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Exopolysaccharides from *Lactiplantibacillus plantarum* C7 Exhibited Antibacterial, Antioxidant, Anti-Enzymatic, and Prebiotic Activities

Taroub Bouzaiene [1,*], Mariem Mohamedhen Vall [1], Manel Ziadi [1], Ines Ben Rejeb [1], Islem Yangui [2,3], Abdelkarim Aydi [4], Imene Ouzari [5] and Hamdi Moktar [1]

[1] Laboratoire d'Écologie et de Technologie Microbienne, Institut National des Sciences Appliquées et de Technologie (INSAT), University of Carthage, Tunis 1080, Tunisia; maryemmed246@gmail.com (M.M.V.); manel_z52@yahoo.fr (M.Z.); ines.benrejeb@insat.ucar.tn (I.B.R.); moktarhamdi@insat.ucar.tn (H.M.)
[2] Higher Institute of Agronomy of Chott-Mariem, University of Sousse, BP. 47, Chott Mariem, Sousse 4042, Tunisia; yanguiislam@gmail.com
[3] Laboratory of Management and Valorization of Forest Resources, National Research Institute of Rural Engineering, Water and Forests (INRGREF), University of Carthage, BP. 10, Ariana 2080, Tunisia
[4] French School Victor Hugo, Gontardstraße 11, 60488 Frankfurt am Main, Germany; aydiabdelkarim@gmail.com
[5] Laboratory of Microorganisms and Active Biomolecules, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis 2092, Tunisia; imene.ouzari@fst.utm.tn
* Correspondence: taroub.bouzayane@insat.ucar.tn

Abstract: Previously, the exopolysaccharides produced by *Lactiplantibacillus plantarum* C7 isolated from the broiler intestine have been shown to possess probiotic potential. In this study, we highlighted the capacity of this strain to produce exopolysaccharide (EPS) endowed with several biological activities. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical scavenging activities of EPS were found to be 30.4% and 68.165%, respectively, at 100 mg/mL. The ferrous reducing potential of EPS was measured to be 25.26%. Furthermore, EPS exhibited antibacterial effects against both Gram-negative and Gram-positive pathogens, including *Escherichia coli* ATCC 10536 and *Bacillus cereus* ATCC 11778, with inhibition zones of 22.5 ± 0.70 mm and 20 ± 1.41, respectively. On the other hand, the exopolysaccharide also exhibited excellent inhibitory activity against butyrylcholinesterase (BChE), achieving 70.99% inhibition at a concentration of 10 mg/mL. FTIR spectra were used to characterize functional groups in EPS. Our findings proved that EPS from *Lactiplantibacillus plantarum* C7 could be explored for various applications, particularly in the health and functional food sectors.

Keywords: exopolysaccharide; *Lactiplantibacillus plantarum*; antioxidant; antibacterial; prebiotic properties

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1. Introduction

The global polysaccharide market is expected to grow at a Compound Annual Growth Rate (CAGR) of 7% from 2023 to 2032, with the market size projected to reach USD 265.1 billion by 2032 [1]. This market focuses on complex carbohydrates composed of multiple monosaccharide units sourced from bacteria, algae, fungi, plants, and other sources, used in various industries like the food and beverage industry, the pharmaceuticals industry, and the personal care and cosmetics industry [1,2].

Among polysaccharide products, the interest in exopolysaccharides (EPSs), especially those produced by lactic acid bacteria (LAB), is being driven by several key factors, mainly the increasing consumer demand for eco-friendly and clean-label food products free from artificial additives [3–6].

Lactiplantibacillus plantarum strains are well recognized as significant producers of EPSs [7], which are considered among the most important bioactive compounds produced by this bacterial species [8]. It is a facultative heterofermentative LAB species colonizing many ecological niches, particularly the gastrointestinal tract and fermented foods. The EPS produced by *L. plantarum* have been associated with numerous functional roles and health benefits, like immunomodulatory effects, prebiotic properties, antioxidant activities, and cholesterol-lowering effects [9,10]. In fact, the immunomodulatory effects of these EPSs have been observed to stimulate the production of pro-inflammatory cytokines such as IL-12 and TNF-α, suggesting a potential role in enhancing the immune response [10]. Additionally, they have been proven to stimulate the growth of probiotic strains contributing to health-promoting benefits because of their prebiotic properties [9]. Furthermore, they have been shown to inhibit α-glucosidase and remove cholesterol [10].

On the other hand, these EPSs have been revealed to possess antioxidant properties and neuroprotective effects [11–13], confirming their potential role in combating cognitive decline and memory impairment associated with Alzheimer's disease [14,15].

The bioactivity of EPS produced by *L. plantarum* strains is related to their structure. Indeed, the monomeric composition, molecular weight, and charge of EPSs are strain-dependent, and so are the biological functions (e.g., antioxidant, antitumor, and antibiofilm activity) [5]. Certain structural features may be associated with specific bioactivities. For example, the presence of α-(1 → 3) and α-(1 → 6) linkages in the main chain and α-(1 → 3) linkages in the branched chains of EPS produced by *L. plantarum* has been associated with immunomodulatory activity [16].

In this context, this study aimed to determine the functional potential of exopolysaccharides extracted from a probiotic LAB strain, *L. plantarum* C7, based on the study of their in vitro antimicrobial, antioxidant, anti-enzymatic, and prebiotic activities.

2. Material and Methods

2.1. Probiotic Bacteria

Lactiplantibacillus plantarum C7 was previously isolated from intestinal broiler chicken and proved as a producer of EPS with probiotic attributes, as mentioned in our previous study [17]. The strain was maintained in the collection of the Laboratory of Ecology and Microbial Technology (LETMI), National Institute of Applied Science and Technology, University of Carthage.

2.2. Extraction of EPS

The extraction of EPS from the *Lactiplantibacillus plantarum* C7 strain was carried out on an MRS broth (Scharlab, Barcelona) modified with 10% sucrose (10 g/100 mL). Briefly, 300 mL of the prepared medium was inoculated with *L. plantarum* C7 in an Erlenmeyer flask and subsequently incubated for 24 h at 37 °C. The extraction of EPS was performed according to Chen et al. [18]. The bacterial suspension was centrifuged at 4000 rpm for 30 min at 4 °C to remove the bacterial cells (FIR Labo SV 11RH). The supernatant was collected and mixed with 2 volumes of ethanol in order to precipitate the EPS. The mixture was kept at 4 °C for 24 h and then centrifuged at 3000 rpm for 5 min. The pellet was then dried at 40 °C for 24 h.

2.2.1. Determination of Total Carbohydrate Content of Exopolysaccharide by Phenol Sulfuric Acid Method

Exopolysaccharide (total carbohydrate) production was determined by the phenol sulfuric acid method (Phillips, PU8625) against a glucose standard [19] prepared with different concentrations ranging from 0 to 300 µg/mL.

2.2.2. Fourier Transform Infrared (FTIR) Analysis of Exopolysaccharides

Functional groups of EPS from *L. plantarum* C7 were examined by Fourier Transformed Infrared (FTIR) spectroscopy. FTIR spectra were recorded at room temperature with a

Nicolet Spectrophotometer (model 560). The spectra were obtained using attenuated total reflectance mode with a diamond crystal ranging from 4000 to 400 cm^{-1}.

2.3. Functional Activities of EPS

2.3.1. Assay of Antibacterial Activity of Exopolysaccharides

The antibacterial activities of the EPS were evaluated against seven indicator bacteria: *Escherichia coli* ATCC 10536, Staphylococcus aureus ATCC 6538 Pseudomonas paraaeruginosa ATCC 9027, Klebsiella pneumonia ATCC 10031, Bacillus cereus ATCC 11778, Staphylococcus epidermis ATCC 12228, and Enterococcus hirae ATCC 10541. The test was performed using the agar well diffusion assay according to Chen et al. [20]. In brief, agar plates containing TSG medium (Merck Co., Darmstadt, Germany) were covered with molten agar (7.5 g/L) containing each indicator strain (10^6 cfu/mL) and left to solidify. Subsequently, a hole was made in the center of the plate, and 70 µL of EPS (100 mg/mL) was deposited in the hole and allowed to diffuse at 4 °C for 2 h. After an overnight incubation at 37 °C, the plates were examined, and the diameter of the clear zone around the hole was measured. Gentamicin and distilled water were used as positive and negative controls, respectively.

2.3.2. Antioxidant Activities

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity of Exopolysaccharides

The DPPH free radical scavenging activity of EPS was determined according to the method of Yin et al. [21]. Briefly, an aliquot 50 µL of EPS (100 mg/mL) was added to 950 µL of DPPH radical solution (60 µM) and incubated for 30 min in the dark. Discoloration was measured at 517 nm. Trolox was used as positive control. The percentage of scavenged DPPH radical was calculated using the following formula:

$$\text{Scavenging ability (\%)} = [(A_{control} - A_{sample}/A_{control}] \times 100$$

where A_{sample} is the absorbance of the sample, and $A_{control}$ is the absorbance of the control. The results were expressed in micrograms of Trolox equivalents per milligram of EPS (µg TE/mg EPS).

Determination of 2,2′-Azino-bis(3-Ethylbenzothiazoline-6-Sulphonic Acid) (ABTS) Radical Scavenging Activity of Exopolysaccharides

The ABTS radical cation decolorization assay was performed according to Ben ElHadj Ali et al. [22]. Briefly, a stock solution of the ABTS cation radical was prepared by combining potassium persulfate ($K_2S_2O_8$; 3 mM) and ABTS (8 mM) solutions (v/v). The oxidation of ABTS was performed away from light over 16 h, resulting in a blue-greenish solution. This solution was subsequently diluted in distilled water to yield the stable radical ($ABTS^+$), and the absorbance was adjusted to 0.7 ± 0.2 at 734 nm. An aliquot of 10 µL from the EPS extract was added to 990 µL of the ABTS solution. The mixture was then vortexed and incubated for 20 min in the dark at room temperature. Absorbance was measured at 734 nm, and the percentage of scavenged ABTS radical was calculated using the following formula:

$$\text{Scavenging ability (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where A_{sample} is the absorbance of the sample, and $A_{control}$ is the absorbance of the control. The results were also expressed as micrograms of Trolox equivalents per milligram of EPS (µg TE/mg EPS).

Determination of Ferric Reducing Antioxidant Power (FRAP) of Exopolysaccharides

The FRAP test was carried out following the method of Messaoud et al. [23]. The FRAP reagent was prepared by mixing a solution of TPTZ (10 mM of 2,4,6-tri-2-pyridyl-s-triazine (TPTZ) in 40 mM HCl), a ferric chloride solution (20 mM, $FeCl_3 \cdot 6H_2O$), and an acetate buffer (300 mM, pH 3.6) in proportions of 1:1:10 (v/v). A volume of 900 µL of the FRAP solution was combined with 90 µL of distilled water and 30 µL of the EPS extract. The

mixture was then incubated at 37 °C for 30 min, after which the absorbance was measured at 405 nm. The ferric reducing power was determined using a calibration curve of the iron sulphate solution ($FeSO_4$, 6 H_2O). Each reaction was carried out in triplicate. The results were expressed as millimoles of Fe^{2+} equivalents per gram of extract (mmol Fe^{2+}/g EPS).

2.3.3. Enzyme Inhibitory Activities

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) Inhibitory Activities

AChE and BChE inhibitory activity assays were performed following the method outlined by Eldeen et al. [24], with some modifications. Initially, three buffers were prepared in the following manner: buffer A (50 mMTris-HCl, pH 8), buffer B (buffer A containing 0.1% BSA (bovine serum albumin)), and buffer C (with buffer A containing 0.1 M NaCl and 0.02 M $MgCl_2$).

A volume of 20 µL from the EPS extract was added to 375 µL of buffer B, along with 25 µL of the enzyme solution (AChE or BChE, 0.28 U/mL of buffer B). Subsequently, the mixture solution was incubated for 15 min at 37 °C. Then, 80 µL of 0.15 mM acetylthiocholine iodide (ATCI) or butyrylthiocholine iodide (BTCI), along with 500 µL of DTNB (5,5′-dithiobis-2-nitrobenzoic acid) (0.3 mM in buffer C) were added. The resulting solution was incubated for 15 min at 37 °C, and the absorbance was measured at 405 nm. A control mixture was prepared without the addition of EPS extract. Each reaction was performed in triplicate, and the results were expressed in micrograms of donepezil equivalents per milligram of EPS (µg eq donepezil/mg EPS).

Alpha-Amylase Inhibitory Activity

The α-amylase inhibitory activity assay was carried out as described by Sekar et al. [25], with minor adjustments. In brief, 40 µL of the EPS was mixed with 40 µL of α-amylase solution (3 U) and 400 µL of phosphate buffer (20 mM with 6.7 M NaCl, pH 6.7), followed by an incubation period of 10 min at 37 °C. Subsequently, 400 µL of phosphate buffer (20 mM with 6.7 M NaCl, pH 6.7) and 50 µL of starch solution (0.4%) were added to the mixture solution, which was then incubated at 37 °C for 15 min. Finally, 50 µL of iodine reagent (2.5 mm) was added, and the absorbance was measured at 630 nm. The results were expressed as micrograms of acarbose equivalents per milligram of EPS (µg EA/mg EPS).

2.3.4. Prebiotic Activities

The prebiotic activity of EPS was evaluated according to Hubner et al. [26] and the following equation:

$$\mathrm{PAS} = \left[\frac{(\textit{probiotic}\ (\log \mathrm{cfu/mL})\ \textit{on the prebiotic at}\ 24\ \mathrm{h} - \textit{probiotic}\ (\log \mathrm{cfu/mL})\ \textit{on the prebiotic at}\ 0\ \mathrm{h})}{(\textit{probiotic}\ (\log \mathrm{cfu/mL})\ \textit{on the glucose at}\ 24\ \mathrm{h} - \textit{probiotic}\ (\log \mathrm{cfu/mL})\ \textit{on the glucose at}\ 0\ \mathrm{h})}\right] - \left[\frac{(\textit{enteric}\ (\log \mathrm{cfu/mL})\ \textit{on the prebiotic at}\ 24\ \mathrm{h} - \textit{enteric}\ (\log \mathrm{cfu/mL})\ \textit{on the prebiotic at}\ 0\ \mathrm{h})}{(\textit{enteric}\ (\log \mathrm{cfu/mL})\ \textit{on the glucose at}\ 24\ \mathrm{h} - \textit{enteric}\ (\log \mathrm{cfu/mL})\ \textit{on the glucose at}\ 0\ \mathrm{h})}\right]$$

The probiotic strain we used was *Lacticaseibacillus rhamnosus GG* (ATCC 53103) and *Escherichia coli* ATCC 10536, belonging to the collection of the Laboratory of Ecology and Microbial Technology (LETMI).

2.3.5. Statistical Analysis

All experiments were performed in triplicate, and values were expressed as mean ± SD. To assess the difference in antibacterial activities between standard and EPS extract, Student's *t*-tests were carried out using SPSS software V.20. Data were considered statically significant when $p < 0.05$.

3. Results and Discussion

3.1. Production of EPS

The total carbohydrate in exopolysaccharide extract was determined as 3.679 g eq glucose/L. Lower amounts ranging from 0.2 to 0.9 g/L have been estimated for EPS from *L. plantarum* strains by Li et al. [27]. Additionally, Senturk et al. [28] reported similar

amounts of EPS from several strains of *L. plantarum*. Even lower amounts have been reported for EPS extracted from *L. plantarum* NTMI05 and *L. plantarum* NTMI20 (197 g/L and 0.187 g/L, respectively), isolated from cow milk [29].

3.2. Infrared Spectrum Analysis of EPS by FTIR Spectroscopy

Functional groups of EPS were determined by FTIR spectroscopy, which revealed typical absorption peaks for exopolysaccharides (Figure 1). As shown in Figure 1, the broad intense peak was shown at 3278 cm^{-1}, which indicated the presence of hydroxyl groups (O-H). According to the literature, this result confirmed the polysaccharide nature of the compound [30]. The second peak was observed at 2930 cm^{-1}, demonstrating the presence of the (C-H) stretching vibration of the aliphatic CH_2 group [31]. The absorption peak at around 1620 cm^{-1} corresponded to the stretching vibration of (C-O), which was the carboxyl group [32]. The prominent absorption at 1200–950 cm^{-1} was considered as the fingerprint region of the exopolysaccharides [33]. The FTIR spectra confirmed the presence of both carboxyl and hydroxyl groups in EPS. These groups were revealed to be associated with biological activities of EPS, including antioxidant and antibacterial activities [34].

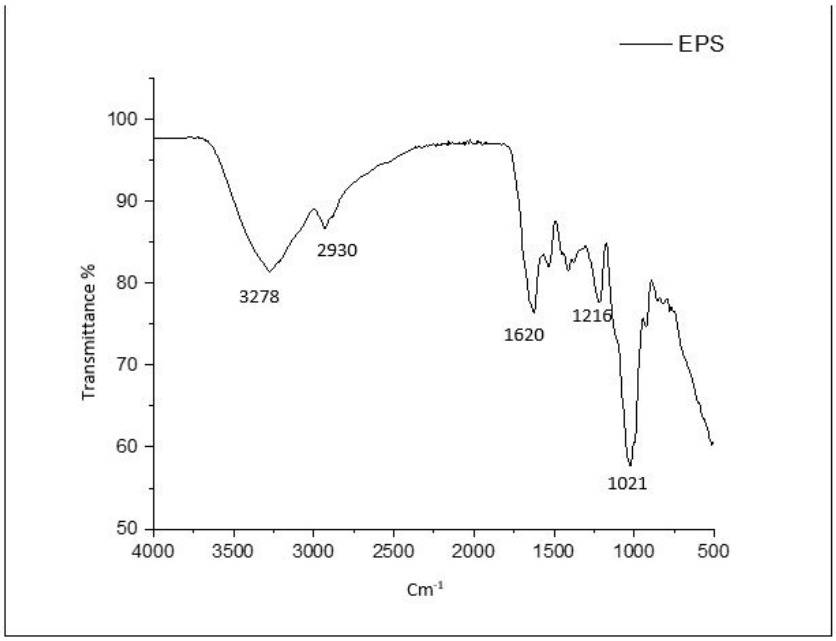

Figure 1. FTIR analysis of EPS extract from *L. plantarum* C7.

3.3. Functional Activities of EPS

3.3.1. Antibacterial Properties of EPS Extract

Various common pathogenic bacteria were used to evaluate antibacterial activities of EPS extracted from *L. plantarum* C7. As shown in Figure 2, the EPS exhibited an antibacterial spectrum against all tested bacteria, including both Gram-Positive and Gram-Negative strains. The greatest antibacterial effect of EPS was observed against *E. coli* ATCC 10536, corresponding to an inhibition zone of 22.5 ± 0.70 mm. This activity was significantly higher than that shown by the Gentamicin (18 mm), as demonstrated by the Student *t*-test ($p < 0.01$). Also, *B. cereus* ATCC 11778, was highly sensitive to the EPS extract, showing an inhibition zone of 20 ± 1.41 mm. However, this inhibition was not significantly different ($p > 0.05$) from that exhibited by the Gentamicin. The antibacterial effect against *K. pneumoniae* ATCC 10031 was significantly lower (9 ± 1.41 mm) in comparison with the Gentamicin ($p < 0.01$). A similar study on EPS from *L. plantarum* isolated from camel milk showed variability in antibacterial effects against *E. coli* and *S. aureus* [35].

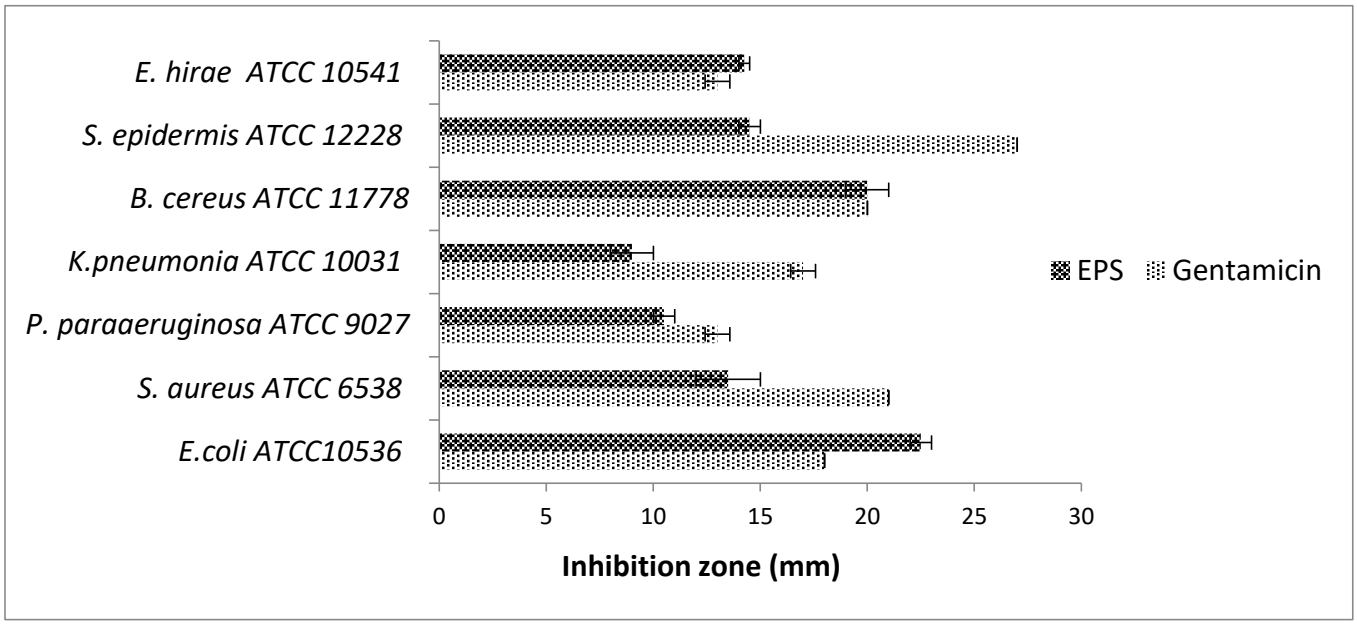

Figure 2. Antibacterial activity of exopolysaccharide extract from *L. plantarum* C7 against tested bacteria compared to Gentamicin.

The antibacterial effect of EPS extracted from LAB has been investigated in numerous studies; even so, the clarifications of the mechanisms are still at a preliminary stage [36,37]. Evidently, the antibacterial activity of EPSs is attributed to their effect on the peptidoglycan layer of the pathogenic bacteria envelope, caused by disrupting its structure [38]. Further, the antibacterial properties of LAB-EPS were essentially due to their functional groups being carbonyl, phosphate, and hydroxyl groups [39]. Additionally, the electrical charge of LAB-EPS might play an important role in the inhibition mechanism of pathogenic bacteria. In this context, Nehal et al. [40] have reported that the negative charge of EPS from *Lactococcus lactis* demonstrated a high inhibitory effect against Gram-positive strains including *B. cereus* ATCC 10702. On the other hand, the presence of EPS could promote the production of secondary metabolites in the growth medium, subsequently affecting the proliferation of both Gram-positive and Gram-negative bacteria [41].

3.3.2. Antioxidant Activity of EPS

The DPPH radical scavenging activity, evaluated in Trolox equivalents per EPS extract, was found to be 292.81 ± 3.28 µg TE/mg Ex, corresponding to a DPPH radical inhibition of 30.4% (Table 1). This result aligns with that of Dilna et al. [42], who reported a DPPH radical scavenging activity of 23.63% for EPS from *L. plantarum* RJF4. Nevertheless, other previous studies have revealed much higher radical scavenging activities for EPS than those found in our study (56.48% and 75.91% from *L. plantarum* R31 and *L. plantarum* C70, respectively) [27,35]. In addition to the DPPH assay, the ABTS radical scavenging assay showed that the EPS extract from *L. plantarum* C7 is capable of scavenging the ABTS radical with an inhibition percentage of 68.165% at a concentration of 100 mg/mL EPS.

This inhibition corresponds to 0.689 ± 0.032 µg TE/mg of EPS. Our findings are consistent with those of Ayyash [43], who demonstrated an ABTS radical inhibition of 49.42% for *L. plantarum* C70. Additionally, our results support previous findings reported by Min et al. [44], who found that the EPS from *L. plantarum* JLAU103 had an ABTS radical inhibition capacity of 65.5%. The ABTS and DPPH radical scavenging activities could be explained by the interaction of EPSs with free radicals, acting as electron donors and thereby converting them into stable forms [45].

Table 1. Biological activities of EPS from *L. plantarum* C7.

Biological Activities		
Antioxidant activities		
DPPH	Inhibition (%)	30.4
	µg TE/mg EPS	292.81 ± 3.28
ABTS	Inhibition (%)	68.16
	µg TE/mg EPS	0.689 ± 0.032
FRAP	Inhibition (%)	25.26
	mmol Fe^{2+}/g EPS	0.0313 ± 0.0027
Enzyme inhibitory activities		
Acetylcholinesterase (AChE) inhibitoryactivity	Inhibition (%)	16.38
	µg DE/mg EPS	0.11 ± 0.01
Butyrylcholinesterase (BChE) inhibitoryactivity	Inhibition (%)	70.99
	µg DE/mg EPS	0.44 ± 0.03
Alpha-amylase inhibitoryactivity	Inhibition (%)	21.078
	µg AE/mg EPS	0.11 ± 0.008
Prebiotic activity		
PAS	0.043	

Regarding the FRAP assay, the EPS extract from *L. plantarum* C7 exhibited a moderate ferrous reducing potential (25.26%) at a concentration of 100 mg/mL of EPS (Table 1). This antioxidant activity, evaluated in mmol Fe^{2+} per gram of extract, was determined to be 0.031 ± 0.0027 mmol Fe^{2+}/g EPS (Table 1). Although, our results differ from those of Bomfim et al. [46], who reported a ferrous ion reducing antioxidant power ranging from 1.1 to 1.2 mmol Fe^{2+}/g for EPS produced by *L. plantarum* CNPC003, they are similar to those of Wang et al. [47]. In their study, antioxidant capacities reaching 0.2 mmol Fe^{2+}/g EPS for *Limosilactobacillus fermentum* S1 were demonstrated [47]. It is worth noting that the values recorded in the ferrous reducing potential assay, which is a rapid method used to measure total antioxidant capacity, varied depending on the studied strain.

Given this, the difference between the antioxidant capacity of EPS measured by DPPH and ABTS radical scavenging activities, as explained by Xu et al. [48], is probably due to the fact that DPPH is a hydrophobic molecule, whereas ABTS is hydrophilic. On the other hand, several authors have attributed the differences in the antioxidant activities of EPS from LAB to EPS concentration, chemical composition, and method(s) of extraction [45]. Additionally, structural characteristics, the presence of active groups, sulfate content, and binding positions are identified as primary factors influencing the antioxidant capacity of bacterial EPS extracts [49,50]. Within the contents of EPS extracts from *L. plantarum* C7, glucose, galactose, glycerol, rhamnose, and ribitol have been previously identified [17]. It has been reported by Zhou et al. [34] that the antioxidant activity of EPS is due to functional groups and monosaccharide compositions such as hydroxyl (OH) groups.

3.3.3. Enzyme Inhibitory Activities of EPS

In the present study, the EPS extract from *L. plantarum* C7 exhibited notable anti-cholinesterase activity, particularly against BChE (70.99% at 10 mg/mL), while showing limited inhibition against AChE (Table 1). The BChE inhibitory activity was determined to be 0.44 ± 0.03 µg eq DE/mg EPS (Table 1). Our results corroborate those of a recent study on EPS from *Bacillus maritimus*, which revealed an excellent BChE inhibitory capacity for EPS, reaching 94.62 ± 2.66% at a concentration of 1000 µg/mL [50]. The observed AChE inhibitory activity was measured to be 16.38% at a concentration of 2 mg/mL, with an

estimated value of 0.11 ± 0.01 µg eq DE/mg EPS (Table 1). Contrasting these findings, Selim et al. [50] demonstrated a more potent AChE inhibitory effect of EPS from *B. maritimus*, ranging from 24.82 ± 0.88% to 60.15 ± 1.85% at concentrations from 100 to 1000 µg/mL. This difference may be attributed to variations in EPS composition, which are known to be highly dependent on the bacterial strains utilized, as well as the sources of carbon and nitrogen present in the growth medium [51].

As it is known, the enzymes AChE and BChE are involved in brain neurotransmission disorders, including Alzheimer disease. Therefore, the EPSs extracted from *L. plantarum* C7, as inhibitors of these enzymes, could be considered as natural products with anti-Alzheimer's properties. These properties seem to be attributed to the high molecular weight of polysaccharides, which exhibit antioxidant and neuroprotective effects against neurotoxicity, thereby safeguarding cells from Alzheimer's disease [52].

Concerning alpha-amylase, our investigation revealed that the EPS extract exhibited moderate inhibitory activity against this enzyme, with a value of 0.11 ± 0.008 µg eq acarbose/mg (Table 1). The inhibition at a concentration of 100 mg/mL was determined to be 21.078%. This finding aligns closely with the result reported by Dilna et al. [42], who observed an alpha-amylase inhibitory activity of 25% at a concentration of 100 mg/mL for EPS from *L. plantarum* RJF4. However, Sasikumar et al. [33] demonstrated a significantly higher capacity of EPS from *L. plantarum* BR2, estimated to be 10% at 100 µg/mL. This inhibitory activity could be attributed to the ability of EPS to obstruct the active site of alpha-amylase, which might lead to a reduction in sugar intake from carbohydrate hydrolysis. Given the involvement of this enzyme in diabetes, the ability of EPS to inhibit it could indicate its potential role in the treatment of the disease [35].

3.3.4. Prebiotic Activity of EPS

EPS from *L. plantarum* C7 showed a positive prebiotic score of 0.043 (Table 1). This finding indicates that the EPS was specifically utilized by the probiotic bacteria *L. s rhamnosus* GG and promoted its growth. Based on the definition of prebiotics as food ingredients that specifically stimulate the growth of gastrointestinal strains, various EPS from lactic acid bacteria have been considered as prebiotic substrates [26,53]. As mentioned by Silva et al. [54], EPS from *L. plantarum* demonstrated a higher prebiotic score by stimulating the growth of probiotic strains, while non-probiotic strains did not exhibit such stimulation.

4. Conclusions

EPS produced by *L. plantarum* C7 exhibited potential functional activities, including antibacterial, antioxidant, prebiotic, and anti-enzymatic activities. Functional groups identified using FTIR spectra revealed the presence of hydroxyl and carboxyl groups that are associated with polysaccharides. These diverse bioactive properties suggest that this EPS has potential applications in food industries, as it could be used as an antioxidant or antibacterial agent, providing a novel product with the health benefits attributed to EPS.

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