choudhury, A. R. (2019). "Production, characterization and bio-emulsifying activity of a novel thermostable exopolysaccharide produced by a marine strain of *Rhodobacter johrii* CDR-SL 7Cii," *International Journal of Biological Macromolecules* 127, 240-249. DOI:

10.1016/j.ijbiomac.2019.01.045

Upadhyaya, C., Patel, H., Patel, I., and Upadhyaya, T. (2025). "Extremophilic exopolysaccharides: Bioprocess and novel applications in 21st century," *Fermentation* 11(1), article ID 16. DOI: 10.3390/fermentation11010016

Vijayaraghavan, P., and Vincent, S. G. P. (2014). "Statistical optimization of fibrinolytic enzyme production by *Pseudoalteromonas* sp. IND11 using cow dung substrate by response surface methodology," *SpringerPlus* 3, 1-10. DOI:10.1186/2193-1801-3-60

Wang, Q., Chen, S., Zhang, J., Sun, M., Liu, Z., and Yu, Z. (2008). "Co-producing lipopeptides and poly-γ-glutamic acid by solid-state fermentation of *Bacillus subtilis* using soybean and sweet potato residues and its biocontrol and fertilizer synergistic effects," *Bioresource Technology* 99(8), 3318-3323. DOI: 10.1016/j.biortech.2007.05.052

Wang, X., Shao, C., Liu, L., Guo, X., Xu, Y., and Lü, X. (2017). "Optimization, partial characterization and antioxidant activity of an exopolysaccharide from *Lactobacillus plantarum* KX041," *International Journal of Biological Macromolecules* 103, 1173-1184. DOI: 10.1016/j.ijbiomac.2017.05.118

Wang, Y., Mehmood, S., Maina, N. H., Katina, K., and Coda, R. (2024). "Synthesis in situ of heteropolysaccharide by *Levilactobacillus brevis* AM7 during fermentation of oat and hemp and its effect on the techno-functional properties of oat yogurt type model," *Food Hydrocolloids* 147, article 109416. DOI: 10.1016/j.foodhyd.2023.109416

Xie, Y., Liu, H., Li, Y., Tian, J., Qin, X., Shabani, K. I., Liao, C., and Liu, X. (2020). "Characterization of Pickering emulsions stabilized by OSA-modified sweet potato residue cellulose: Effect of degree of substitute and concentration," *Food Hydrocolloids* 108, article 105915. DOI: 10.1016/j.foodhyd.2020.105915

Yalmanci, D., Dertli, E., Tekin-Cakmak, Z. H., and Karasu, S. (2023). "Utilization of exopolysaccharide produced by *Leuconostoc lactis* GW-6 as an emulsifier for low-fat mayonnaise production," *International Journal of Biological Macromolecules*226, 772-779. DOI: 10.1016/j.ijbiomac.2022.12.069

Yang, S., Xu, X., Peng, Q., Ma, L., Qiao, Y., and Shi, B. (2023). "Exopolysaccharides from lactic acid bacteria, as an alternative to antibiotics, on regulation of intestinal health and the immune system," *Animal Nutrition* 13, 78-89. DOI: 10.1016/j.aninu.2023.02.004

Yokoi, H., Saitsu, A., Uchida, H., Hirose, J. U. N., Hayashi, S., and Takasaki, Y. (2001). "Microbial hydrogen production from sweet potato starch residue," *Journal of Bioscience and Bioengineering* 91(1), 58-63. DOI: 10.1016/S1389-1723(01)80112-2

Zhang, L., Liu, C., Li, D., Zhao, Y., Zhang, X., Zeng, X., Yang, Z., and Li, S. (2013). "Antioxidant activity of an exopolysaccharide isolated from *Lactobacillus plantarum* C88," *International Journal of Biological Macromolecules* 54, 270-275. DOI: 10.1016/j.ijbiomac.2012.12.037

Zhao, F., Guo, C., Cui, Q., Hao, Q., Xiu, J., Han, S., and Zhang, Y. (2018). "Exopolysaccharide production by an indigenous isolate *Pseudomonas stutzeri* XP1 and its application potential in enhanced oil recovery," *Carbohydrate Polymers* 199, 375-381. DOI: 10.1016/j.carbpol.2018.07.038

Zhou, Y., Cui, Y., and Qu, X. (2019). "Exopolysaccharides of lactic acid bacteria: Structure, bioactivity and associations: A review," *Carbohydrate Polymers* 207, 317-332. DOI: 10.1016/j.carbpol.2018.11.093

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