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أتقدم بخالص الشكر وعظيم الامتنان إلى الأستاذ الدكتور بوبكري، مشرف هذا العمل، على ما بذله من جهد في تأطيري ومتابعة هذا البحث، وعلى توجيهاته العلمية القيمة، وصبره وتفانيه في المرافقة الأكاديمية طيلة فترة إعداد هذه المذكرة.

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Abstract

Although certain lactic acid bacteria (LAB) strains producing exopolysaccharides (EPS) are considered unsuitable for fermented meat production which may be misinterpreted as spoilage criteria. Nevertheless, the EPS from meat-borne LAB can be explored for their bio-functional properties that make them valuable in industrial, pharmaceutical and biotechnological applications.*Kaddid*-borne LAB strains were evaluated for theirEPS production phenotypically based on colony texture ropy or mucoid. The selected EPS-producers*Weissella (We.). Cibaria* BK2, *We. Cibaria* BK4 and *Pediococcus (Pe.). Acidilactici* BK14 were cultured in optimized modified-MRS medium, followed by extraction and quantification of EPS-production yield by phenol-sulfuric acid method. *W. cibaria* BK2 demonstrated the highest EPS production reaching the concentration of 471.66 mg/L. In addition, exhibited the highest DPPH scavenging activity with a removal rate value of 52.02%, while ascorbic acid (1 mg/mL) used as positive control reach ~ 105% of DPPH scavenging activity. Meanwhile, hydroxyl radical scavenging assay, *W. cibaria* BK2achieved 159.06% inhibition, highlighting its remarkable capacity to neutralize highly reactive oxygen species (ROS). In contrast, the EPS from *Pe.Acidilactici* BK14 demonstrated the lowest antioxidant performance with DPPH and hydroxyl radical removal rates of 32.2 and 35.63%respectively. The FTIR analysis provided further confirmation of EPS composition by revealing characteristic absorption bands. A broad peak at approximately 3300 cm indicated O-H or N-H stretching vibrations, suggesting the presence of hydroxyl or amine groups. A strong peak near 1700 cm was associated with C=O stretching, typical of carbonyl-containing groups such as ketones or carboxylic acids. Additionally, the region between: 1000-1200 cm showed C-O stretching vibrations, indicative of esters or ethers. These spectral features confirm the presence of high-molecular-weight bioactive compounds, such as polysaccharides, proteins, and organic acids, produced by the tested strains. These finding suggest the potential of EPS from *We. Cibaria*BK2 to be used as naturalsource of antioxidants and textural agent for food, pharmaceuticaland agriculturalapplications.

Key words: *Kaddid*, *We. Cibaria*,exopolysaccharides, antioxidants, FFTIR.

Résumé

Bien que certaines souches de bactéries lactiques (LAB) productrices d'exopolysaccharides (EPS) soient considérées comme inappropriées pour la production de viandes fermentées – ce qui peut être interprété à tort comme un signe d'altération – les EPS issus des LAB d'origine carnée peuvent être valorisés pour leurs propriétés bios fonctionnelles, utiles dans les domaines industriel, pharmaceutique et biotechnologique. Les souches de LAB isolées du Kaddid ont été évaluées phénotypiquement pour leur production d'EPS, basée sur la texture des colonies (filamenteuse ou mucoïde). Les souches sélectionnées productrices d'EPS, Weissella (We.) cibaria BK2, We. Cibaria BK4 et Pediococcus (Pe.) acidilactici BK14, ont été cultivées dans un milieu MRS modifié optimisé. L'extraction et la quantification des EPS ont été réalisées par la méthode de l'acide phénol-sulfurique. W. cibaria BK2 a montré la plus haute production d'EPS, atteignant 471,66 mg/L. De plus, elle a présenté la plus grande activité antioxydant DPPH, avec un taux d'élimination de 52,02 %, tandis que l'acide ascorbique (1 mg/ml), utilisé comme témoin positif, a atteint ~105 %. Par ailleurs, dans le test de piégeage des radicaux hydroxyles, W. cibaria BK2 a atteint 159,06 % d'inhibition, démontrant une capacité remarquable à neutraliser les espèces réactives de l'oxygène (ROS). En revanche, les EPS de Pe. Acidilactici BK14 ont montré la plus faible activité antioxydant, avec des taux d'élimination de 32,2 % (DPPH) et 35,63 % (radicaux hydroxyles). L'analyse FTIR a confirmé la composition des EPS, révélant des bandes caractéristiques : une large bande autour de 3300 cm^{-1} (liaisons O-H ou N-H), une bande forte près de 1700 cm^{-1} (liaison C=O), et des bandes entre 1000–1200 cm^{-1} (liaisons C-O). Ces signatures spectrales confirment la présence de composés bioactifs de haut poids moléculaire, comme les polysaccharides, protéines et acides organiques. Ces résultats suggèrent le potentiel de We. Cibaria BK2 comme source naturelle d'antioxydants et agent texturant dans les domaines alimentaires, pharmaceutiques et agricoles.

Mots-clés : Kaddid, We. Cibaria, exopolysaccharides, antioxydants, FTIR.

ملخص

على الرغم من أن بعض سلالات بكتيريا حمض اللاكتيك (LAB) المنتجة للسكريات الخارجية (EPS) تُعد غير مناسبة لإنتاج اللحوم المخمّرة، مما قد يُساء تفسيره كعلامة على فساد المنتج، فإن السكريات الخارجية المستخلصة من LAB المرتبطة باللحوم يمكن استكشافها من حيث خصائصها الحيوية الوظيفية التي تجعلها ذات قيمة في التطبيقات الصناعية والدوائية والتقنية الحيوية. تم تقييم سلالات LAB المعزولة من الكديد من حيث إنتاج EPS ظاهريًا، اعتمادًا على مظهر المستعمرات اللزجة أو المخاطية. وقد تم اختيار السلالات المنتجة للـEPS، وهي Weissella (We.) Cibaria 4BK2، We. Cibaria BK و14Pediococcus (Pe.) acidilactici BK، وزُرعت في وسط MRS معدّل ومحسَّن، ثم تم استخلاص السكريات الخارجية وقياس كميتها باستخدام طريقة الفينول-حمض الكبريتيك. أظهرت السلالة 2W. Cibaria BK أعلى إنتاج للـEPS بتركيز بلغ 471.66 ملغ/لتر. كما سجلت أعلى نشاط مضاد للأكسدة في اختبار DPPH بنسبة إزالة بلغت 52.02٪، بينما وصل حمض الأسكوربيك (1 ملغ/مل) المستخدم كعنصر ضابط إلى نحو 105٪. أما في اختبار التخلص من الجذور الهيدروكسيلية، فقد بلغت نسبة التثبيط لـ 2W. Cibaria BK حوالي 159.06٪، مما يُبرز قدرتها العالية على تحييد أنواع الأوكسجين التفاعلية (ROS). في المقابل، أظهرت السكريات الخارجية المستخلصة من 14Pe. Acidilactici BK أضعف نشاط مضاد للأكسدة، حيث بلغت نسب إزالة DPPH والجذور الهيدروكسيلية 32.2٪ و35.63٪ على التوالي. كما أكدت تحاليل FTIR تركيب السكريات الخارجية من خلال ظهور نطاقات امتصاص مميزة، حيث ظهرت قمة عريضة عند 3300 سم⁻¹ تشير إلى اهتزازات O-H أو N-H، مما يدل على وجود مجموعات هيدروكسيل أو أمين، وقمة قوية عند حوالي 1700 سم⁻¹ مرتبطة باهتزازات C=O، النموذجية للمركبات المحتوية على مجموعة كربونيل مثل الكيتونات أو الأحماض الكربوكسيلية، بالإضافة إلى نطاق بين 1000–1200 سم⁻¹ يُظهر اهتزازات C-O، مما يدل على وجود إسترات أو إيثرات. تؤكد هذه السمات الطيفية وجود مركبات حيوية عالية الوزن الجزيئي مثل السكريات، البروتينات، والأحماض العضوية التي تنتجها السلالات المختبرة. وتشير هذه النتائج إلى إمكانية استخدام السكريات الخارجية المنتجة من 2We. Cibaria BK كمصدر طبيعي لمضادات الأكسدة وكمُحسّن قوام في الصناعات الغذائية، الدوائية والزراعية.

الكلمات المفتاحية: كديد، We. Cibaria، سكريات خارجية، مضادات أكسدة، FTIR

Liste of Tables

Table of contents

Liste of figures i

Liste of tables ii

Introduction 1

Bibliographic study 4

I.Meat born Lactic acid bacteria (LAB) 4

I.1Characterization of LAB isolates 4

I.2 Functional properties of meat-borne LAB starters 5

I.2.1Antioxidant and GABA-producing capacities of meat-borne LAB 5

I.2.2.Antimicrobial of LAB: 6

I.2.3.Acidification of LAB: 6

I.2.4.Proteolysis and lipolysis: 7

I.3Exopolysaccharide (EPS) from meat-borne LAB 7

I.3.1Definition of EPS 7

I.3.2Conditions of production from LAB 8

I.3.3Structure of EPS 9

I.3.3Biosynthetic pathways 12

I.3.4Methods of microbial EPS Screening, isolation andcharacterization 12

I.3.5Physicochemical properties, biological activities and applications of the EPS 13

II. Material and methods 17

II.1Culture medium 17

II.2LAB strains and growth conditions 18

II.3Phonotypical detection EPS production 20

II.4EPSlevel production by selected LAB 20

II.4.1Extraction of EPS-producers LAB 20

Liste of Tables

II.4.2 Quantification of EPS-producers LAB.....21
II.5 Evaluation of antioxidant activity of EPS-producer LAB.....21
II.5.1DPPH Free Radical Scavenging Activity.....21
II.5.2Evaluation of Hydroxyl Radical (HO•) Scavenging Activity.....22
II.6FourierTransform Infrared (FTIR) Spectroscopy for Solid-State analysis: methodology and functional group identification.....22
II.7 Statistical analysis.....23
III. Resultats and discussion:.....24
III.1. Phenotypical evaluation of EPS production from *Kaddid*-borne LAB:.....24
III.2.Quantification of EPS from selected mucoid-producers *Kaddid*-borne LAB....27
III.3.Evaluationof antioxidative activity of purified EPS from LAB.....29
III.3.1.DPPH scavenging activity (DPPH-SA).....29
III.3.2. Hydroxylradical scavenging activity.....31
III.4.Chemical characterization of functional groupe of LAB EPSs by FTIR analysis 33
Conclusion:.....37
References:.....III

Liste of figures

Figure 1 : chemical structure of A: Inulin and B: curdlan.....10
Figure 2 : classification of bacterial EPSs.....11
Figure 3: Biological activities of EPS.....15
Figure 4: Stringy and viscous appearance of EPS-producing LAB strains cultivated on sucrose-containing media.The images illustrate the pronounced mucoid formation observed in strains(A): *We.cibaria*BK2, (B): *We. confusa*BK4and (C):*Pe. acidilactici* BK14.....26
Figure 5: Evaluation of EPS production by selected ropy-producers*Kaddid*-borne LAB strains.....27

Liste of Tables

Figure 6:standard curve of glucose.....28
Figure 7: Evaluation of the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH˙) scavenging activity (%) for Kaddid-borne EPS-producers LAB strains and ascorbic acid (1 mg/mL).....30
Figure 8 :Evaluation of the Hydroxyl radical (HO˙) scavenging activity (%) of EPS from *Kaddid*-borne LAB strains and ascorbic acid (1 mg/mL) was used as positive control.....32
Figure 9:FTIR spectra analysis of the purifiedEPSs produced by, (A): *We. cibaria* BK2, (B): *We. confusa* BK4 and (C): *Pe. acidilactici* BK14.....36

Liste of tables

Table 1 : Functional and sensory characteristics of selected lactic acid bacteria (LAB) strains.....4
Table 2 : Standard MRS Broth composition.....18
Table3 : Lactic acid bacteria strains used in this study.....19
Table 4 :Evaluation of EPS production by observing *ropy*or *mucoid*colonies.....25

Liste of Tables

Introduction

Introduction

Lactic acid bacteria (LAB) are among the most important microorganisms used in the food industry, particularly in fermentation processes, due to their valuable technological and functional properties. Although certain LAB strains isolated from meat may exhibit spoilage-related characteristics—such as slimy texture or off-odors—and are often considered indicators of product deterioration, this does not diminish the potential biological value of the compounds they produce, especially exopolysaccharides (EPS)(Fadda et al., 2010).

Not all LAB strains isolated from meat are EPS producers, but those that are can serve as promising sources of these high-molecular-weight biopolymers. EPS are known for their diverse structures and multifunctional roles. In addition to their uses in food systems as thickeners or stabilizers, EPS have demonstrated various bioactive properties, including antioxidant, anti-inflammatoryand digestive-regulating activities, which make them attractive for health-related applications(Carneiro et al., 2024).

The characterization of EPS is a critical step in understanding their structure and functionality. This involves:Identification and quantification, often using methods such as the phenol-sulfuric acid assay.Physicochemical analysis, including monosaccharide composition, molecular weight, solubility, and viscosity.Biological evaluation, focusing on activities such as antioxidant potential, anti-inflammatory effects, and digestive modulation(Donot et al., 2012; Silva et al., 2019).

Even thought, EPS-producing LAB are considered inappropriate for meat fermentation due to their slime production, which is generally seen as a spoilage indicator. However, these strains can still be valuable for other industrial and agricultural applications. In this study, LAB strains isolated from *Kaddid* (Boubakri et al., 2022; Boubakri et al., 2025) were screened for their EPS production potential. In the first stage, the isolates were phenotypically evaluated for ropiness and mucoid colony formation. The selected EPS-producers LAB were subjected to optimization of production process, followed by extraction, purificationand quantitative analysis. Furthermore, the purified LAB's EPS were assayed for their antioxidant activity by using DPPH and Hydroxyl radical tests. Finally, the structure of the EPS was estimated by FTIR technique.

Introduction

Bibliographic study

Bibliographic study: Exopolysaccharides from meat-borne Lactic acid bacteria

I. Meat born Lactic acid bacteria (LAB)

LAB are regarded as among the safest microorganisms, playing a significant role in the food and food-processing industries. The biofilms formed by LAB inhibit the growth of undesirable microorganisms on meat and meat products. Numerous studies have shown that LAB produce antimicrobial metabolites that effectively target food-spoiling pathogens, thereby ensuring safety and extending shelf life. This review focuses on the application of LAB as bio preservatives to enhance the shelf life and fortification of meat and meat products.**(Lahiri et al., 2022)**

Table 1 : Functional and sensory characteristics of selected lactic acid bacteria (LAB) strains(Lahiri et al., 2022).

Type de LAB	Technological Role	Sensory impact
W.cibaria BK2	Fermentation, preservation	Enhances flavor and aroma
W.cunfusa BK4	Fermentation, preservation	Balances flavor, improves aroma
Pe.aidilactici BK14	Fermentation, antimicrobial	Improves texture and flavor

I.1Characterization of LAB isolates

The identification of LAB isolates was conducted through morphological and biochemicalcharacterizationtechniques.

I.1.1 Preliminary morphological characterization

Morphological features of the isolates were assessed using Gram staining, spore staining, capsule staining, and motility tests, following the standard procedures described by Fawoleand Oso 2020.

I.1.2Biochemical Characterization

Biochemical identification involved several tests, including the catalase test (Fawole and Oso 2020) ,coagulase test, oxidase test, hydrogen sulfide (H_2S)

production test, and methyl red test, all performed according to Cheesbrough2020 Additional tests such as the Voges-Proskauer (VP) test and sugar fermentation assays were also carried out using Cheesbrough's methods 2020 Sugar fermentation tests were performed to evaluat the ability of the isolates to ferment different sugars, which helps in distinguishing between bacterial species.The ability of the isolates to grow at different temperatures was evaluated as described by(Adelekan et al., 2020).

I.1.3Molecular Characterization

Molecular markers represent essential tools in the study of microbial communities, as they provide critical information regarding both the taxonomic identification of isolates and their intraspecific genetic variability. Among the various molecular techniques, randomly amplified polymorphic DNA (RAPD-PCR) is widely recognized for its simplicity, cost-effectiveness, and efficiency in generating DNA fingerprints, particularly for the genetic characterization of lactic acid bacteria (LAB). The reproducibility of RAPD primers at optimized annealing temperatures ensures reliable and consistent results. Given the functional variability among LAB strains due to their genetic heterogeneity, molecular markers are commonly employed to evaluate both inter- and intraspecific differences. Cluster analysis, often applied in this context, allows for the identification of genetically distinct groups and proves especially useful in exploratory research where no prior classification exists. This strategy facilitates the selection of genetically diverse strains from different clusters or sub clusters, thereby optimizing the number of isolates required for subsequent technological and functional assessments(Aktepe et al., 2024).

I.2Functional properties of meat-borne LAB starters

I.2.1Antioxidant and GABA-producing capacities of meat-borne LAB

An important criterion when screening probiotic meat-derived LAB strains is their ability to produce health-promoting compounds, such as antioxidants and γ-amino butyric acid (GABA). The antioxidant properties of lactic acid bacteria LABare linked to their surface molecules and metabolic activities, which help neutralize oxidative compounds like free radicals ($R^{\cdot}$) and reactive oxygen species (ROS), or inhibit their formation during meat fermentation and within the intestinal tract(Boubakri et al., 2025).

Moreover, many LAB strains can synthesize GABA, a non-proteingenic amino acid produced through the decarboxylation of glutamate by the enzyme glutamate decarboxylase (GAD). GABA is a bioactive compound broadly distributed across living organisms. In mammals, it serves as a key inhibitory neurotransmitter in the central nervous system, helping to lower blood pressure, regulate neuronal excitability, and potentially exert anti-diabetic effects. Additionally, GABA plays a role in gut-brain communication by interacting with immune cells, enteroendocrine cells, and enteric neurons(Boubakri et al., 2025).

Due to its wide-ranging health benefits, GABA holds potential for the development of functional foods, including GABA-enriched fermented meat products. Given that red meat is naturally high in glutamate (approximately 10–15% of total amino acids in muscle proteins), fermented meat provides a suitable environment for substantial GABA production by Lactobacillus strains exhibiting GAD activity(Boubakri et al., 2025).

I.2.2.Antimicrobial of LAB

Lactic acid bacteria (LAB) in fermented foods exhibit antimicrobial properties, making them valuable as natural bio preservatives to inhibit the growth of pathogenic and spoilage bacteria and fungi (Gyawali & Ibrahim, 2014). In addition to preserving food safety, LAB help maintain the nutritional quality of various foods (Ammor et al., 2006; Ahmadova et al., 2013). Over the past century, significant efforts have focused on identifying and characterizing the wide range of antagonistic compounds produced by LAB (Liu, 2003).

The preservative effects of LAB arise from the production of various active metabolites, including organic acids (e.g., lactic, acetic, formic, and propionic acids), which lower the pH of the medium, enhancing antimicrobial activity. LAB also produce other antimicrobial agents such as fatty acids, acetoin, hydrogen peroxide, diacetyl, antifungal compounds (e.g., propionate, phenyl-lactate, hydroxyphenyl-lactate, cyclic dipeptides, and 3-hydroxy fatty acids), and a variety of bacteriocins (e.g., nisin, reuterin, reutericyclin, pediocin, lacticin, enterocin). Additionally, they produce bacteriocin-like inhibitory substances (BLIS) (Favaro et al., 2015).

I.2.3.Acidification of LAB:

Lactic acid bacteria (LAB) rapidly produce lactic acid, leading to environmental acidification that suppresses the growth of competing microorganisms. These bacteria exhibit a high glycolytic capacity; in Lactococcus species, glycolytic enzymes account for approximately 20% of the cytosolic proteome [1]. This enables efficient ATP production and rapid growth, compensating for their lack of respiratory pathways. Beyond their long-standing role in the food industry, LAB are also promising candidates for the bioconversion of renewable feedstocks into valuable products such as biofuels and biochemicals, owing to their resilience and tolerance to harsh industrial conditions.(Martinussen et al., 2013).

I.2.4.Proteolysis and lipolysis

The biochemistry of fermented foods is intricate, involving numerous enzymatic reactions. A key outcome of fermentation is the drop in pH and the suppression of undesirable microorganisms, driven by the production of antimicrobial metabolites like lactic acid, hydrogen peroxide, diacetyl, acetaldehyde, reuterin, and bioactive peptides (Papagianni 2012). Fermentation also generates metabolic by-products such as lipolytic and proteolytic enzymes (Burgain et al. 2014).

Lipolysis refers to the enzymatic breakdown of triglycerides into free fatty acids, glycerol, and mono- or diglycerides. These intermediates enhance emulsification and contribute to the final texture of fermented products. The extent of lipolysis varies among LAB strains and is a critical factor in selecting starter cultures, particularly for plant-based fermentations, baked goods, and dairy products (Esteban-Torres et al. 2014; Tanasupawat et al. 2015).

Proteolysis involves the cleavage of protein peptide bonds, resulting in peptides and free amino acids. These amino acids may undergo further reactions (e.g., decarboxylation, deamination), significantly influencing flavor, aroma, and texture. Proteases are thus essential in shaping sensory qualities and moisture levels in fermented foods, especially in products like aged cheeses (Savijoki et al. 2006).

I.3Exopolysaccharide (EPS) from meat-borne LAB

I.3.1Definition of EPS

EPS are extracellular polymeric substances produced by bacteria, archaea, and eukaryotes, playing a key role in the formation of microbial aggregates. Although

EPS commonly refers to ‘exopolysaccharides’ the extracellular matrix also includes proteins, nucleic acids, phospholipids, and glycoproteins in notable amounts.(Jurášková et al., 2022a) Microbial EPS are typically water-soluble or soluble in dilute salt solutions and are categorized into two types:homopolysaccharides (HoPS), synthesized mainly from sucrose, and heteropolysaccharides (HePS), produced from various substrates. These compounds create a three-dimensional matrix that can influence the texture and structure of food products. EPS production is often triggered by suboptimal or stress conditions, serving as a protective mechanism for microorganisms (Jurášková et al., 2022a)

I.3.2Conditions of production from LAB

EPS production in LAB depends on both environmental conditions and bacterial strain. Thermophilic LAB usually produce EPS during growth (growth-associated), while mesophilic LAB, common in meat fermentation, often show growth-independent EPS production. In growth-associated strains, EPS is mainly synthesized during the exponential phase, whereas growth-independent strains may produce EPS at various stages, including the stationary phase, especially under stress. EPS production patterns can differ even among strains of the same species. Over time, EPS degradation occurs, likely due to cell lysis and enzyme activity in the culture medium(“Usage of in situ exopolysaccharide-forming lactic acid bacteria in food production: Meat products—A new field of application? - Loeffler - 2020 - Comprehensive Reviews in Food Science and Food Safety - Wiley Online Library,” n.d.).

*I.3.2.1*Availability of carbon and nitrogen

EPS production by LAB is influenced by the availability and type of carbon and nitrogen sources. Sucrose enhances HoPS production and viscosity in fermented products. Both the amount and type of carbon source can affect EPS yield and structure. An optimal carbon-to-nitrogen ratio is critical for maximizing EPS output, as shown by increased yields when nitrogen levels were raised in culture media(“Usage of in situ exopolysaccharide-forming lactic acid bacteria in food production: Meat products—A new field of application? - Loeffler - 2020 - Comprehensive Reviews in Food Science and Food Safety - Wiley Online Library,” n.d.).

*I.3.2.2*Temperature

Growth temperature can significantly inhibit EPS synthesis. Temperature plays a crucial role in the microbial synthesis of EPS. For LAB and Bifidobacteria, optimal EPS production generally occurs between 26 to 31 °C A reduction of 10 °C below the optimum(“Usage of in situ exopolysaccharide-forming lactic acid bacteria in food production: Meat products—A new field of application? - Loeffler - 2020 - Comprehensive Reviews in Food Science and Food Safety - Wiley Online Library,” n.d.).

*I.3.2.3*pH

Optimal EPS production by LAB typically occurs at pH 5.5–6.5, though this may not align with the optimal pH for microbial growth. Very low or high pH values inhibit both growth and EPS synthesis. Maintaining a constant, controlled pH during fermentation increases EPS yields(“Usage of in situ exopolysaccharide-forming lactic acid bacteria in food production: Meat products—A new field of application? - Loeffler - 2020 - Comprehensive Reviews in Food Science and Food Safety - Wiley Online Library,” n.d.).

I.3.3Structure of EPS

Extracellular polymeric substances (EPSs) are high-molecular-weight carbohydrate biopolymers produced by microorganisms—such as bacteria, fungi, yeasts, and microalgae—mainly during their growth and metabolism. These secondary metabolites play crucial roles in microbial survival, including forming protective microenvironments, supporting biofilm development, aiding pathogenicity, promoting cell adhesion, and maintaining structural integrity. EPS production is typically triggered during the late exponential or stationary growth phases in response to environmental stresses like extreme temperatures, pH imbalances, salinity, radiation, toxic chemicals, antibiotics, heavy metals, and oxidative agents. However, some EPSs are also produced under normal metabolic conditions.

Once synthesized, EPSs exist in two main forms:

- Capsular polysaccharides: tightly bound to the cell surface, forming a protective layer.
- Slime polysaccharides : loosely associated or secreted into the environment, aiding in adhesion, colonization, and defense against

stress(“Microbial Exopolysaccharides: Structure, Diversity, Applications, and Future Frontiers in Sustainable Functional Materials,” n.d.).

*I.3.2.1*Chemical structure

EPS sexhibit complex chemical structures that range from linear to highly branched heteropolysaccharides, due to diverse combinations of sugar-based monomeric units. Recent analytical methods assess the bonding patterns of these monomers by converting EPSs into partially methylated alditol acetates, which are then analyzed using gas chromatography–mass spectrometry (GC-MS). To study their structure in detail, EPSs can be selectively fragmented through Smith degradation. The use of linkage-specific enzymes and nonspecific mild acid hydrolysis can further reduce branching and molecular weight. These resulting low-molecular-weight fragments are then characterized by GC-MS and nuclear magnetic resonance (NMR) spectroscopy. Additionally, matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS) can be employed for structural analysis (“A Study on Isolation and Identification of Exopolysaccharide (EPS ...,” n.d.).

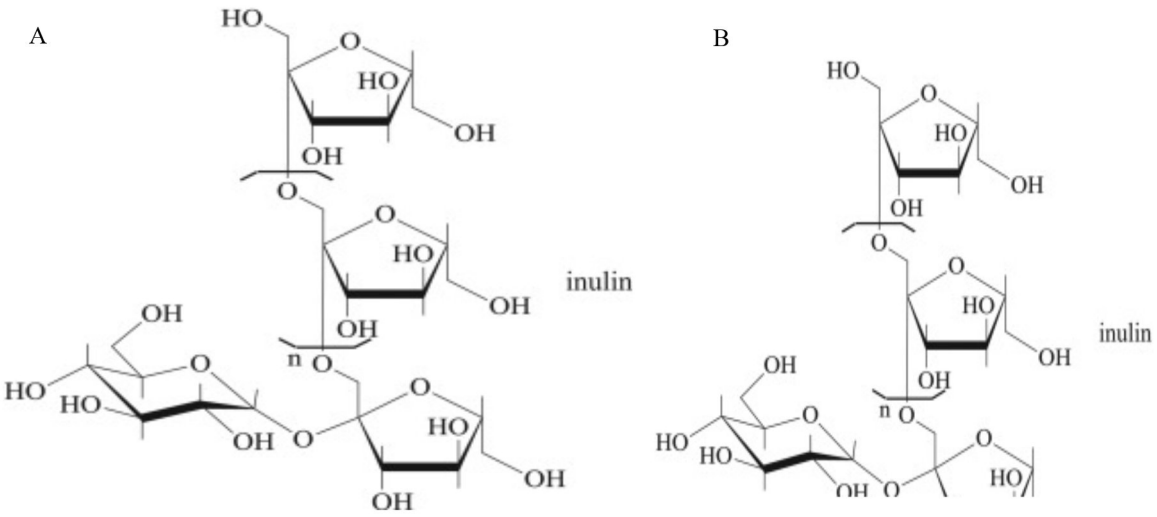


Figure 1:chemical structure of A: Inulin and B: curdlan

I.3.2.2Classification and chemical composition of EPSs

Microbial EPSs produced by LAB are broadly categorized into two main types: HoEPS and HeEPS. Figure 1 provides an overview of the classification of EPS derived from LAB.

In microbial cells, both heteropolysaccharides and some homopolysaccharides are synthesized and secreted into the extracellular environment. Notably, the synthesis of certain homopolysaccharides occurs outside the cell, following the secretion of specific enzymes.Homo-exopolysaccharides are further classified into four subgroups:

. α - D - g l u c a n s

. β - D - g l u c a n s

.Fructans

.Polygalactans

Among α-D-glucans, dextrans are produced by *Leuconostoc (Le.) mesenteroides* subsp. *mesenteroides* and/ subsp. *dextranicum*, while alternans are derived from *Le. Mesenteroides*, and mutans from *Streptococcus (St.) mutans* and *St. Sobrinus*.These glucans mainly consist of glucose units linked *via*α-1.6 and α-1.3 bonds,withoccasional branching at positions2 and 4β-D-glucans, produced by species of *Pediococcus (Pe.)* and *Streptococcus*, are composed of glucose residues connected predominantly *via*β-1.3 linkages, with branching at β-1.2 positions.

Fructans, produced by *St.Salivarius*, consist of fructose units linked mainly by β-2.6 bonds,withβ-2,1branchingattheO1position.Polygalactans, another type of HoEPS, are composed of repeating galactose units with identical chemical structures connected byvaryingglycosidicbonds.

HeEPS are produced by both mesophilic and Thermophilic LAB. The main mesophilic LAB include *Lactococcus (Lc.) lactis* subsp. *lactis*, *Lactobacillus (Lab.) rhamnosus*, *Lc. lactis* subsp. *cremoris*, *Latilactibacillus (Lat.) sakei*, and *Lacticaseibacillus (Lcb.) casei*. In contrast, key thermophilic LAB species are *Lactobacillus (Lab.)*subsp. *bulgaricus*, *Lab. Acidophilus*,*Lab. Helveticus*and*St.thermophilus*.The sugar linkage patterns of selected EPS produced by LAB are illustrated in Figure 2.***("(PDF) Exopolysaccharide and lactic acid bacteria," 2025)***

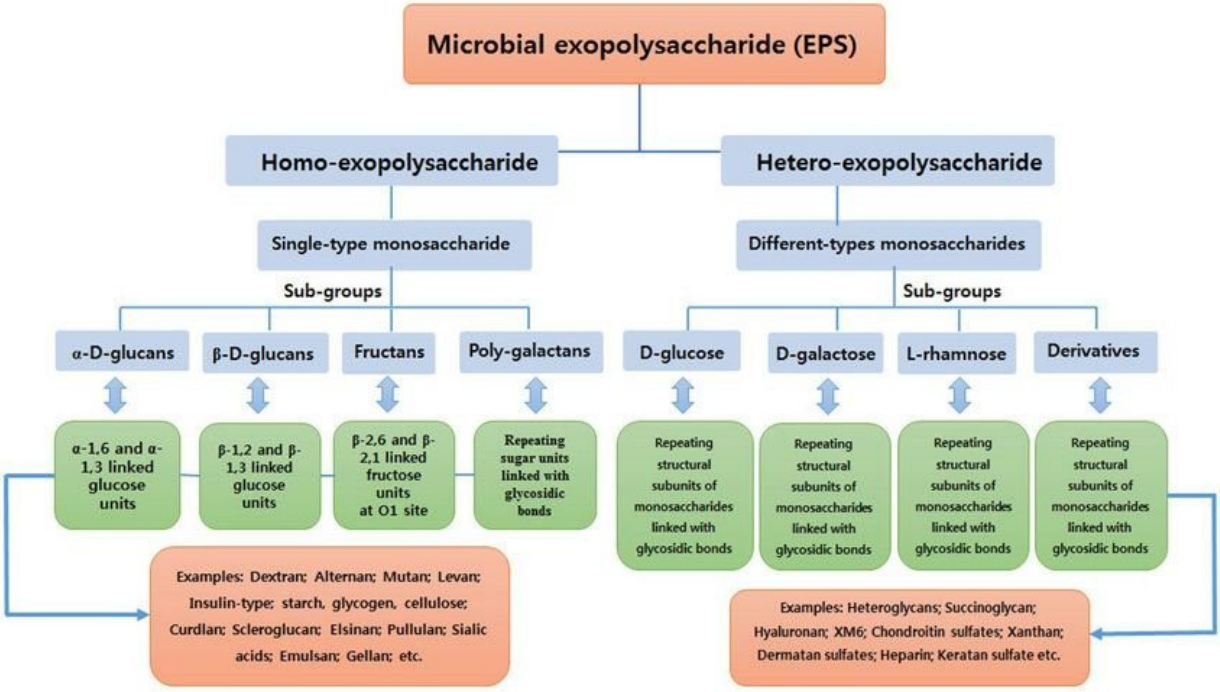


Figure 2 :classification of bacterial EPSs(“Classification of bacterial exopolysaccharide | Download ...,” n.d.).

I.3.3Biosynthetic pathways

EPSs are produced through conserved biosynthetic pathways among microbes. HoPS aresynthesized by a single enzyme, while HePS require multipleenzymes and regulatory genes. Two enzyme groups are involved: one for sugar nucleotideprecursors and another for EPS-specific functions. HoPS synthesis is simpler, occurringextracellularly with sucrose or other substrates, and catalyzed by glycosylhydrolases. Incontrast, HePS synthesis is complex and energy-intensive, involving sugar uptake,precursor formation, membrane transport, and polymerization. HePS vary in physicalproperties due to compositional differences. LAB typically produce more HoPS, andfactors like pH, temperature, and medium influence EPS yields, which can be greatly improved through optimization(Jurášková et al., 2022).

I.3.4Methods of microbial EPS Screening, isolation andcharacterization

Although many researchers have investigated EPS production by LAB, knowledge regarding the kinetics of EPS synthesis remains limited. Variations in

production yield and EPS composition can result in a complete loss of production, as numerous factors influence both the quantity and structure of EPS. These factors include cultivation time, environmental conditions, and the type of carbon source used, all of which can significantly affect the chemical composition, structural integrity, and biological activity of the produced EPS. Moreover, each microbial strain has unique physiological requirements that influence its capacity to synthesize EPS.(Jurášková et al., 2022a) Given the strain-specific nature of EPS production and its dependence on medium composition and incubation parameters (e.g., temperature, humidity), selecting an appropriate methodology for screening, isolation, and purification is essential. In recent years, a variety of techniques have been developed and refined to study EPS derived from LAB, covering aspects such as detection, extraction, and compositional and structural analysis. Screening for EPS-producing strains typically involves cultivating LAB in media supplemented with various sugars—such as glucose, fructose, sucrose, galactose, or lactose.(Jurášková et al., 2022a) A simple and commonly used approach to evaluate EPS production is the visual assessment of colony morphology. EPS-producing colonies often display distinct phenotypic traits: slimy colonies exhibit a mucilaginous texture, while ropy colonies produce thread-like filaments that stretch when an inoculation loop is lifted from the colony or cell pellet (Jurášková et al., 2022a)

I.3.5Physicochemical properties, biological activities and applications of the EPS

*I.3.5.*1Fermented products

The use of LAB in meat fermentation has ancient roots and remains essential in producing a wide range of traditional foods. Today, lactobacilli and pediococcus are the most common LAB used to improve food safety in raw meat by producing lactic and acetic acids, competing for nutrients, and releasing bacteriocins. These actions also enhance meat’s texture, moisture, and color. Some meats are suitable carriers for probiotics due to minimal heat processing, though high salt, low pH, and low water activity challenge bacterial survival. EPS produced by certain LAB strains can offer protective benefits under these conditions. Research shows that fermented meats may better preserve probiotic viability than dried forms. For example, EPS-producing *Pediococcus* strains have

been found in traditional Turkish sausages. As demand for lower-fat meat products rises, LAB-derived EPS has been explored as a natural texture enhancer. In fat-reduced products like German Teewurst, EPS improved spread ability and overall quality. Recent reviews highlight LAB capable of forming EPS in various meat types, including cooked and fermented sausages. Although promising, consistent EPS production must be carefully controlled to ensure uniform quality, posing a challenge for broader industrial application(Korcz and Varga, 2021).

*I.3.5.2*Technological features of EPS

EPS exhibit valuable technological features—such as stabilizing, gelling, thickening, and water-binding—determined by their physicochemical properties, including degree of polymerization, molecular weight, and structural configuration. In the food industry, EPS serve as emulsifiers, viscosity enhancers, stabilizers, gelling agents, and moisture retainers in a variety of products like beverages, yogurt, jams, bread, jellies, and salad dressings, enhancing consistency, texture, mouth feeland reducing syneresis. Beyond food applications, their unique characteristics and health-promoting benefits support their use in diverse industries such as pharmaceuticals, cosmetics, textiles, adhesives, and wastewater treatment. Additionally, EPS offer functional bioactivities, including antimicrobial, antioxidant, cholesterol-lowering, anticoagulant, antitumor, and immunoregulatory effects(Ali et al., 2023).

*I.3.5.3*Agricultural Applications

LAB’s EPSs show promising potential in agriculture for promoting plant growth and enhancing disease resistance. Research indicates that applying LAB EPSs to soil can significantly improve root development by forming a protective matrix around the roots. This matrix enhances soil structure, improves water retention, and facilitates better nutrient uptake. In greenhouse trials, plants treated with LAB EPSs exhibited longer and more branched roots compared to untreated controls. Moreover, LAB EPSs can trigger systemic resistance in plants against pathogens by activating defense mechanisms, such as the production of pathogenesis-related proteins and phytoalexins,thereby lowering the incidence and severity of plant diseases.

LAB EPSs demonstrates broad utility across various sectors, ranging from improving human health and food quality to advancing drug delivery systems and boosting agricultural productivity. Their adaptability and advantageous properties continue to support innovation and research across multiple industries.

I.3.5.4Biological Activities of Exopolysaccharides (EPSs)

EPSs have demonstrated a range of significant health benefits, including antioxidant, cholesterol-lowering, antitumor, antiviral, and immunomodulatory effects. Reactive oxygen species (ROS) such as hydroxyl radicals ($OH^{\bullet}$), superoxide ($O2^{\bullet-}$), and nitric oxide ($NO^{\bullet}$) are associated with the development of serious conditions like Parkinson's disease, atherosclerosis, cancer, and rheumatoid arthritis. EPSs possess strong antioxidant and free radical scavenging activities, which help neutralize these ROS.The antioxidant properties of EPSs can also be applied to inhibit the oxidation of vegetable oils. For instance, EPS isolated from *Rhizobium meliloti* strain has been shown to effectively inhibit lipid oxidation in sunflower oil emulsions during a 50-hour holding period at 60°C.EPSs from probiotic bacteria have also demonstrated anti-ulcer activity. Studies using acetic acid-induced gastric ulcers and ethanol-induced erosion models in rats revealed the anti-ulcer effects of *Bifidobacteriumbreve* strains YIT4014 and 4043, and *Bifidobacterium bifidum* YIT4007. Both the bacteria and their polysaccharide fractions (PSFs) were effective, particularly those rich in rhamnose. Polysaccharides with rhamnose content exceeding 60% were especially potent in promoting gastric ulcer healing.
Furthermore, EPSs have shown the ability to stimulate the immune system and exert anticancer effects, making them promising candidates for therapeutic applications.

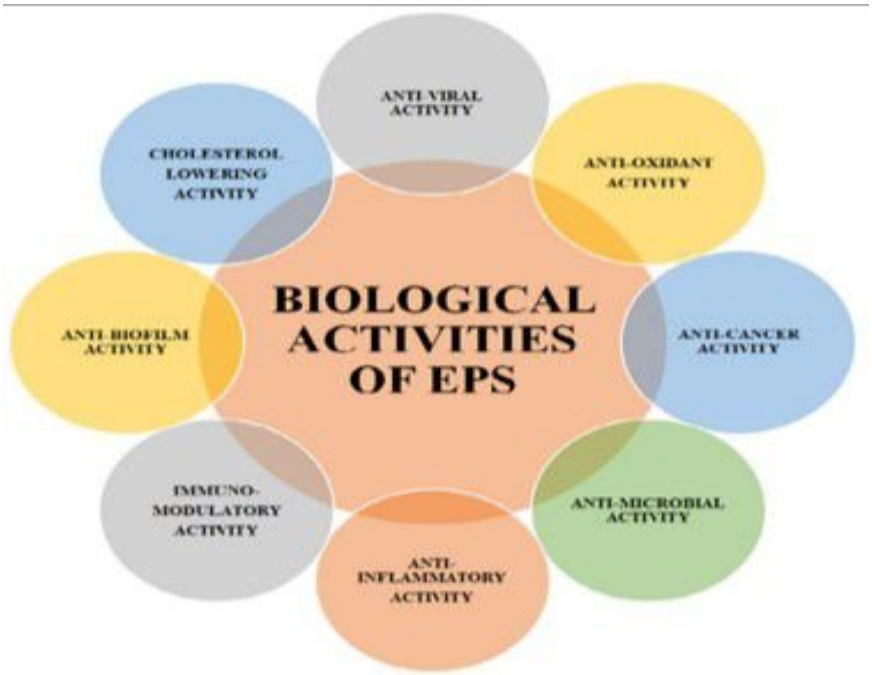


Figure 3:Biological activities of EPS("A Study on Isolation and Identification of Exopolysaccharide (EPS ...," n.d.).

*I.3.5.5*Antioxidant Activity of EPS fromLAB

LAB have attracted significant scientific interest due to their potential health benefits,particularly their antioxidant capabilities. A growing body of research has demonstrated that LAB exhibit various antioxidant mechanisms, including free radical scavenging, inhibition of lipid peroxidation, metal ion chelation, and the enhancement of antioxidant enzyme activities. These properties play an important role in reducing oxidative stress, which is implicated in the pathogenesis of numerous chronic diseases.Among LAB, strains belonging to the *Bifidobacterium* and lactobacilli genera have been extensively studied and are reported to effectively scavenge free radicals such as 2.2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals. These findings suggest their potential application in functional foods and therapeutic formulations aimed at oxidative stress mitigation. To comprehensively evaluate the antioxidant activity of a specific LAB strain, commonly employed assays include DPPH and ABTS radical scavenging tests, as well as nitric oxide inhibition analysis. These assays provide a reliable indication of the strain's ability to neutralize reactive species and support its potential health-promoting effects(Vougiouklaki et al., 2023).

- **DPPH radical scavenging activity:** The 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay is one of the most widely recognized and frequently used methods for evaluating antioxidant capacity. This review adopts a general perspective on the application of the DPPH assay. Numerous studies have explored its use, including adaptations for different analyses, efforts to identify compounds with the highest antioxidant activity, and attempts to optimize measurement procedures. Given its broad usage, it is crucial to implement standardized protocols for the DPPH assay, particularly regarding the choice of reaction media and assay conditions to ensure consistency and comparability of results(“DPPH Radical Scavenging Assay,” n.d.).
- **Hydroxyl radical scavenging activity:** The hydroxyl radical scavenging assay is a laboratory method used to assess the ability of compounds to neutralize hydroxyl radicals—highly reactive species known to cause extensive cellular damage. This assay evaluates the interaction between test substances and hydroxyl radicals, emphasizing their antioxidant potential. By quantifying a compound’s capacity to scavenge these radicals, the assay provides valuable insights into its effectiveness in mitigating oxidative stress and its potential therapeutic relevance(“DPPH Radical Scavenging Assay,” n.d.).

Material and methods

II. Material and methods

This research was conducted between February and May 2025 at the Pedagogical Laboratories of Biology (PLB), Faculty of Natural and Life Sciences, Yahia Fares University, Ouzera Campus, Médéa, Algeria.The study aimed to investigate the production of exopolysaccharides (EPS)by lactic acid bacteria (LAB) isolated from meat. In addition, the quantification of EPS by selected strains, the determination of its nature as well as to evaluate its antioxidant activity. EPS production was quantified using the phenol-sulfuric acid method. Antioxidant activity EPS was evaluated based on ability to scavenge reactive oxygen species (ROS) and reduce oxidative stress.
This work seeks to explore the relationship between EPS from LAB and its antioxidant activity.

II.1Culture medium

The bacterial culture medium is an artificial environment used in laboratories to provide optimal conditions for bacterial growth and reproduction. This medium consists of nutrients such as carbon sources (like sugars), nitrogen (from proteins or meat extracts), and mineral salts, and may include specific additives to stimulate or inhibit the growth of certain bacteria. One of the most well-known media is MRS medium, specially designed for the growth of lactic acid bacteria, as it contains a balanced mixture that meets their nutritional needs while suppressing the growth of competing microorganisms.

To prepare MRS medium we Mix the ingredients (in the table 1) and dissolve them in a container containing 1 liter of distilled water, after preparing the MRS broth, dispense it into falcons or test tubes and sterilize them.

Table 2 :Standard MRS Broth composition

Ingredients	G/L
Peptone	10
Meat extract	10
Yeast extract	5
Glucose	20
Sodium acetate	5
Magnesium Sulfate	0.1
Manganese sulfate	0.05
Dipotassium hydrogen phosphate	2
Ammonium sulfate	2
Tween 80	1
Final PH (at 25°C)	6.5+-0.2

II.2LAB strains and growth conditions

LAB strains previously isolated from laboratory-made Kaddid and artisanal *Kaddid*meat (Boubakri et al., 2022; Boubakri et al., 2025) are listed in Table 2. Stock cultures were stored at –80 °C in de Man, Rogosa, and Sharpe (MRS) broth containing 25 % (w/v) glycerol. Purity was confirmed by verifying a catalase-negative reaction and Gram-positive staining. Once purity was established, new pure-strain stocks were prepared and conserved at both +4 °C (short term) and –80 °C (long term).

Before each experiment, strains were subcultured three times (1 %; v/v) in MRS at 30 °C for 18 h under static conditions. At the third subculture, mid-exponential growth (18 h) was confirmed by measuring the optical density at 600 nm.

Table 3 : Lactic acid bacteria strains used in this study

LAB strains	Accession number	Origine
Enterococcus (E.)foecium ***K11*** *E. faecium* ***K19***	AP019394.1 AP019394.1	Laboratory-prepared *kaddid*
Kaddid K1 *K2* *K3* *K4* *K5* *K6* *K9* *K10* *K11* *K12* *K13* *K15* *K16* *K17* *K18* *K19* *K20* *K21* *K22* *K23* *K24* *K25* *K26*	JQ653151.1	Laboratory-prepared *Kaddid* (Boubakri et al., 2022)

K27 *K28* *K29*		
porapantarurn K7 ***Lpb. paraplantarum K8***	CP053337.1 CP053337.1	Laboratory-prepared *kaddid*
Pediococcus acidilactici BK14 *Pediococcus acidilactici BK17*	CP050079.1 CP050079.1	Traditional South-Western Algerian *kaddid* samples (Boubakri et al. 2022)
We.cibaria *We.confusa*	CP059407.1	Laboratory-prepared *kaddid*

II.3Phonotypical detection EPS production

The ropy or mucoidphenotypeswere evaluated from three times refreshed LAB culture in MRS broth, then the 4th activation was made for preadaptation in modified-sugar-MRSby replacing glucose with varying concentrations of sucrose (10 g, 25 g, and 50 g)dissolved in 0.5 L of distilled water. Each culture was spotted in the same modified- sugar-MRS agar and incubated at 30 °C during 48h. Repines or mucoid aspects were examined by touching the colony with a loop. *Latilactibacillus (Lat.) sakei*CRL1407 was used as positive control.

II.4EPSlevel production by selected LAB

II.4.1Extraction of EPS-producers LAB

Fresh 18-hour LAB subcultures were inoculated at 1% (v/v) into 50 mL of modified MRS broth supplemented with 50g/L sucrose. The cultures were incubated statically at 30 °C for 72 hours. Following incubation, the samples were centrifuged at 6000 × g for 30 minutes to recover the supernatants.

EPS extraction was performed using a modified method based on Geel-Schutten G (1998). Briefly, each collected supernatant was mixed with two volumes of cold ethanol (4 °C) and incubated overnight at 4 °C to allow EPS

precipitation. The resulting mixture was centrifuged at 2000 × g for 15 minutes, and the EPS pellet was collected. To improve purity, the pellet was resuspended in distilled water, re-precipitated by adding two volumes of cold ethanol, and centrifuged again at 2000 × g for 15 minutes. The EPS was washed with absolute ethanol to remove residual medium components, and the final precipitates were dried at room temperature.

II.4.2 Quantification of EPS-producers LAB

The quantification of exopolysaccharides (EPS) was carried out using the phenol-sulfuric acid method, a widely used colorimetric technique known for its simplicity, rapidity, and accuracy. This method was selected for its effectiveness in determining total carbohydrate content in EPS samples.(Yadav et al., 2024a) . In this study, 500 µL of the EPS-containing sample was mixed with 500 µL of 5% phenol solution (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), followed by the rapid addition of 2.5 mL of concentrated sulfuric acid (98%, VWR Chemicals, Radnor, PA, USA). The mixture was incubated in the water Bath at 30°C for 10 min, then allowed to cool to room temperature. The absorbance of the resulting yellow-orange complex was measured at 490 nm using a spectrophotometer. D-glucose (Sigma-Aldrich, Merck KGaA, Darmstadt and Germany) was used to construct a standard calibration curve. EPS concentrations were expressed as milligrams of glucose equivalents per plate(“Characterization of Bacterial Exopolysaccharides Produced from Different Fruit-Based Solid Media,” n.d.).

II.5 Evaluation of antioxidant activity of EPS-producer LAB

II.5.1DPPH Free Radical Scavenging Activity

The DPPH (2.2-diphenyl-1-picrylhydrazyl) radical scavenging activity of intact Lactobacillus cells was assessed using a modified method based on(Li et al2020). Briefly, 1 mL of a bacterial cell suspension was mixed with 1 mL of freshly prepared DPPH solution (0.05 mM in ethanol) in Eppendorf tubes and incubated in the dark for 1 hour. A solution of ascorbic acid (1 mg/mL in distilled water)served as the positive control.

Following incubation, samples were centrifuged at 10,000 × g for 10 minutes at 20°C, and the absorbance of the supernatant was measured at 600 nm using a

SAFAS MP 96 micro plate reader. The scavenging activity (%) was calculated as follows:

Scavenging activity (%) = [1 − ((A_sample − A_blank) / A_control)] × 100

Where:

A_sample=absorbance of the sample

A_blank = absorbance of SS with DPPH

A_control = absorbance of SS alone

II.5.2Evaluation of Hydroxyl Radical (HO•) Scavenging Activity

Hydroxyl radical scavenging activity was determined using the Fenton reaction. Intact cells suspended in 1 mL of SS were added to glass tubes containing 1 mL of brilliant blue (0.435 mM) and 2 mL of $FeSO_4$ (0.5 mM). The reaction was initiated by adding 1.5 mL of 3% H_2O_2 and incubating the mixture at 30°C for 1 hour.

Ascorbic acid (1 mg/mL) served as the positive control, while SS without cells was used as the negative control. After incubation, samples were centrifuged at 3,000 × g for 10 minutes at 20°C, and the absorbance of the supernatant was recorded at 600 nm. The scavenging activity (%) was calculated using the formula:

Scavenging activity (%) = [(A_s − A_0) / (A − A_0)] × 100

Where:

A_s = absorbance of the sample

A_0 = absorbance of the control (without sample)

A = absorbance of the blank (without H_2O_2).

II.6FourierTransform Infrared (FTIR) Spectroscopy for Solid-State analysis methodology and functional group identification

In the context of Fourier-transform infrared (FTIR) spectroscopy, solid sample preparation begins by finely grinding the sample with dry potassium bromide (KBr) in a ratio of approximately 1–2% of the sample weight. The mixture is then pressed to form a transparent pellet suitable for measurement. The instrument is turned on and allowed to warm up if necessary, followed by a background scan to eliminate any interferences. Instrument parameters should be

set as follows: a spectral range of 4000 to 400 cm^{-1}, a resolution of around 4 cm^{-1}, and a number of scans between 8 and 32 depending on the required precision. During spectrum acquisition, the prepared sample is placed in the designated compartment of the spectrometer, and the measurement is initiated. Once the spectrum is obtained, it is saved for analysis. For spectrum interpretation, characteristic absorption bands are identified: a broad O–H stretch between 3200 and 3600 cm^{-1}, a sharper N–H stretch around 3300 cm^{-1}, C–H stretching between 2800 and 3100 cm^{-1}, C≡C or C≡N between 2100 and 2260 cm^{-1}, a strong C=O stretch between 1650 and 1750 cm^{-1}, C=C or C=N between 1500 and 1650 cm^{-1}, and C–O or C–N between 1000 and 1300 cm^{-1}. For more accurate identification, the obtained spectrum is compared with reference spectra or spectral databases to deduce the probable molecular structure or determine the functional groups present in the sample.

II.7 Statistical analysis

Graph Pad Prism 7.04 software was used to assess the statistical analysis of the results by the use of two-way ANOVA tests, followed by *Tukey* test multiple comparison between samples or *Dunnett's* multiple comparison test when comparing to the controls,where $P < 0.05$ was considered significant difference unless otherwise indicated. All the test values were the means of at least two independent assays with the adequate technical repetitions and expressed as mean ± standard deviation.

Results and Discussion

III. Results and discussion

III.1. Phonotypical evaluation of EPS production from *Kaddid*-borne LAB

EPSs are high-molecular-weight, complex carbohydrates that are secreted by various microorganisms, notably LAB, as extracellular bio-polymers, simplest method to assess EPS production is through visual inspection of colony phenotypes, specifically looking for slimy(mucoid) or ropy appearances(Yadav et al., 2024b). A *mucoid* phenotype is indicated by mucilaginous, sticky colonies, whereas a ropy phenotype is identified by the formation of long, thread-like filaments when an inoculation loop is lifted from the surface of the colony or cell pellet(Jurášková et al., 2022b).In addition,EPS-producing strains can exhibit two distinct phenotypes like*ropy* or *non-ropy*. These phenotypes are determined by the localization of the exopolysaccharides—either secreted into the surrounding medium as free EPSs (fEPSs) or retained on the bacterial surface as capsular EPSs (cEPSs), respectively(Yadav et al., 2024b) Both forms of EPSs have been shown to enhance technological properties such as viscosity, creaminess, shear resistanceand water-holding capacity. However, *ropy* cultures typically exhibit higher apparent viscosity than *non-ropy* ones (Mende et al., 2016; Zeidan et al., 2017). According to Cirrincione et al. (2018), *ropy* strains contribute to a smoother texture and greater viscosity in fermented dairy products. Additionally, EPSs act as effective viscosity enhancers, improving the sensory experience by prolonging the product’s presence in the mouth (Nampoothiri et al., 2017).

Table4: Evaluation of EPS production by observing *ropy* or*mucoid*colonies

LAB	EPS aspect	
	ropiness	**mucoid**
Lat.sakei K5	+	-
K25	+	-
Lat.sakei subsp. sakeiK21	+	-
K22	+	-
K24	+	-
K26	+	-
Lpb.paraplantarum K17	+	-
K19	+	-
K21	+	-
E.faecium K15	+	-
K19	-	++
K21	+	-
Pe.acidilactici BK14	-	+++
BK17	+	-
We.cibariaBK2	-	+++
BK3	-	++
BK4	-	+++
We.confusaBK6	+	-
BK11	+	-
Lat. Sakei CRL 1407	+++	-

Positive test (+, ++, +++); negative test (-). *Lat. sakei* CRL1407 (CERELA culture collection) was used as positive control for gas and EPS production.

During the phenotypic characterization of the isolated LAB, particular attention was given to the physical appearance of colonies grown on MRS agar. Notably, the strains *Weissella cibaria* BK2, *Weissella confusa* BK4and *Pediococcus acidilactici* BK14 displayed a distinct mucoidphenotype. This characteristic was especially evident in the formation of shiny, viscous colonies, suggestive of significant EPS production. The presence of the mucoid appearance is typically associated with the secretion of extracellular polysaccharides, which play a crucial role in biofilm

formation, stress resistance and potential functional applications in food matrices. These observations are consistent with the visual evidence presented in Figure 5, supporting the hypothesis that these strains are strong EPS-producers.

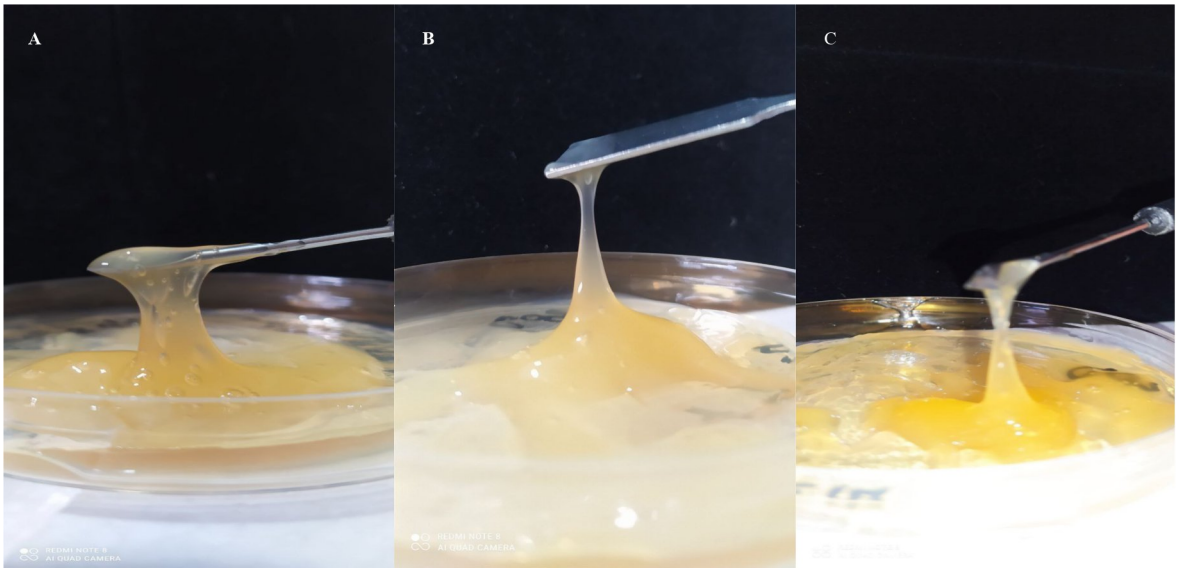


Figure 4 : Stringy and viscous appearance of EPS-producing LAB strains cultivated on sucrose-containing media.The images illustrate the pronounced mucoid formation observed in strains(A): *We.cibaria*BK2, (B): *We. confusa*BK4and (C):*Pe. Acidilactici* BK14.

In the study reported byJin et al. (2019) , all 156 LAB colonies grown on sucrose-containing media exhibited pronounced sticky and viscous characteristics, distinguishing them from those cultivated with other carbohydrate sources. From these, ten putative EPS-producing strains were selected based on their mucoid, slimy, and string-forming appearance. Among them, *we. Confusa* VP30 demonstrated the highest EPS production, reaching 59.99 ± 0.91 g/L in mMRS broth, while the other strains produced less than 0.3 g/L. This high EPS yield was consistent with its significant mucoid phenotype. These findings align with previous reports highlighting the role of *Weissella* species, in spontaneous lactic acid and alcoholic fermentations, and their growing interest in research due to their remarkable EPS-producing capabilities.

III.2.Quantification of EPS from selected mucoid-producers *Kaddid*-borne LAB

The figure 5 evaluates the production of EPS by three (3) selected mucoid-producing LABstrains isolated from traditional *Kaddid*. EPS production was measured by the absorbance level at OD_{480} using saccharose as the carbon source. Each bar in the chart represents the mean ± standard deviation from three technical replicates. According to one-way ANOVA (nonparametric) followed by *Tukey*'s multiple comparison test, no statistically significant differences were observed among the three strains (*Pe. acidilactici*BK14, *We. cibaria* BK2, and *We. confusa* BK4). This is indicated by the shared letter "a" above each bar and the label "ns" (not significant), suggesting similar EPS production levels across the strains. The statistical analysis was conducted with a high confidence level (99.9%), and significant differences were considered at $P< 0.0001$. Overall, the results show that all three strains exhibited comparable EPS production under the same experimental conditions.

Figure 5: Evaluation of EPS production by selected ropy-producers*Kaddid*-borne LAB strains

Results representing the absorbance (OD_{480}) level of EPS from saccharose.Each bar represents the mean ± SD from duplicate performed in 3 technical repetitions.

According to One-way ANOVA (nonparametric) followed with *Tukey* testmultiple comparison between groups (*P*<0.05) with99.9% confidence interval, bars not sharing similar letters differ significatively (*P*-value<0.0001).

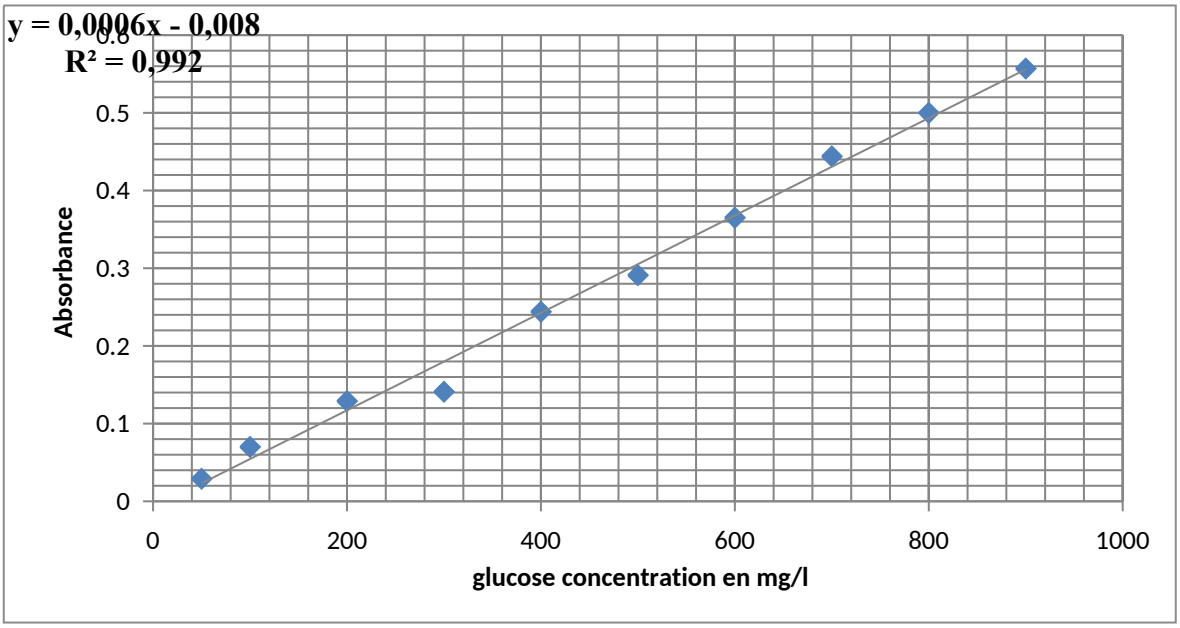


Figure 6: standard curve of glucose

In the study published by Di Cagno et al. (2006), the production of EPS was examined in various strains of *We. Cibaria*, *Lactiplantibacillus (Lpb.) plantarum*, and *Pe. Pentosaceus* isolated from sourdough. After six days of incubation at 30 °C in an MRS-based medium supplemented with sucrose as the sole carbon source, EPS production ranged from 5.54 to 7.88 mg/mL. The strains produced glucans during active growth, followed by fructans in the later stages of incubation.

Both *W. cibaria* and *Lpb. plantarum* demonstrated strong EPS-producing capabilities, reaching concentrations of approximately 2.5 g/kg during sourdough fermentation. Importantly, EPS synthesis was found to be strictly dependent on sucrose, with no EPS formation observed when glucose or fructose was used. Genetic analysis revealed the presence of *eps*D-like glycosyltransferase genes in both species, indicating the involvement of conserved biosynthetic pathways in EPS production.

In a study conducted by Valerio et al. (2020), the ability of different strains of *We. Cibaria* and *Lpb. plantarum* to produce EPS using sucrose as a carbon source

was investigated in a semi-industrial liquid medium containing 10% sucrose (mMRS_S). Contrary to previous findings, such as those reported by Di Cagno (2006) and the results obtained in my own experiment, the *Lpb. plantarum* ITM21B strain showed no ability to produce EPS under the same conditions. In contrast, certain *we. Cibaria* strains, such as C43-11, exhibited high EPS production levels exceeding 18 g/L, while other *we. Confusa* strains produced lower amounts, ranging from 1 to 2 g/L. These results suggest that EPS production is not solely dependent on the bacterial species but is significantly influenced by the specific strain, even within the same species. This variability contradicts my findings, in which the three studied strains (*Pe. acidilactici* BK14, *We. cibaria* BK2and *We. confusa* BK4) did not show significant differences in EPS production. Therefore, the study by Valerio et al. (2020) highlights that the physiological behavior regarding EPS production can vary markedly between strains, underscoring the importance of individual strain characterization before their use in food or industrial applications.

III.3.Evaluationof antioxidative activity of purified EPS from LAB

III.3.1.DPPH scavenging activity (DPPH-SA)

The DPPH assay is a common method for evaluating antioxidant activity by measuring the ability of compounds to scavenge free radicals, thereby indicating their potential to inhibit lipid oxidation(Škrovánková et al., 2012). Certain LAB, particularly those from the genera *Bifidobacterium* and *Lactobacillus*, have been shown to reduce levels of free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Therefore, assessing the antioxidant potential of a specific strain can be effectively carried out through DPPH radical scavenging and nitric oxide inhibition assays (Vougiouklaki et al., 2023).

The DPPH radical scavenging activity of *Kaddid*-borne LAB strains was found to be strain-dependent, ranging from 32.2%to 52.02%. These results are illustrated in Figure 7.

Figure 7: Evaluation of the 2.2-diphenyl-1-picrylhydrazyl free radical ($DPPH^{\cdot}$) scavenging activity (%) for Kaddid-borne EPS-producers LAB strains and ascorbic acid (1 mg/mL)

Was used as positive control. Each bar represents the mean ± SD from duplicate performed in 3 technical repetitions. One-way ANOVA (nonparametric) with $P<0.05$ and 99.9% confidence interval was used, when it followed by Tukey test multiple comparison between the groups, bars not sharing the same letters differ significantly. While when it followed by Dunnett's multiple comparison test to the control positive, bars superscript asterisks differ significantly.

The results of the DPPH assay showed that ascorbic acid (1 mg/mL) used as a positive control, exhibited the highest free radical scavenging activity(105.23%), reflecting its strong antioxidant capacity as a standard compound. In comparison, the EPS from LAB strains isolated from *Kaddid* demonstrated significantly lower antioxidant activity. *Dunnett*'s test confirmed a highly significant difference ($P< 0.001$) between ascorbic acid and all tested strains. Among the strains, *Pe.aidilactici* BK14 showed the lowest activity(32.75%), while. *We. Cibaria* BK2and *We. Cibaria* BK4 displayed moderate activity with no statistically significant difference between them52.02 and 40.86%respectively. According to *Tukey*'s test, the difference in letters (a, b) indicates statistically significant variation among groups, with ascorbic acid forming a distinct group from

the LAB strains. Although the antioxidant activity of the EPS from bacterial strains was lower than that of the positive control, the presence of measurable scavenging activity suggests their potential in reducing free radicals.

Similar results were observed byAkinyemi et al. (2024)*We. cibaria* GM 93m3 showed the least (6–57%),also (Badea et al., n.d.) 2024 Observed that *We. Cibaria* Recorded percentage of 49.82%,while*Pe. Acidilactici* recorded between49.08 to 50.51%,It is higher than the percentage of results we have.

III.3.2. Hydroxyl radical scavenging activity

Hydroxyl radicals (HO•) can be generated through the Fenton reaction, which involves the interaction of hydrogen peroxide (H_2O_2) with reduced transition metals like Fe^{2+}. These radicals are considered the most reactive among the reduced oxygen species and are believed to play a key role in initiating cellular damage *in vivo* (Duan et al., 2007). Due to their extreme reactivity, hydroxyl radicals can attack a wide variety of biomolecules, including sugars, amino acids, lipids and nucleotides (Wang et al., 2008). Therefore, scavenging these radicals is a crucial antioxidant mechanism for protecting living organisms from oxidative stress(Sowndhararajan and Kang, 2013).

The hydroxyl radical scavenging activity of EPS from *Kaddid*-borne LAB strains was found to be strain-dependant, ranging from 35.63to 159.06% these results are illustrated in figure8.

Figure 8: Evaluation of the Hydroxyl radical ($HO^{\cdot}$) scavenging activity (%) of EPS from *Kaddid*-borne LAB strains and ascorbic acid (1 mg/mL) was used as positive control.

Each bar represents the mean ± SD from duplicate performed in 3 technical repetitions. One-way ANOVA (nonparametric) with $P< 0.05$ and 99.9% confidence interval were used, when it followed by *Tukey* test multiple comparison between the groups, bars not sharing the same letters differ significantly. No significative difference comparison to the control positive when it followed by *Dunnett*'s multiple comparison test.

The evaluation of hydroxyl radical ($HO^{\cdot}$) scavenging activity of purified EPSfrom three different LAB strains isolated from the *Kaddid*, using ascorbic acid (1 mg/mL) as a positive control, revealed significant differences in antioxidant potential. The EPS of the strain *we. Cibaria* BK2 exhibited the highest scavenging activity (159.06%), followed by ascorbic acid (82.29%), while EPS of*Pe. acidilactici* BK14 showed the lowest effectiveness (35.63%). The scavenging activity of EPSproduced by*We. Cibaria* BK4 was moderate (59.32%). Statistical analyses indicated that certain strains, particularly EPS of*We. Cibaria* BK2, demonstrated strong antioxidant activity and hold potential as natural sources of antioxidants, whereas others were less effective. Similar results were observed byŁepecka et al.

(2023) the *Pe.aidilactici*from fermented meat products record a value of 33.33%.However,Yu et al.(2018) reported that EPS produced by*We.Cibaria* exhibited hydroxyl radical scavenging activity of 51.66% similar to *We. Confusa* BK4, but significatively much less compared to *We. Cibaria* BK.

III.4.Chemical characterization of functional group of LAB EPSs by FTIR analysis

FTIR spectrum of the exopolysaccharide (EPS) extract. Characteristic absorption bands were observed at 3032 cm^{-1} (O–H stretching), 1639 cm^{-1} (C=O stretching), representing carbonyl groups such as uronic acids or amides, 1021 cm^{-1} (C–O–C and C–O–H stretching of glycosidic linkages), and 614 cm^{-1} (sugar ring deformation). These signals confirm the polysaccharidic nature of the EPS, in agreement with previously reported microbial exopolysaccharides spectra(Mohite et al., 2019a; Gieroba et al., 2023).

The FTIR spectrum obtained for the isolated compound is consistent with the structural profile of a typical microbial EPSs (Fig10). A broad absorption band centered around 3400 cm^{-1} corresponds to O–H stretching vibrations, indicating the abundance of hydroxyl groups commonly found in carbohydrate chains. The peak at approximately 2920 cm^{-1} reflects aliphatic C–H stretching, associated with methylene and methyl groups in the sugar backbone. Strong absorption bands observed at 1004 and 1104 cm^{-1} are attributed to C–O–C and C–O–H stretching vibrations, confirming the presence of glycosidic linkages that connect monosaccharide units. A weaker band near 1636 cm^{-1} is indicative of C=O stretching, potentially arising from uronic acid residues or amide group. Altogether, these spectral features validate the identification of the substance as a polysaccharide rich in hydroxyl functionalities, likely composed of sugar monomers linked *via* glycosidic bondstypical of EPSs produced by bacterial or fungal strains(Mohite et al., 2019a; Gieroba et al., 2023).

Description:

1. ~3400 cm^{-1} (Broad Peak)
    - O–H stretching vibration

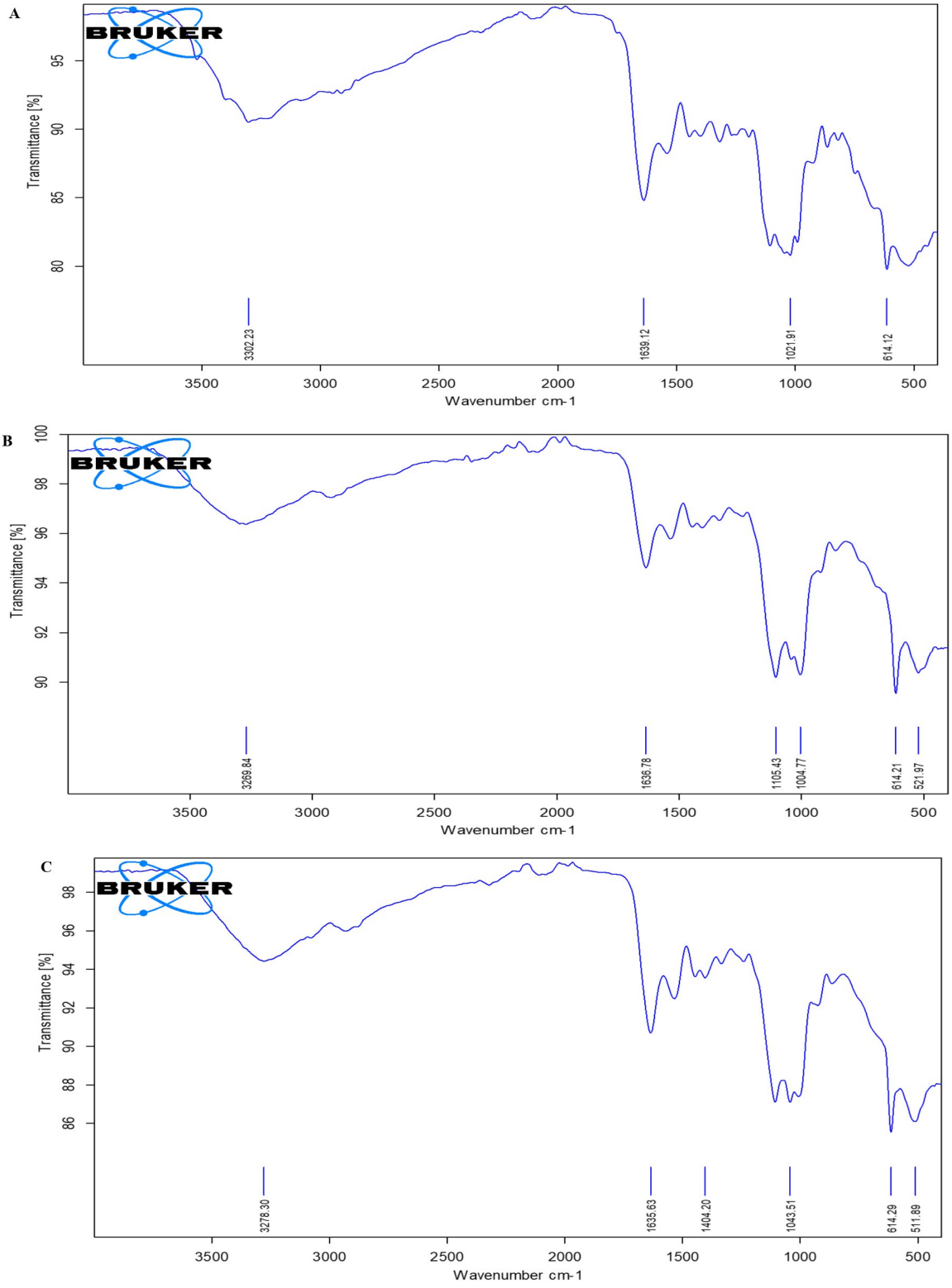
- Strong and broad: characteristic of polysaccharides due to abundant hydroxyl (–OH) groups in sugar rings.

2. ~2920 cm^{-1} (Shoulder/Medium Peak)
    - C–H stretching vibrations
    - Corresponds to –CH and $–CH_2$ groups in sugar backbones.
3. ~1640–1650 cm^{-1} (Weak to Medium Peak)
    - C=O stretching (Amide I or uronic acids) or bound water
    - Could indicate presence of proteins or uronic acid groups (often found in acidic polysaccharides).
4. ~1400 cm^{-1}
    - C–H bending and possibly O–H bending
    - Typical for polysaccharide skeleton.
5. ~1050–1150 cm^{-1} (Strong, Sharp Peaks)
    - C–O–C and C–O stretching vibrations
    - Strong signals in this region are diagnostic for glycosidic linkages (C–O–C) and ring structures.
6. ~890–950 cm^{-1} (Anomeric region)
    - Indicates β- or α-glycosidic linkages
    - A peak near 890 cm^{-1} often corresponds to β-linkages (typical for bacterial EPS).

The FTIR analysis of the third spectrum confirms the polysaccharide nature of the extracted compound. The broad and strong absorption around 3400 cm^{-1} indicates abundant hydroxyl (O-H) groups, which are typical in carbohydrate structures. The peak near 2920 cm^{-1} corresponds to aliphatic C-H stretching vibrations from methyl and methylene groups within the sugar backbone. A weak to medium band around 1640-1650

Cm-¹ suggests the presence of carbonyl (C=O) groups, possibly from uronic acids or amide linkages, or associated water. The signal at approximately 1400 cm-¹ is related to C-H and O-H bending, commonly found in polysaccharide frameworks. Strong and sharp peaks in the range of 1050-1150 cm-¹ confirm the presence of glycosidic (C-O-C) linkages essentialfor sugar polymerization. Finally, the absorption near 890 cm-¹ indicates B-glycosidic

Linkages, which are typical for bacterial exopolysaccharides. Overall, the spectrum strongly supports the identification of the compound as a microbial



exopolysaccharide.

Figure 9: FTIR spectra analysis of the purifiedEPSs produced by, (A): *We. Cibaria* BK2, (B): *We. Confusa* BK4 and (C): *Pe. Acidilactici* BK14.

Results and Discussion

The result has been shown to(Mohite et al., 2019) As part of the structural analysis, FTIR-spectroscopy revealed the presence of several characteristic functional groups, including hydroxyl and carbonyl groups, as well as ether, glycosidic bonds, and sulfate groups. The recorded absorption peaks indicate the presence of organic compounds such as glucose, glucuronic acid, pyruvic acid, succinic acid, and sulfate compounds, confirming the complex, polysaccharide-rich nature of this biopolymer. These components contribute to the negative charge of the EPS, enhancing its ability to bind heavy metal ions. Moreover, the presence of both glucuronic acid and sulfate groups suggests their potential role in biofilm stability and biosorption mechanisms. These findings are consistent with scientific literature on extracellular polymers produced by *Bacillus subtilis* species, highlighting the promising potential applications of these polymers in environmental remediation, particularly in the removal of toxic metals from polluted environments.

Results and discussion

Conclusion

EPS-producers LAB are considered unappropriated for meat fermentation, due to their slime production which is spoilage indicator. However, they can be used for other industrial and/or agricultural applications.In This study, *Kaddid*-borne LAB (Boubakri et al., 2022; Boubakri et al., 2025) were screened for their EPS production potential. At the first stage, LAB were evaluated for their phonotypical ropiness or mucoid colony production. Therefore, three mucoid-producers LAB *We. Cibaria* BK2, *We. Confusa* BK4 and *Pe. Acidilactici* BK14 were selected for further EPS characterization.The EPS of selected LAB strains were subjected to optimization production, extraction and quantification of EPS, the values were yielding to 390.00, 421.11 and 441.66 mg/L for. *We. Cibaria*BK2, *Pe.acidilactici*BK14 and *We. Confusa* BK4 respectively.

The biological effects of purified LAB's EPS were evaluated by in vitro analyses revealed strong antioxidant activity, particularly in scavenging DPPH and hydroxyl radicals. These findings highlight the ability of the selected LAB's EPS to mitigate oxidative stress. Indeed, the EPS from *We. Cibaria* BK2 exhibited the highest DPPH scavenging activity, with a notable removal rate of 52.02%. While EPS from *We. Confusa* BK4 exhibited an activity of 40%. Whereas, *Pe. Acidilactici* BK14 showed moderate scavenging activity at 32.2%. These outcomes point to *We. Cibaria* BK2 as the most promising strain in terms of antioxidant efficacy, making it a potential candidate for use in functional food formulations or natural antioxidant systems.

In the hydroxyl radical scavenging activity, EPS of *We.cibaria*BK2 is the highest with a notable removal of 159.06%, and the EPS from *We. Confusa* BK4 exhibited an activity of 56%,While *Pe.aidilactici* BK14 it was the lowest scavenging activity at 35.63%. This result indicates the strain's high ability to neutralize hydroxyl radicals, which are among the most reactive and damaging oxygen species. From above the efficiencies scavenging of HO• and DPPH• from EPS produced by *W. cibaria* BK2 suggests its potential as a promising candidate for use in food or pharmaceutical applications as a natural antioxidant agent.

Based on the analysis of the FTIR spectra, several characteristic absorption bands indicate the presence of specific functional groups in the samples. The broad peaks around 3300 cm^{-1} suggest the presence of O–H or N–H stretching

vibrations, indicating hydroxyl or amine groups. The strong absorption near 1700 cm^{-1} is typically associated with C=O stretching, confirming the presence of carbonyl-containing groups such as ketones or carboxylic acids. Additionally, bands in the region of 1000–1200 cm^{-1} correspond to C–O stretching, indicating the presence of esters or ethers. As a whole, the FTIR results confirm the presence of various functional groups, reflecting the chemical composition of the sample and supporting the success of the synthesis, extraction, or chemical modification performed on the pure EPS of the tested stains.

Reference

Reference

A Study on Isolation and Identification of Exopolysaccharide (EPS ... [WWW Document], n.d. www.google.com. URL https://www.google.com/imgres?imgurl=https://www.ijraset.com/images/text_version_uploads/image%25201_1349.png&tbnid=gRenNzNHdyxjSM&vet=1&imgrefurl=https://www.ijraset.com/research-paper/isolation-and-identification-of-exopolysaccharide-eps-roducing-bacteria-from-soil&docid=LcInVhiCgJWTgM&w=376&h=381&hl=fr-FR&source=sh/x/im/can/1&kgs=6ccb4e0be504d1ac&sfr=vfe (accessed 5.14.25).

Adelekan, A.O., Olurin, T.O., Ezeani, A.O., 2020. Antioxidant Activities of Exopolysaccharides Produced by Lactic Acid Bacteria Isolated from Commercial Yoghurt Samples. Adv. Microbiol. 10, 359–374. https://doi.org/10.4236/aim.2020.108026

Akinyemi, M.O., Ogunremi, O.R., Adeleke, R.A., Ezekiel, C.N., 2024. Probiotic Potentials of Lactic Acid Bacteria and Yeasts from Raw Goat Milk in Nigeria. Probiotics Antimicrob. Proteins 16, 163–180. https://doi.org/10.1007/s12602-022-10022-w

Aktepe, Y., Aydın, F., Bozoğlu, T., Özer, G., Çakır, İ., 2024. Molecular characterization and multifunctional evaluation of lactic acid bacteria isolated from traditional sourdough. Int. J. Food Microbiol. 423, 110845. https://doi.org/10.1016/j.ijfoodmicro.2024.110845

Ali, A.H., Bamigbade, G., Tarique, M., Esposito, G., Obaid, R., Abu-Jdayil, B., Ayyash, M., 2023. Physicochemical, rheological, and bioactive properties of exopolysaccharide produced by a potential probiotic *Enterococcus faecalis* 84B. Int. J. Biol. Macromol. 240, 124425. https://doi.org/10.1016/j.ijbiomac.2023.124425

Badea, F., Pristavu, M.C., Aldea, C.A., Israel-Roming, F., Matei, F., n.d. IN VITRO SCREENING OF LACTIC ACID BACTERIA AS BIOCONTROL AGENTS FOR BIOPRESERVATION OF PERISHABLE AGRO-FOOD PRODUCTS.

Boubakri, K., Melian, C., Cataldo, P.G., Hebert, E.M., Idoui, T., Vignolo, G., 2025. Exploring the functional and probiotic potential of bacteriocin-producing

Reference

lactobacilli from Algerian *Kaddid*. Biocatal. Agric. Biotechnol. 66, 103597. https://doi.org/10.1016/j.bcab.2025.103597

Carneiro, K.O., Campos, G.Z., Scafuro Lima, J.M., Rocha, R. da S., Vaz-Velho, M., Todorov, S.D., 2024. The Role of Lactic Acid Bacteria in Meat Products, Not Just as Starter Cultures. Foods 13, 3170. https://doi.org/10.3390/foods13193170

Characterization of Bacterial Exopolysaccharides Produced from Different Fruit-Based Solid Media [WWW Document], n.d. URL https://www.mdpi.com/2311-5637/9/7/657 (accessed 6.22.25).

Classification of bacterial exopolysaccharide | Download ... [WWW Document], n.d. . www.google.com. URL https://www.google.com/imgres?imgurl=https://www.researchgate.net/publication/287151403/figure/fig1/AS:614171045531654@1523441196763/Classification-of-bacterial-exopolysaccharide.png&tbnid=XIaCM4jKAD9XQM&vet=1&imgrefurl=https://www.researchgate.net/figure/Classification-of-bacterial-exopolysaccharide_fig1_287151403&docid=bGlrkCrfuM6U6M&w=850&h=486&hl=fr-FR&source=sh/x/im/can/1&kgs=c9da0405e51651e0&sfr=vfe (accessed 5.17.25).

Di Cagno, R., De Angelis, M., Limitone, A., Minervini, F., Carnevali, P., Corsetti, A., Gaenzle, M., Ciati, R., Gobbetti, M., 2006. Glucan and Fructan Production by Sourdough Weissella cibaria and Lactobacillus plantarum. J. Agric. Food Chem. 54, 9873–9881. https://doi.org/10.1021/jf061393+

Donot, F., Fontana, A., Baccou, J.C., Schorr-Galindo, S., 2012. Microbial exopolysaccharides: Main examples of synthesis, excretion, genetics and extraction. Carbohydr. Polym. 87, 951–962. https://doi.org/10.1016/j.carbpol.2011.08.083

DPPH Radical Scavenging Assay [WWW Document], n.d. URL https://www.mdpi.com/2227-9717/11/8/2248 (accessed 6.19.25).

Fadda, S., López, C., Vignolo, G., 2010. Role of lactic acid bacteria during meat conditioning and fermentation: Peptides generated as sensorial and hygienic biomarkers. Meat Sci., Special Issue: 56th International Congress of Meat Science and Technology (56th ICoMST), 15-20 August 2010, Jeju, Korea 86, 66–79. https://doi.org/10.1016/j.meatsci.2010.04.023

Reference

Gieroba, B., Kalisz, G., Krysa, M., Khalavka, M., Przekora, A., 2023. Application of Vibrational Spectroscopic Techniques in the Study of the Natural Polysaccharides and Their Cross-Linking Process. Int. J. Mol. Sci. 24, 2630. https://doi.org/10.3390/ijms24032630

Jin, H., Jeong, Y., Yoo, S.-H., Johnston, T.V., Ku, S., Ji, G.E., 2019. Isolation and characterization of high exopolysaccharide-producing Weissella confusa VP30 from young children's feces. Microb. Cell Factories 18, 110. https://doi.org/10.1186/s12934-019-1158-1

Jurášková, D., Ribeiro, S.C., Silva, C.C.G., 2022a. Exopolysaccharides Produced by Lactic Acid Bacteria: From Biosynthesis to Health-Promoting Properties. Foods 11, 156. https://doi.org/10.3390/foods11020156

Jurášková, D., Ribeiro, S.C., Silva, C.C.G., 2022b. Exopolysaccharides Produced by Lactic Acid Bacteria: From Biosynthesis to Health-Promoting Properties. Foods 11, 156. https://doi.org/10.3390/foods11020156

Korcz, E., Varga, L., 2021. Exopolysaccharides from lactic acid bacteria: Techno-functional application in the food industry. Trends Food Sci. Technol. 110, 375–384. https://doi.org/10.1016/j.tifs.2021.02.014

Lahiri, D., Nag, M., Sarkar, T., Ray, R.R., Shariati, M.A., Rebezov, M., Bangar, S.P., Lorenzo, J.M., Domínguez, R., 2022. Lactic Acid Bacteria (LAB): Autochthonous and Probiotic Microbes for Meat Preservation and Fortification. Foods 11, 2792. https://doi.org/10.3390/foods11182792

Łepecka, A., Szymański, P., Okoń, A., Zielińska, D., 2023. Antioxidant activity of environmental lactic acid bacteria strains isolated from organic raw fermented meat products. LWT 174, 114440. https://doi.org/10.1016/j.lwt.2023.114440

Martinussen, J., Solem, C., Holm, A.K., Jensen, P.R., 2013. Engineering strategies aimed at control of acidification rate of lactic acid bacteria. Curr. Opin. Bioethanol, Food biotechnology • Plant biotechnology 24, 124–129. https://doi.org/10.1016/j.copbio.2012.11.009

Microbial Exopolysaccharides: Structure, Diversity, Applications, and Future Frontiers in Sustainable Functional Materials [WWW Document], n.d. URL https://www.mdpi.com/2673-4176/5/3/18 (accessed 5.14.25).

Mohite, B.V., Koli, S.H., Rajput, J.D., Patil, V.S., Agarwal, T., Patil, S.V., 2019a. Production and characterization of multifacet exopolysaccharide from an

Reference

agricultural isolate, Bacillus subtilis. Biotechnol. Appl. Biochem. 66, 1010–1023. https://doi.org/10.1002/bab.1824

Mohite, B.V., Koli, S.H., Rajput, J.D., Patil, V.S., Agarwal, T., Patil, S.V., 2019b. Production and characterization of multifacet exopolysaccharide from an agricultural isolate, Bacillus subtilis. Biotechnol. Appl. Biochem. 66, 1010–1023. https://doi.org/10.1002/bab.1824

(PDF) Exopolysaccharide and lactic acid bacteria: Perception, functionality and prospects, 2025. . ResearchGate. https://doi.org/10.3329/bjp.v11i1.23819

Sowndhararajan, K., Kang, S.C., 2013. Free radical scavenging activity from different extracts of leaves of Bauhinia vahlii Wight & Arn. Saudi J. Biol. Sci. 20, 319. https://doi.org/10.1016/j.sjbs.2012.12.005

Usage of in situ exopolysaccharide-forming lactic acid bacteria in food production: Meat products—A new field of application? - Loeffler - 2020 - Comprehensive Reviews in Food Science and Food Safety - Wiley Online Library [WWW Document], n.d. URL https://ift.onlinelibrary.wiley.com/doi/full/10.1111/1541-4337.12615 (accessed 5.14.25).

Vougiouklaki, D., Tsironi, T., Tsantes, A.G., Tsakali, E., Van Impe, J.F.M., Houhoula, D., 2023. Probiotic Properties and Antioxidant Activity In Vitro of Lactic Acid Bacteria. Microorganisms 11, 1264. https://doi.org/10.3390/microorganisms11051264

Yadav, M.K., Song, J.H., Vasquez, R., Lee, J.S., Kim, I.H., Kang, D.-K., 2024a. Methods for Detection, Extraction, Purification, and Characterization of Exopolysaccharides of Lactic Acid Bacteria—A Systematic Review. Foods 13, 3687. https://doi.org/10.3390/foods13223687

Yadav, M.K., Song, J.H., Vasquez, R., Lee, J.S., Kim, I.H., Kang, D.-K., 2024b. Methods for Detection, Extraction, Purification, and Characterization of Exopolysaccharides of Lactic Acid Bacteria—A Systematic Review. Foods 13, 3687. https://doi.org/10.3390/foods13223687

Yu, H.-S., Lee, N.-K., Choi, A.-J., Choe, J.-S., Bae, C.H., Paik, H.-D., 2018. Antagonistic and antioxidant effect of probiotic Weissella cibaria JW15. Food Sci. Biotechnol. 28, 851–855. https://doi.org/10.1007/s10068-018-0519-6

Reference

Annexes

