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Cellulolytic lactic acid probiotic bacteria from cricket gut: antioxidant and *α*-amylase inhibitory activities of their exopolysaccharide

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Abstract

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This study aimed to isolate cellulolytic lactic acid bacteria (LAB) with probiotic properties from the gut of crickets and to investigate the antioxidant and α-amylase inhibitory activities of the exopolysaccharide (EPS) produced by the selected bacterial strains. Cricket samples were collected from four farms in Ubon Ratchathani Province, Thailand. A total of 14 LAB isolates were obtained and characterized based on their morphological and biochemical properties. The isolates were subsequently screened for their cellulolytic activity, and only one isolate, designated FF02041, exhibited cellulase enzyme production. Comparative analysis of the 16S rRNA gene sequence (1,282 bp) identified FF02041 as *Lactobacillus* sp. This isolate was further evaluated for probiotic potential and demonstrated tolerance to both acidic conditions and bile salts. The EPS extracted from isolate FF02041 exhibited DPPH and ABTS free radical scavenging activities of 15.93% and 25.59%, respectively, at a concentration of 10 mg mL^{-1}. Additionally, the EPS showed ferric reducing antioxidant power (FRAP) of 21.74 ± 0.06 mg Fe^{2+} equivalents per gram of EPS. Furthermore, at the same concentration (10 mg mL^{-1}), the EPS displayed α-amylase inhibitory activity with an inhibition rate of 49.61%, corresponding to 22.74 ± 0.09 mg acarbose equivalents (AE) per gram of EPS. These findings highlight the cricket gut represents a novel reservoir of functional probiotics and bioactive EPS with strong potential for nutraceutical and functional food development.

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1. Introduction

Probiotics are live microorganisms that confer health benefits to the host when consumed in adequate amounts, such as promoting gut microbiota balance and aiding lactose digestion [1]. They are commonly isolated from dairy products, fermented foods, and the digestive tracts of humans and animals. Lactic acid bacteria (LAB) are among the most widely used probiotics, including *Lactobacillus* spp., *Bacillus* spp., *Lactococcus* spp., *Bifidobacterium* spp., and *Streptococcus* spp. [2–7]. To exert probiotic functionality, candidate strains must demonstrate resilience to gastrointestinal stresses, including low pH and bile salts [8].

Crickets are emerging as an economically important insect and a promising protein source for future food production. They are herbivorous insects rich in cellulose-containing plant matter. Herbivores rely on gut-associated microorganisms producing cellulase to degrade cellulose into simple sugars for nutrient absorption. Cellulolytic microorganisms have applications in animal husbandry for improving feed digestibility [9]. For example, cellulolytic LAB from soymilk waste have been proposed as poultry probiotics [10]. Additionally, supplementation of *Bacillus cereus* var. toyoi to sows has been shown to reduce the transmission of *Escherichia coli* to piglets, decrease post-weaning diarrhea, and improve growth performance, with a higher gain-to-feed ratio (680 g kg^{-1}) compared to controls (628 g kg^{-1}) [11, 12].

Microbial metabolites such as exopolysaccharides (EPS) exhibit diverse biological activities, including antioxidant, antimicrobial, immunomodulatory, and gut microbiota modulating effects [13–15]. Type 2 diabetes mellitus (T2DM), characterized by hyperglycemia and impaired carbohydrate metabolism, can be managed partly by inhibiting α-amylase to slow carbohydrate digestion [16, 17]. Acarbose is effective but often causes gastrointestinal side effects [17, 18]. Oxidative stress, driven by excessive reactive oxygen species (ROS) and weakened antioxidant defenses, contributes to diabetic complications. Therefore, strategies that mitigate oxidative stress either by reducing ROS production or enhancing antioxidant capacity are of considerable interest in T2DM prevention and management [19].

EPS from LAB have recently attracted attention for their potential health benefits, particularly their antioxidant and α-amylase inhibitory activities. Sasikumar *et al.* [20] reported that EPS from *Lactobacillus plantarum* BR2 exhibited strong free radical scavenging and α-amylase inhibitory properties, while Ramchandran & Shah [21] demonstrated EPS mediated α-amylase inhibition. Although EPS from conventional LAB sources have been extensively studied, EPS produced by LAB isolated from cellulose-degrading insects remain largely unexplored.

Therefore, this research aims to isolate cellulolytic lactic acid probiotic bacteria from cricket gut and evaluate the antioxidant and α-amylase inhibitory activities of the EPS derived from the selected bacterial strains.

2. Materials and Methods

Sample Collection

Four frozen cricket samples were collected from a commercial cricket farm located in Ubon Ratchathani Province, Thailand. The farm was selected based on its consistent production and availability of harvest-ready crickets during the sampling period. All crickets were 30 - 35 days old, corresponding to the typical age range at which they are harvested for commercial use. The samples were labeled HR, FF, MT, and JD, as summarized in Table 1.

Table 1 Sources of cricket samples in Ubon Ratchathani Province, Thailand.

Sample Source	Location	Code
Hero Farm	90 Moo 15, Rai Noi Subdistrict, Mueang District, Ubon Ratchathani	HR
Fifa Farm	5 Moo 6, Don Khae Alley 11, Kham Yai Subdistrict, Mueang District, Ubon Ratchathani	FF
Kru Maem Cricket Farm	59 Moo 8, Ban Nong Ku, Non Kha Long Subdistrict, Phibun Mangsahan District, Ubon Ratchathani	MT
Jedi Farm	44 Moo 8, Ban Nong Ku, Non Kha Long Subdistrict, Phibun Mangsahan District, Ubon Ratchathani	JD

Isolation and Screening of LAB

LAB were isolated with modifications based on the method of Peng *et al.* [22]. The abdomen was dissected, and the intestines of crickets weighing 1 gram were separated and soaked in 0.85 % (w/v) NaCl solution. Serial dilutions were performed using sterile distilled water. Dilutions from 10^{-4} to 10^{-6} were spread onto the MRS agar and incubated at 28 °C for 48 hours under anaerobic conditions. After incubation, colonies exhibiting characteristics typical of LAB were selected. To obtain pure isolates, purification was carried out via repeated streaking on MRS agar.

Preliminary Identification of LAB

The morphology of the isolated bacteria was studied under a microscope, examining shape, arrangement, and Gram staining. Biochemical properties were also investigated, including catalase activity, indole production, motility, and fermentation of sugars (glucose, cellobiose, lactose, maltose, mannitol, and xylose) [23].

Cellulolytic LAB Screening

Cellulase enzyme activity was assessed using the method described by Mandel & Weber [24]. The isolated LAB strains were cross-streaked onto CMC agar and kept at 28 °C for 72 hours of incubation. The surface was then flooded with iodine and left to stand for 15 minutes. The iodine was removed, rinsed with sterile distilled water, and then flooded with 1M NaCl for 15 minutes. The clear zone formed around the colonies was observed.

Identification of cellulolytic LAB

The 16S rRNA gene of the isolated cellulolytic LAB was amplified using polymerase chain reaction (PCR) with universal bacterial primers targeting the region between positions 27 and 1492 of the 16S rRNA gene [25]. The forward primer 27F (5′-AGA GTT TGA TCT GGC TCA G-3′) and reverse primer 1492R (5′-TAC GGT ACC TTG TTA CGA CTT-3′) were employed under standard amplification conditions, comprising 25 cycles with an annealing temperature of 50 °C. PCR amplicons were purified using the Montage PCR Clean-up Kit (Millipore) and subsequently subjected to Sanger sequencing using the ABI PRISM® BigDye™ Terminator v3.1 Cycle Sequencing Kit in conjunction with AmpliTaq® DNA Polymerase [26]. Sequence data were visually inspected and aligned with reference bacterial lineages available in GenBank (National Center for Biotechnology Information, NCBI) using the BLASTN algorithm, following the analytical approach described by Torkian *et al.* [27].

Phylogenetic analysis

Phylogenetic analysis of the obtained amplicon sequence was conducted using MEGA XI software [28]. The 10 top-ranking BLAST hits for the 16S rRNA sequence of the isolate were selected for phylogenetic reconstruction employing the neighbor-joining method based on the Kimura 2-parameter model [29]. Statistical support for the tree topology was assessed through 1,000 bootstrap

replicates. The resulting phylogenetic tree was rooted and subsequently analyzed.

Evaluation of Probiotic Properties of cellulolytic LAB

Acid Tolerance Test

Fifty milliliters of the isolated LAB culture were incubated for 12 hours at 28 ± 2 °C. The culture was then transferred into MRS broth adjusted to pH 6.7 (control) and pH 3.0 (test). The samples were kept at 28 ± 2 °C for an additional 12 hours of incubation under static (non-shaking) conditions. Every 3 hours, a spectrophotometer (Genesys 20, Thermo Fisher Scientific, USA) was used to measure the optical density at 620 nm to monitor bacterial growth [30].

Bile Salt Tolerance Test

Fifty milliliters of the isolated LAB culture were incubated for 12 hours at 28 ± 2 °C. The culture was then inoculated into MRS broth without bile salts (control) and MRS broth supplemented with 0.3% (w/v) bile salts (test). Incubation continued at 28 ± 2 °C for 12 hours under static (non-shaking) conditions. Every 3 hours, a spectrophotometer (Genesys 20, Thermo Fisher Scientific, USA) was used to measure the optical density at 620 nm to monitor bacterial growth [31].

Extraction of EPS

EPS was extracted following a previously described method [32] with slight modifications. The selected strain was inoculated into MRS medium and incubated at 28 °C for 24 hours. The culture was centrifuged at 4,000 × g for 20 minutes to pellet the cells. The supernatant was carefully transferred to a new tube and boiled at 100 °C for 10 minutes to inactivate enzymes. After cooling to 28 ± 2 °C, 2% (v/v) Sevag reagent (chloroform:n-butanol, 4:1) was added to remove protein contaminants, followed by incubation at 4 °C for 24 hours. The mixture was centrifuged at 8,000 × g for 20 minutes, and the clear supernatant was collected. EPS was precipitated by adding three volumes of chilled absolute ethanol to the supernatant, then incubating at 4 °C for 24 hours. The precipitate was recovered by centrifugation at 8,000 × g for 20 minutes, and the resulting pellet was vacuum-dried to yield purified EPS for further analysis.

Determination of Total Carbohydrate Content of EPS extracts

The phenol-sulfuric acid method was employed to determine the total carbohydrate content in EPS extracts, using D-glucose standard prepared with different concentrations ranging from 156.25 to 5,000 mg L^{-1}. In brief, 150 µL of concentrated sulfuric acid was combined with 50 µL of the extract, followed by the addition of 30 µL of 5% phenol solution. The reaction mixture was incubated at 90 °C for 5 minutes, and the absorbance was measured at 470 nm using a microplate reader (EZ read 2000, Waterbeach, Cambridge, UK).

Assay of α-Amylase Inhibition

The α-amylase inhibition assay was performed according to a previously described method [33]. Briefly, 20 µL of phosphate buffer (pH 6.9), 20 µL of EPS extract, and 20 µL of α-amylase (0.25 U mL^{-1}; Sigma-Aldrich, St. Louis, MO, USA) were mixed and incubated at 37 °C for 10 minutes. Subsequently, 400 µL of 0.5% (w/v) soluble starch and 160 µL of distilled water were added, and the mixture was incubated for an additional 3 minutes at 37 °C. The reaction was stopped by adding 250 µL of dinitrosalicylic acid (DNS) color reagent, followed by boiling for 15 minutes. The mixture was then cooled to 28 ± 2 °C, and absorbance was measured at 540 nm using a microplate reader (EZ Read 2000, Waterbeach, Cambridge, UK). An analytical blank was prepared by following the same procedure but substituting distilled water for the enzyme solution. Acarbose (Thermo Fisher Scientific, Fair Lawn, NJ, USA) served as the positive control. The results were reported as milligrams of acarbose equivalents per gram of EPS (mg AE g^{-1} EPS).

Determination of antioxidant property

DPPH Assay

The DPPH assay using a modification of the method described by Xiang *et al.* [34]. Briefly, 180 µL of 0.1 mM DPPH solution was added into 96 well plate, followed by the addition of 20 µL of EPS (10 mg mL^{-1}) and then incubated in the dark at 28 ± 2 °C for 30 minutes. Subsequently, the absorbance was measured at a 517 nm using a microplate reader (EZ Read 2000, Waterbeach, Cambridge, UK). The positive control was ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) at different concentrations (7.81, 15.62, 31.25, 62.5 and 125 mg L^{-1}). The formula below was used to determine the percentage of DPPH radical scavenging activity.

$$\text{Inhibition \%} = [(\text{A control} - \text{A sample})/\text{A control}]\text{x}100$$

A control is the absorbance of the control reaction (DPPH solution without sample) and *A sample* is the absorbance of the test sample. AAE g^{-1} EPS, or milligrams of ascorbic acid equivalents per gram of EPS, was used to express the results.

ABTS Assay

The ABTS radical scavenging activity of EPS was determined using a modified protocol based on Kim *et al.* [35]. Briefly, the ABTS radical solution ($ABTS^{\bullet+}$) was generated by reacting 2.45 mM potassium persulfate with 7 mM ABTS at a 1:0.5 ratio. The reaction mixture was subsequently stored for 16 hours in the dark at 28 ± 2 °C. Following this, the absorbance at 734 nm was adjusted to 0.7 ± 0.02 by diluting the ABTS radical solution with 95% ethanol. For the ABTS assay, 180 µL of the adjusted ABTS radical solution was mixed with 20 µL of EPS in a 96-well plate. After a 6 minutes incubation in the dark, the absorbance of the mixture was measured at 743 nm using a

microplate reader (EZ read 2000, Waterbeach, Cambridge, UK), with ethanol as the control. The positive control was Trolox (Sigma-Aldrich, St. Louis, MO, USA) at different concentrations (15.62, 31.25, 62.5, 125 and 250 mg L^{-1}). ABTS radical scavenging activity was determined as a percentage inhibition, calculated with the equation below:

$$\text{Inhibition \%} = [(\text{A control} - \text{A sample})/\text{A control}]\text{x}100$$

A control is the absorbance of the control reaction (ABTS radical solution without sample) and *A sample* is the absorbance of the test sample. Additionally, the data were presented as milligrams of Trolox equivalents per gram of EPS (mg TE g^{-1} EPS).

FRAP Assay

The FRAP assay was conducted with modifications based on the method described by Benzie & Strain [36]. To prepare the FRAP reagent, 10 mM TPTZ in 40 mM HCl, 20 mM ferric chloride, and 0.2 M acetate buffer (pH 3.6) were mixed at a ratio of 10:1:1 (v/v/v). To perform the assay, 25 µL sample of the extract was added to 175 µL of the FRAP reagent, followed by incubation at 28 ± 2 °C for 6 minutes. The absorbance was then measured at 593 nm. The reducing power of the samples was calculated using a standard curve of $FeSO_4$ ranging from 31.25 to 500 mg L^{-1}. The FRAP value was expressed as milligrams of Fe^{2+} equivalents per gram of extract (mg Fe^{2+} eq. g^{-1} EPS).

Statistical Analysis

Data are presented as mean ± standard deviation (S.D.) from three independent replicates ($n = 3$). Statistical significance was determined at $P < 0.05$ using one-way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons.

3. Results and Discussion

Isolation and Screening of LAB

A total of 14 lactic acid bacteria (LAB) isolates were obtained from the gut of crickets using MRS agar. The colony morphology of the isolated bacteria was predominantly round and convex with smooth edges, and most colonies were white in color (Fig. 1).

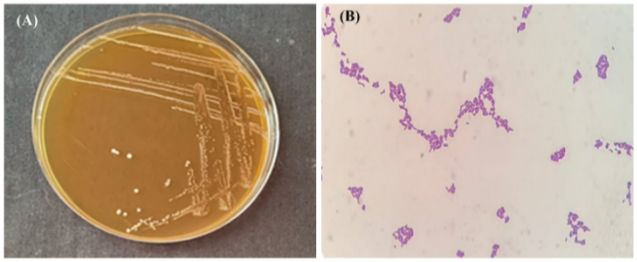

Fig. 1 (A) Colony morphology of isolate FF01062 on MRS agar, and (B) its morphology after Gram Staining and observation under a light microscope at 1,000x magnification.

Morphological and Biochemical Characterization

Gram staining revealed that all LAB isolates were Gram-positive. Of the 14 isolates, seven were cocci (HR0103, FF01042, FF02012, JD0104, MT0101, MT0204, and MT0205), while the remaining seven were rods (FF01011, FF02041, FF01062, MT0106, MT0107, JD0203, and MT0207), each displaying distinct cellular morphologies (Table 2).

Biochemical characterization, including catalase activity, indole production, motility, and sugar fermentation, was conducted (Table 3). All isolates were catalase-negative. The indole test, which detects tryptophanase activity, was also negative for all isolates, indicating the absence of this enzyme. None of the isolates exhibited motility, as evidenced by the absence of spreading growth from the inoculation site.

Sugar fermentation patterns varied among the isolates, consistent with the findings of Thanananta *et al.* [37], who also reported diversity in sugar utilization among LAB strains.

Cellulolytic LAB Screening

Cellulolytic bacteria produce cellulase enzymes that degrade cellulose into simpler sugars, primarily glucose, by hydrolyzing β-1,4-glycosidic bonds [38]. Selecting a cellulase-producing LAB strain can deliver dual functional benefits: probiotic effects and fiber-degrading capacity, both of which can enhance gut health and feed utilization efficiency. Cellulase activity promotes the hydrolysis of dietary cellulose into fermentable oligosaccharides, improving fiber digestibility and generating prebiotic substrates that support both the host and its gut microbiota. Previous studies have shown that cellulolytic *Bacillus cereus* strains can produce short-chain fatty acids *in vivo* while promoting a balanced gut microbiota, highlighting their potential as feed probiotics [39].

The combination of cellulolytic and probiotic traits facilitates bacterial colonization and metabolic activity in the gastrointestinal tract, with practical applications in probiotic-enriched silage and fiber-targeted feed supplements for livestock production.

In the present study, screening for cellulolytic activity revealed that only isolate FF02041 was positive. This strain produced a distinct clear zone on CMC agar following Gram's iodine staining (Fig. 2B-C), indicating hydrolysis of carboxymethyl cellulose (CMC) and suggesting cellulase production [40]. Such clear zone formation is a recognized indicator of cellulase activity and is consistent with previous findings reported by Ghazanfar *et al*. [41]. Based on these results, isolate FF02041 was selected for further characterization and evaluation of its functional properties.

Table 2 Morphological characteristics of LAB isolated from cricket gut.

Sample No.	Code of LAB isolate	Gram stain	Cellular morphology	Cell arrangement
1	HR0103	+	Cocci	Grape-like clusters
2	FF01011	+	Rod	Single
3	FF01042	+	Cocci	Grape-like clusters
4	FF01062	+	Rod	Single
5	FF02012	+	Cocci	Diplococci
6	FF02041	+	Rod	Single
7	MT0101	+	Cocci	Diplococci
8	MT0106	+	Rod	Single
9	MT0107	+	Rod	Single
10	MT0204	+	Cocci	Diplococci
11	MT0205	+	Cocci	Diplococci
12	MT0207	+	Short rod	Paired rod
13	JD0104	+	Cocci	Diplococci
14	JD0203	+	Rod	Paired rod

Table 3 The physiological and biochemical properties of LAB isolated from cricket gut.

No.	LAB isolate code	Catalase	Indole	Motility	Fermentation of sugar					
					Cellobiose	Lactose	Maltose	Mannitol	Xylose	Glucose
1	HR0103	-	-	-	+	-	+	-	-	+
2	FF01011	-	-	-	+	+	+	+	+	+
3	FF01042	-	-	-	+	+	+	-	-	+
4	FF01062	-	-	-	+	-	+	-	+	+
5	FF02012	-	-	-	+	-	+	-	+	+
6	FF02041	-	-	-	+	+	+	+	+	+
7	MT0101	-	-	-	-	-	-	-	+	+
8	MT0106	-	-	-	+	-	-	-	+	+
9	MT0107	-	-	-	+	+	-	-	+	-
10	MT0204	-	-	-	+	-	+	-	+	+
11	MT0205	-	-	-	-	-	-	-	+	-
12	MT0207	-	-	-	-	-	-	-	-	-
13	JD0104	-	-	-	-	-	-	-	+	-
14	JD0203	-	-	-	-	-	-	-	-	-

Identification of cellulolytic LAB

Based on comparative analysis of the 16S rRNA gene sequence of strain FF02041 (1,282 bp), the closest match was found to *Lactobacillus paraplantarum*, partial 16S rRNA sequence from strain CW11, with a similarity of 86.0%. Since species-level identification based on 16S rRNA typically requires ≥98.7–99% sequence identity [42], the isolate could not be confidently assigned to any known species. Therefore, the strain was classified at the genus level as *Lactobacillus* sp. (Fig. 3)

Evaluation of Probiotic Properties of cellulolytic LAB

Acid Tolerance Test

Based on the probiotic properties test, isolate FF02041 demonstrated the ability to survive under acidic conditions for 3 hours, with a survival rate of 74.05%. Hassanzadzar *et al*. [43] indicated that incubation at pH levels of 2 and 3 leads to a decrease in the viability of LAB. The difference in survival rates may be attributed to variations in bacterial strains, sources of isolation, or testing methodologies. For instance, a study on LAB isolated from traditional Persian pickled vegetables identified strains like *Lactobacillus plantarum* exhibiting strong acid tolerance, highlighting the influence of specific strains and their origins on acid resistance [44]. These observations underscore the importance of acid tolerance in selecting probiotic strains, as the ability to withstand low pH environments, such as those found in the human stomach, is crucial for their survival and efficacy.

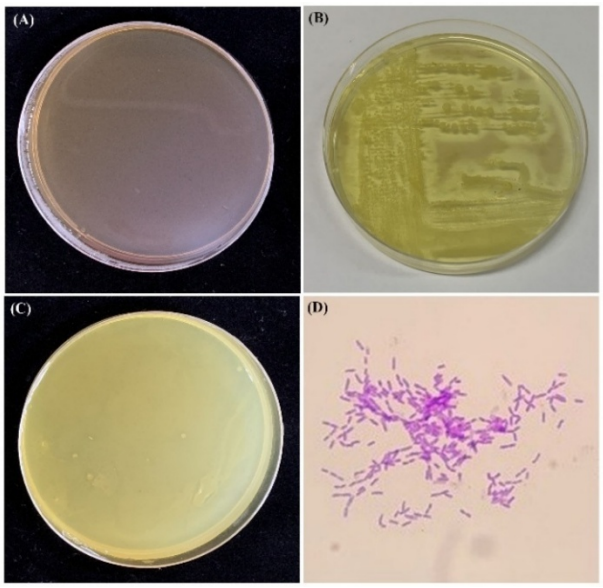

Fig. 2 Cellulolytic activity and morphological characterization of LAB isolate FF02041.
(A) CMC agar plate stained with Gram's iodine.
(B) Clear hydrolysis zone formed by isolate FF02041 on CMC agar after Gram's iodine staining.
(C) Enhanced visibility of the clear zone after flooding the plate with 1 M NaCl.
(D) Cell morphology of isolate FF02041 observed under a light microscope after Gram staining (1,000x magnification).

Bile Salt Tolerance Test

The isolate FF02041 survived in 0.3% (w/v) bile salts for 6 hours with a survival rate of 35.63%, lower than values reported for some probiotic strains. For example, *Lactobacillus acidophilus* TISTR1338 showed 97.68% survival after bile salt adaptation [45], and *Lactobacillus paracasei* and *Lactobacillus curvatus* from bovine colostrum tolerated 0.3 - 0.5% bile salts while maintaining high viability [46].

Crickets lack a liver and thus do not produce bile salts [47, 48], meaning their gut microbiota is not naturally exposed to these compounds and may not have developed tolerance mechanisms such as bile salt hydrolase activity. This likely explains the low bile salt resistance observed in cricket-derived LAB.

For probiotic use in mammals, where intestinal bile salt concentrations typically range from 0.3% to 0.5%, such sensitivity may limit strain survival and functionality. Therefore, bile salt tolerance should be considered a key selection criterion, and strategies like microencapsulation or genetic enhancement may be required to improve viability in the gastrointestinal environment [49].

EPS Production

The total carbohydrate content in the EPS extract from isolate FF02041 was measured at 0.311 g glucose eq. L^{-1}. This value aligns with previous findings by Li *et al.* [50], who reported EPS yields from *Lactiplantibacillus plantarum* strains ranging between 0.2 and 0.9 g L^{-1}. Similarly, Imran *et al*. [51] reported comparable EPS levels for *L. plantarum* NTMI05 and NTMI20 strains isolated from milk, achieving yields of 0.197 g L^{-1} and 0.187 g L^{-1}, respectively. In contrast, Bouzaiene *et al*. [52], reported a significantly higher EPS production of 3.679 g glucose eq. L^{-1} in *L. plantarum* C7.

α-Amylase Inhibitory Activity

The evaluation of the α-amylase inhibitory activity of the EPS from isolate FF02041 revealed that at a concentration of 10 mg mL^{-1}, the EPS exhibited moderate α-amylase inhibitory activity with an inhibition rate of 49.61%. When compared to the standard curve of Acarbose the inhibitory effect corresponds to 22.74 ±0.09 mg AE g^{-1} EPS (Table 4). The α-amylase inhibitory activity of the EPS from isolate FF02041 demonstrates superior efficacy compared to EPS derived from *Lactiplantibacillus plantarum* RJF4 and C7. Specifically, at a concentration of 10 mg mL^{-1}, the EPS from isolate FF02041 exhibited an inhibition rate of 49.61%, corresponding to 22.74 ± 0.09 mg AE g^{-1} EPS. In contrast, the EPS from *L. plantarum* RJF4 exhibited 25% inhibition but at the higher concentration of 100 mg mL^{-1}[53]. Similarly, the EPS from *L. plantarum* C7 demonstrated an inhibition rate of 21.078% at 100 mg mL^{-1} [52]. However, Sasikumar *et al.* [54], demonstrated a remarkably higher capacity for EPS derived from *L. plantarum* BR2, achieving approximately 10% inhibition at just 100 µg mL^{-1}. Further purification and structural characterization of EPS-FF02041 may enhance its inhibitory efficacy and provide insights into its mechanism of action.

Antioxidant Activity

The DPPH radical scavenging activity of the EPS from isolate FF02041, evaluated at a concentration of 10 mg mL^{-1}, was found to be 0.90 ± 0.01 mg AAE g^{-1} EPS, corresponding to a DPPH radical inhibition of 15.93% (Table 4). The observed activity surpasses that previously reported for EPS derived from *L. plantarum* C7, which demonstrated a 30.4% inhibition at a concentration of 100 mg mL^{-1} [52]. In contrast, other studies have documented higher DPPH radical scavenging activities for EPS from different *L. plantarum* strains. For instance, EPS from *L. plantarum* R301 demonstrated a scavenging activity of 97.69% at 4 mg mL^{-1} [55].

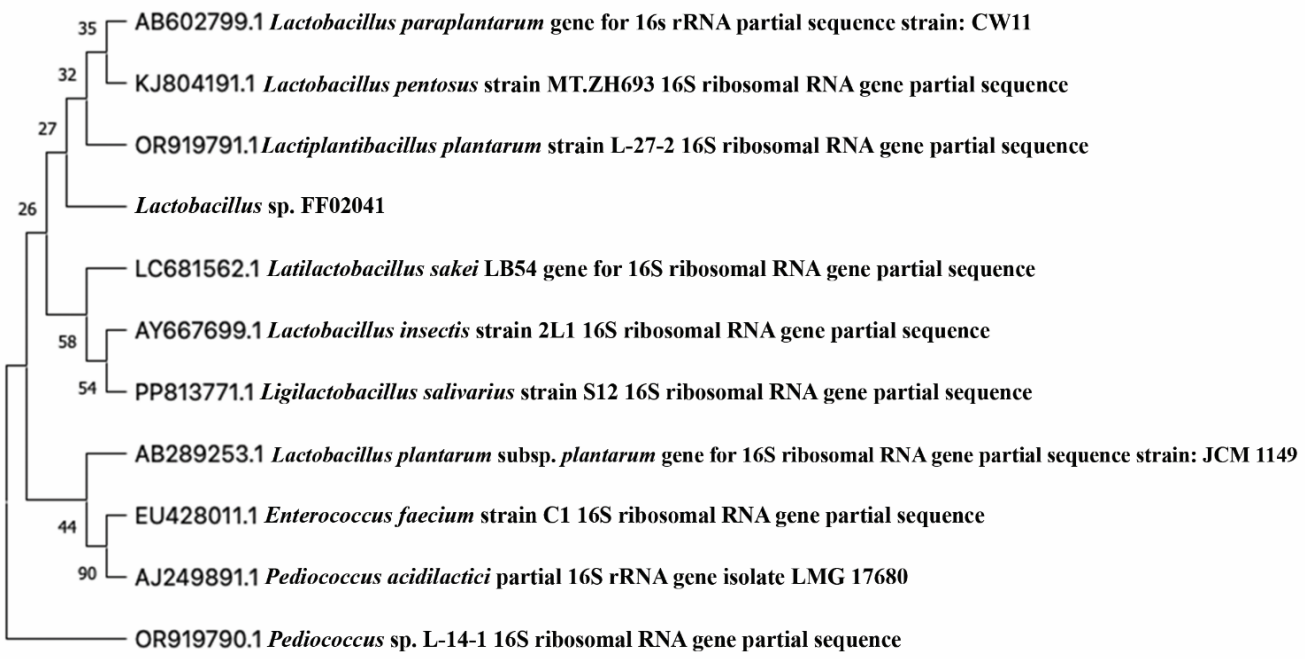

Fig. 3 A phylogenetic tree was constructed using the Neighbor-Joining method in MEGA XI with 1,000 bootstrap replicates.

The ABTS radical scavenging activity of the EPS extracted from isolate FF02041 was evaluated at a concentration of 10 mg mL^{-1}, resulting in an inhibition percentage of 25.59%. This activity corresponds to 3.83 ± 0.14 mg TE g^{-1} EPS. These findings are higher than those reported by Bouzaiene *et al.* [52], who observed an ABTS radical inhibition of 68.165% at a concentration of 100 mg mL^{-1} for EPS derived from *L. plantarum* C7. It is possible to explain the observed DPPH and ABTS radical scavenging activities to EPS's capacity to interact with free radicals, serving as electron donors and stabilizing them into non-reactive forms. This process emphasizes EPS's potential as a naturally occurring antioxidant [56].

The antioxidant capacity of the EPS from isolate FF02041 was assessed using the FRAP assay. The results indicated a reducing power of 21.74 ± 0.06 mg Fe^{2+} eq. g^{-1} EPS, suggesting a moderate antioxidant potential. This value is lower compared to some EPS from other strains. For instance, EPS from *Lactiplantibacillus plantarum* R301 exhibited a reducing power, which was about 30% that of ascorbic acid when both tended to be stable with the increased concentration [55]. These comparisons highlight that while EPS from isolate FF02041 possesses antioxidant properties, its efficacy is moderate relative to certain other *L. plantarum* strains.

The antioxidant and enzyme-inhibitory activities observed may be related to structural features of the EPS, including molecular weight, branching, and functional groups such as hydroxyl, carboxyl, or sulfate [57, 58]. Greater branching or the presence of uronic acids can increase active sites for radical scavenging [59], while α-amylase or α-glucosidase inhibition may result from steric hindrance or binding affinity linked to polysaccharide conformation [60]. Detailed structural characterization in future studies could clarify these relationships and guide the development of targeted EPS-based applications.

Table 4 Bioactivities of EPS from isolate FF02041.

Bioactivities		
α-amylase inhibitory activity	Inhibition (%)	49.61
	mg AE g^{-1} EPS	22.74 ±0.09
Antioxidant activities		
DPPH	Inhibition (%)	15.93
	mg AAE g^{-1} EPS	0.90 ± 0.01
ABTS	Inhibition (%)	25.59
	mg TE g^{-1} EPS	3.83 ± 0.14
FRAP	mg Fe^{2+} eq. g^{-1} EPS	21.74 ± 0.06

4. Conclusion

This study reports the isolation and characterization of a novel cellulolytic LAB from the gut of a cricket, which exhibits probiotic properties along with the production of an EPS demonstrating significant antioxidant and α-amylase inhibitory activities. These findings not only broaden the functional potential of insect-derived LAB but also highlight the cricket gut as a promising niche for the discovery of bioactive microbial candidates. To the best of our knowledge, this is the first report of a cellulase-producing LAB isolated from the gut of a cricket, with

an EPS that exhibits dual bioactivities relevant to metabolic health.

Despite these promising results, the study has several limitations. The current analyses are restricted to *in vitro* experiments. Furthermore, the structural composition of the EPS has not been fully elucidated, and the observed bioactivities have yet to be validated *in vivo*. Future research should therefore focus on (1) whole-genome sequencing to confirm strain identity and identify genes involved in cellulase production and EPS biosynthesis; (2) detailed structural analysis of the EPS to determine functional moieties; (3) *in vivo* assessment of probiotic efficacy and safety; and (4) evaluation of the scalability and stability of the EPS for applications in functional food or feed products.

5. Acknowledgement

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6. Declaration of generative AI in scientific writing

The authors declare that no content in this manuscript was generated or written by generative artificial intelligence (AI) tools in a manner that affects the scientific content or conclusions of the study. AI tools were used solely for spell-checking and minor language polishing. All content was thoroughly reviewed and verified by the authors to ensure academic accuracy.

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8. Research involving human and animals rights

This study was conducted in accordance with ethical principles for research involving humans and animals, following the guidelines of relevant institutional regulations to ensure the protection, safety, and welfare of participants and animals.

9. Ethics Approval and Consent to Participate

This research was approved by the Research Ethics Committee of Ubon Ratchathani Rajabhat University, approval number AN663005. All participants provided informed consent prior to their inclusion in the study.

10. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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