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Assessment of Antioxidant and Antibacterial Activities of *Lactobacillus farciminis* HN11 as a Probiotic Candidate

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Abstract

Background and Objective: *Lactobacillus farciminis* is an obligate homofermentative bacterial species in fermented foods. Although other species such as *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus rhamnosus* in *Lactobacillus* genus have been well characterized, probiotic characteristics of *Lactobacillus farciminis* still need to investigate. Thus, the objective of this study was to investigate probiotic characteristics and antibacterial activity of *Lactobacillus farciminis* HN11 isolated from *Solanum macrocarpon* sauces in Hue city, Vietnam.

Material and Methods: *Lactobacillus farciminis* HN11 was cultured in Rogosa and Sharpe media and antibacterial activity of the free-cell suspension was assessed against *Escherichia coli*, *Vibrio parahaemolyticus* K5, *Vibrio parahaemolyticus* KS-02, *Vibrio owensii* KS-05, *Vibrio alginolyticus* KS-08, *Vibrio alginolyticus* A1-1 and *Staphylococcus aureus* using agar well diffusion method. Various probiotic characteristics of the isolate including antibacterial and antioxidant activities, autoaggregation and coaggregation were assessed. Furthermore, hemolytic and amino-acid dercarboxylase activities were assessed for biosafety assessment. The strain abilities to resist sodium chloride and bile salts were assessed as well.

Results and Conclusion: *Lactobacillus farciminis* HN11 exhibited significant resistance to NaCl and bile salts. The strain expressed high coaggregation abilities for *Escherichia coli*, significant autoaggregation abilities and antibacterial activities against *Vibrio* spp. and *Escherichia coli*. Antioxidant assessment showed that *Lactobacillus farciminis* HN11 contained high antioxidant activities. This strain was negative for hemolytic and amino-acid dercarboxylase activities. Moreover, ampicillin and chloramphenicol inhibited growth of *Lactobacillus farciminis* HN11. This study assessed characteristics of *Lactobacillus farciminis* HN11 and showed its great potentials as a probiotic in fermented foods, enhancing antioxidant and decreasing harmful foodborne bacteria. Although, encapsulation of the strain within acid resistance materials is suggested to better protect it against high-acid contents of the stomach.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Probiotics are living microorganisms that can benefit host health, including gastrointestinal microflora improvement, microbiota balancing in intestine, immune system boosting, serum cholesterol decreasing and cancer prevention [1]. Since probiotics should adapt to complex conditions of the gastrointestinal tract (GIT) of the host, probiotic species are often isolated from the hosts such as humans. Moreover, fermented foods are good sources for the isolation of probiotic species [2]. Fermented foods are widely incorporated in daily foods in Asia as healthy foods. Fermentation is a technique to preserve and enhance taste, shelf life, texture and functional characteristics of foods [3]. Lactic acid bacteria (LAB), including several genera such as *Lactobacillus*, *Streptococcus* and *Leuconostoc*, are dominant in fermented foods [4]. These bacteria have been used as starters to ferment yogurts, meats, fishes and shrimps. The LAB play key roles in fermentation by rapidly acidifying raw materials through the production of organic acids [5].

Living starter microorganisms are used to assist the beginning of fermentation, leading to changes in sensorial and chemical composition characteristics of the food sources and homogeneous products [5]. Appropriate characteristics of LAB as starters for fermenting foods include (a) ability to produce antibacterial compounds (bacteriocins and organic acids) to eliminate harmful bacteria, (b) ability to resist high concentrations of salts in foods, acids and bile salts in GIT with health benefits and (c) inability to produce amino-acid decarboxylase (AADC) (no bioaccumulation of amines) with no overall hemolytic activities and sensitivities to antibiotics [6]. Previous studies have shown that use of LAB as starter cultures could control harmful foodborne bacteria with sources of meats, shrimps and fishes [5]. In particular, fermentation products can directly be produced from raw materials of fishes and shrimps without cooking or pasteurization steps to remove pathogens. However, fermented fish and shrimp products from low market-value raw materials that are processed in unsafe fish-processing facilities may lead to foodborne illness risks [7]. Use of LAB with antibacterial activity as starters is an effective way to increase food safety of these products.

Lactobacillus farciminis is an obligate homofermentative species. This strain has used in starter cultures when rapid growth of lactic acid (LA) and decrease of pH are desired [8]. The *L. farciminis* has been identified in fermented foods such as rye sourdoughs [8] and sausages [9]. Although other species such as *L. plantarum*, *L. acidophilus*, *L. casei* and *L. rhamnosus* in *Lactobacillus* genus have been well characterized, probiotic characteristics of *L. farciminis* still need further investigations [10]. Therefore, the aim of this study was to investigate antibacterial and antioxidant characteristics of *L. farciminis* HN11 isolated from local fermented foods (*Solanum macrocarpon* sauces) of Vietnam.

2. Materials and Methods

2.1. Lactic acid bacteria culture

The *L. farciminis* HN11 was isolated from traditionally fermented *Solanum macrocarpon* sauces and registered in NCBI with the accession number of MT472126. This strain was stocked and stored at -40 °C in de Man, Rogosa and Sharpe (MRS) media (Merck, Germany), supplemented with glycerol (25% v v^{-1}). The *L. farciminis* HN11 was inoculated onto MRS-agar plate and incubated at 37 °C for 24 h. A single colony from the MRS agar plate was spread onto a new MRS agar plate. Batch culture was then carried out at 37 °C for 18 h with agitation (180 rpm) using 250-ml flasks with 50 ml of MRS media. Supernatants were collected after centrifuging at 8000× g for 10 min at 4 °C.

2.2. Antibacterial activity assessment

Antibacterial activity of *L. farciminis* HN11 was assessed using cell-free supernatant (CFS) on agar plate. The pathogenic bacterial strains included *Vibrio parahaemolyticus* K5, *V. parahaemolyticus* KS-02, *V. owensii* KS-05, *V. alginolyticus* KS-08, *V. alginolyticus* A1_1, *Escherichia coli* ATCC 85922 and *Staphylococcus aureus* ATCC 25023. *Vibrio* species were cultured in alkaline peptone water media. Moreover, *E. coli* and *S. aureus* were inoculated into 5 ml of LB (lysogeny broth) media and incubated at 37 °C for 18 h. Then, 50 µl of each pathogenic strain ($OD_{600\ nm}$ = 1) were spread onto LB agar plate. Fifty microliters of *L. farciminis* HN11 CFS were added to a 6-mm well and incubated at 37 °C for 24 h. Then, diameters of the inhibition zones were reported. Antibacterial activity was expressed at arbitrary unit (AU ml^{-1}) based on Eq. 1 [11]:

$$\text{Antibacterial activity (AU ml}^{-1}) = \frac{\text{Inhibition circle diameter (mm)} \times 1000}{\text{Volumne taken in the well (µL)}} \quad \text{Eq.1}$$

2.3. Salt tolerance

Salt tolerance was assessed by culturing 100 µL of *L. farciminis* HN11 cell suspension into 900 µL of MRS media containing 5, 10, 15 and 20% (w v^{-1}) NaCl at 37 °C for 24 h. Salt tolerance was analyzed by measuring the optical density at 600 nm. NaCl-free MRS media were used as control. Then, survival proportion of *L. farciminis* HN11 was calculated [12].

2.4. Acid resistance

Acid resistance was assessed based on the previous methods with some modifications [13]. Briefly, *L. farciminis* HN11 was cultured in MRS media at 37 °C for 18 h. Cells were then harvested by centrifugation at 8000× g for 10 min. Cells were washed twice with physiological saline and the OD_{600nm} was adjusted to 1. One milliliter of the cell suspension was added into 24.5 ml of 0.2% NaCl solution at pH 3, 4, 5 and 6 and incubated at 37 °C. Survived bacterial

cells were counted at 0, 3, 6, 9 and 12 h by spreading culture samples onto MRS agar plates.

2.5. Bile-salt tolerance

Briefly, *L. farciminis* HN11 was inoculated into 10 ml of MRS media containing 0.3, 0.5 and 1% (w v^{-1}) bile salts. Cultures were incubated at 25 °C for 24 h. Optical density of the cultures was measured at 600 nm. Control culture was prepared with no bile-salt supplementations. Survival proportion of the bacteria was calculated based on the number of bacterial cells in the samples with and without bile salts [14].

2.6. Autoaggregation and coaggregation assessments

Autoaggreation was assessed based on a method described by Del Re et al. [15]. Briefly, *L. farciminis* HN11 was inoculated into MRS media and incubated at 37 °C for 18 h. Cells were harvested by centrifugation at 8000× g for 10 min, washed with phosphate-buffered saline (PBS) three times and diluted to a cell density of approximately 4×10^8 CFU ml^{-1} using PBS buffer. Sample was incubated at room temperature. One hundred milliliters of the upper suspension were spectrophotometrically measured at various time points (0, 1, 2, 3, 4 and 5 h). Autoaggregation proportion was calculated using Eq. 2:

$$\text{Autoaggregation (\%)} = \frac{1-OD_t}{OD_o} \times 100 \qquad \text{Eq.2}$$

Suspensions of the bacteria were prepared for the assessment of coaggregation using a similar method to that described previously. Then, 2 ml of the suspensions of *L. farciminis* HN11 and *E. coli* ATCC 85922 were mixed and incubated at room temperature. Absorbance of the mixture was recorded at various time points (0, 1, 2, 3, 4 and 5 h). Coaggregation proportion was calculated using Eq. 3 as follows:

$$\text{Coaggregation (\%)} = \frac{(OD_x+OD_y)/_2 - OD_m}{(OD_x+OD_y)/_2} \times 100 \qquad \text{Eq.3}$$

Where, OD_x was the absorbance of *L. farciminis* HN11, OD_y was the absorbance of *E. coli* and OD_m was the absorbance of a mixture of *L. farciminis* HN11 and *E. coli*.

2.7. Antioxidant activity

The *L. farciminis* HN11 cells were washed twice with PBS and diluted to a final concentration of 10^9 CFU ml^{-1} using sterile distilled water. The *L. farciminis* HN11 CFS and cells were assessed for antioxidant activities. Briefly, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) reagent was prepared by incubating 7-mM ABTS solution with 2.45-mM $(NH_4)_2S_2O_8$ at a ratio of 1:1 (v v^{-1}) at room temperature for 12-16 h in dark. The ABTS solution was then adjusted to reach an optical density of 0.70 ±0.02 at 734 nm. One hundred milliliters of each sample were added into 900 µL of ABTS solution and incubated at room temperature for 6 min in dark. Then, absorbance value was recorded. Ascorbic acid was used as reference antioxidant. The ABTS radical scavenging activity was calculated using Eq. 4 [16]:

$$\text{ABTS radical scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100 \qquad \text{Eq.4}$$

Where, A_c was the absorbance of the control at 734 nm and A_s was the absorbance of the sample at 734 nm.

2.8. Antibiotic resistance

Antibiotic resistance was carried out based on a method described by Argyri et al. with slight modifications [17]. Antibiotic resistance of *L. farciminis* HN11 was assessed by cultivating colonies of this strain on 5 ml of MRS media containing various antibiotics (oxytetracyclin hydrochloride, sulfapiridine, sulfamethoxazole, kanamycin, sulfathiazole, chlortetracyclin hydrochloride, ampicillin and chloramphenicol). Antibiotic concentrations ranged 25–500 µg ml^{-1}. Cell density was measured at OD_{600mn} to assess antibiotic resistance.

2.9. Hemolytic activity

To assess hemolytic activity, *L. farciminis* HN11 was cultured in MRS at 37 °C for 18 h and sprayed onto blood agar plates containing 5% of rabbit blood. Plates were incubated at 37 °C for 24 h. Then, β-hemolysis activity (clean zone), α-hemolysis activity (greenish zone) and γ-hemolysis activity (no such zone) were studied [13].

2.10. Amino-acid decarboxylase activity

The *L. farciminis* HN11 was routinely cultured at 37 °C for 72 h in static conditions using MRS broth with 0.1% (w v^{-1}) of L-tyrosine. Fifty microliters of the free-cell supernatant were transferred into 6-mm wells on 1% L-tyrosine MRS agar plates containing 0.006% of bromothymol blue. Plates were incubated at 30 °C for 72 h under anaerobic conditions. Observation of clear purple halos revealed AADC activity [18].

3. Results and Discussion

3.1. Antibacterial activity assessment

Antibacterial activities of the CFS were assessed for *L. farciminis* HN11 against *E. coli* ATCC 85922, *Vibrio* spp. and *S. aureus* ATCC 25023. Results showed that HN11 strain resisted to *Vibrio* spp. and *E. coli* ATCC 85922. Interestingly, *L. farciminis* HN11 showed the strongest activity against *V. parahaemolyticus* K5 known to cause acute hepatopancreatic necrosis disease in shrimps with an antagonistic activity of 380 AU ml^{-1} ±9.5. Moreover, antagonistic activities of the CFS for *L. farciminis* HN11 against *V. parahaemolyticus* KS-02, *V. owensii* KS-05, *V. alginolyticus* KS-08, *V. alginolyticus* A1_1 and *E. coli* ATCC 85922 consisted of 126.67 ±11.5, 43.33 ±5.8, 120, 186.67 ±11.5 and 280 ±10 AU ml^{-1}, respectively. However, CFS for *L. farciminis* HN11 showed no activities against *S. aureus* ATCC 25023 (Table 1, Fig. 1).

Table 1. Antibacterial activity of *Lactobacillus farciminis* HN11 against the pathogenic bacteria

Pathogenic strains	Antagonistic activity (AU ml^{-1})
V. parahaemolyticus K5	380 ± 9.5
V. parahaemolyticus KS-02	126.67 ± 11.5
V. owensii KS-05	43.33 ± 5.8
V. alginolyticus KS-08	120 ± 0
V. alginolyticus A1_1	186.67 ± 11.5
E. coli ATCC 85922	280 ± 10
S. aureus ATCC 25023	0

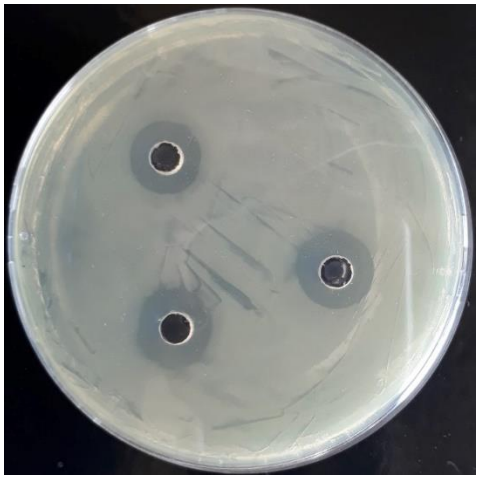

Figure 1. Inhibition of *Vibrio parahaemolyticus* K5 by *Lactobacillus farciminis* HN11. Three 6-mm wells were used to assess inhibition of *Vibrio parahaemolyticus* K5 by CFS *Lactobacillus farciminis* HN11

Antibacterial resistance is an important feature when selecting probiotic strains. Previous studies have shown that *L. farciminis* can inhibit *Listeria monocytogenes*, *Pencillium* spp. and *Aspergillus niger* with diameters of inhibition zones < 12 mm [8,19]. In contrast, *L. farcimins* HN11 inhibited *E. coli* and *Vibrio* spp. with antagonistic activities up to 380 AU ml^{-1}, which was equal to a 19-mm diameter of the inhibition zone. Salleh et al. have reported that LAB isolates, including *L. plantarum, L. buchneri, L. brevis* and *L. acidophilus*, from fermented durian flesh were active against *V. parahaemolyticus* ATCC 17802 with antagonistic activities of 200–275 AU ml^{-1} [20]. Although, reports have been published on antibacterial and antifungal activities of *L. farciminis.* Information of antibacterial and antifungal compounds secreted by *L. farciminis* are still unclear. Stanzer et. al suggested that organic acids such as LA and phenyllactic acid may play importance roles for *L. farciminis* antagonistic activity [8]. In addition, a study by Kaur et. al showed that *Lactobacillus* spp. inhibited *Vibrio* spp. and *E. coli* by preventing the bacterial biofilm formation as well as secreting organic acids to the environment. Thus, pH neutralization of CFS completely abrogated antibacterial activity while other treatments showed no effects on their antagonistic activities [21]. Similarly, CFS of *L. farciminis* HN11 lost antimicrobial activity against *V. parahaemolyticus* K5 after neutralization to pH 6.5 using 1 M NaOH. Therefore, *L. farciminis* HN11 might inhibit *E. coli* and *Vibrio* spp. by similar mechanisms; in which, organic acid production together with elimination of biofilm formation are key roles for its antimicrobial characteristics. Organic acids are well demonstrated to inhibit various pathogenic bacteria by modifying concentrations of proton and associated anions in cytoplasm of cells [22]. However, further investigations are needed for the identification of antibacterial substances to elucidate inhibition mechanisms by this isolate.

3.2. Salt tolerance

To assess salt tolerance, *L. farciminis* HN11 was cultured in media containing various concentrations of NaCl. Results showed that *L. farciminis* HN11 grew faster in media supplemented with 5-20% of NaCl than control media with no supplementation of NaCl. The highest growth rate was 153.14% ±2.4 in media containing 5% of NaCl. Increasing salt concentration decreased the growth rate. Growth rate reached 121.99% ±3.1 at 20% salt concentration, compared to the control. These results showed that *L. farciminis* HN11 could grow well in culture media with high salt concentrations (Fig. 2A). Sodium chloride is widely used as substance in food processing. It is known that high salt concentrations can inhibit growth of harmful microorganisms. Sodium chloride enhances the flavor by decreasing or enhancing activity of certain enzymes associated with the development of organoleptic parameters or by affecting various biochemical mechanisms [23]. Halophilic LAB are the most prevailing bacteria in fermented foods containing high levels of salt that need NaCl for their growth. Typically, halophilic LAB can tolerate high-salt environments of 10-20% NaCl by maintaining their osmotic pressure in cytoplasm through accumulation of potassium and chloride or biosynthesis and/or accumulation of organic osmotic solutes [12,24,25]. Thus, these bacteria are considered good candidates as starter cultures for high salinity fermentation production. Moreover, halophilic LAB could improve characteristics of the fermented food products through the production of flavor and aroma components and organic acids [26]. In the present study, *L. farcimins* HN11 grew well in high salt concentrations of 5-20%, demonstrating that this isolate could be used as starter to prepare fermented foods with high salinity.

3.3. Acid resistance

In general, *L. farciminis* HN11 was assessed for acid tolerance in various pH. Results shown in Fig. 2B indicated that pH greatly affected the viability of this bacterial strain.

The *L. farciminis* HN11 could grow at pH 5.0 (survival rate of 59.37% ±8.9). It grew optimally at pH 6.0 with a survival rate of 89.32% ±10.5 after 12 h. Strong acidic condition (pH 3.0-4.0) inhibited the bacterial cell growth. The pH resistance characteristics of LAB are differently attributed, ranging from low pH level of 2.0-4.0 to medium pH level of 5.0-6.0 [27]. Particularly, LAB include numerous mechanisms to adapt low pH environment such as cell surface hydrophobicity adaption, cell component protection, neutralization, proton pumps, metabolic alteration to stabilize intracellular pH and use of protective substances [28]. Acid tolerance ability of LAB can be enhanced through various ways such as modification of media components by adding soybean lecithin, whey protein and trehalose [29,30]. These potential strategies may improve moderate pH tolerant LAB such as *L. farciminis* HN11.

3.4. Bile-salt tolerance

In general, *L. farciminis* HN11 was assessed for resistance to bile salts at concentrations of 0.3, 0.5 and 1%. Results showed that the growth rate of HN11 strain after 24 h of culture decreased gradually when bile-salt concentration increased. The bacterial growth rates were 57.48% ±10.8, 48.01% ±9.6 and 32.50% ±10.6 in presence of bile salts of 0.3, 0.5 and 1%, respectively (Fig. 2C). Bile salt is a biological cleaner that destroys bacterial cell membranes in the upper GIT. Thus, bacteria living in the intestines must include intrinsic resistance mechanisms to resist bile salts. In general, *Lactobacillus* spp. resist bile salts through activation of molecular machinery to counteract oxidative and acid stresses, as well as modification and utilization of bile efflux systems through bile salt hydrolases [31]. A previous report has shown that the growth rate of *L. farciminis* HM2001, *L. casei* HM3701, *L. acidipiscis* JAM3706, *L. alimentarius* EM2001 and *L. plantarum* JAB2001 were respectively 18.3% ±1.9, 7.2% ±4.0, 37.9% ±19.0, 47.1% ±4.0 and 41.0% ±18.0 in MRS media supplemented with 0.3% of bile salts [32]. Furthermore, growth rate of *L. farciminis* HN11 was 57.48% at a similar bile-salt concentration, indicating advantage of the bacterial strain to survive in the host GIT.

3.5. Autoaggregation and coaggregation assessments

Results showed that the adhesive activity of *L. farciminis* HN11 increased over time. Autoaggregation and coaggregation rates of this bacterial strain were relatively high, with the autoaggregation rate reaching 31.36% ±1.65 and the coaggregation rate with *E. coli* reaching 62.64% ±1.48 after 5 h (Fig. 3A). Tareb et al. reported autoaggregation rates of *L. rhamnosus* CNCM-I-3698 and *L. farciminis* CNCM-I-3699 as 22.1% ±1.4 and 11.07% ±0.8, respectively.

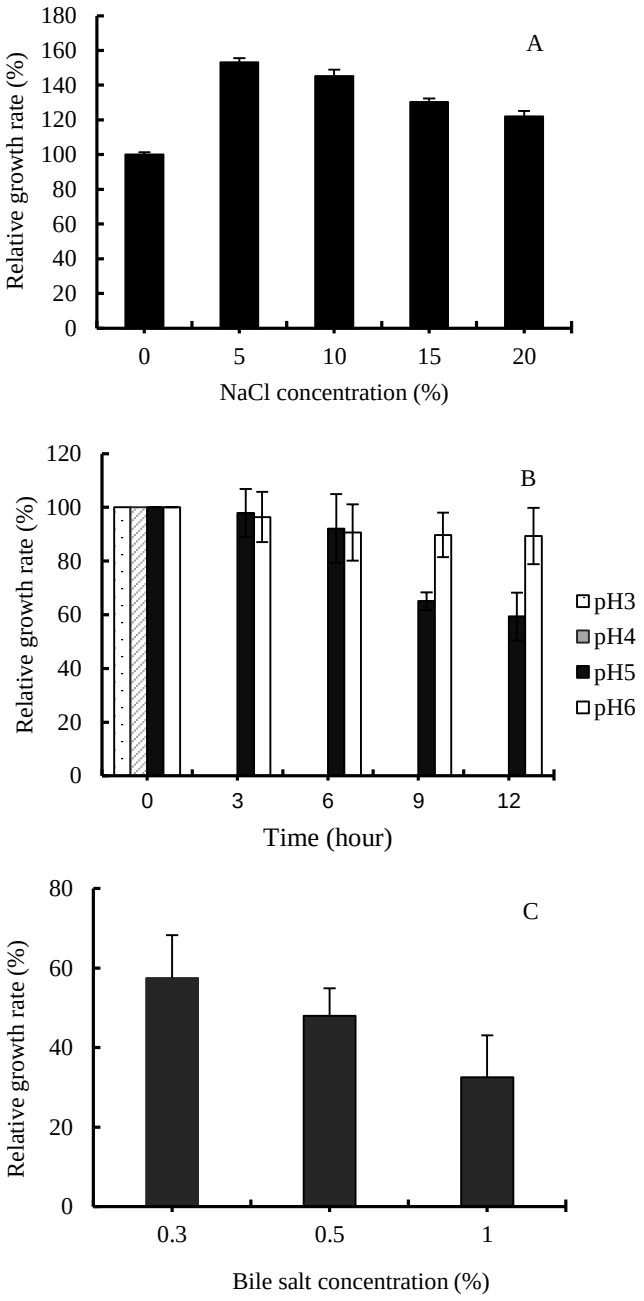

Figure 2. Effects of sodium chloride (A), pH (B) and bile salts (C) on growth of *Lactobacillus farciminis* HN11. Data are presented as mean ±SD of three experimental replicates

Their coaggregation rates with *E. coli* were respectively 7.9% ±1.8 and 8.0% ±1.2 after 4 h of coculture [33]. A study by Tou et al. demonstrated that autoaggregation activities of 22 *Lactobacillus* strains after 5 h of incubation at 37 °C ranged 24.16–41.39%, while their coaggregation with *E. coli* ranged 21.36–32.16% [34]. In contrast, *L. farciminis* HN11 showed higher autoaggregation and coaggregation rates of 31.36% ±1.65 and 62.64% ±1.48, respectively. Thus, *L. farciminis* HN11 could enhance host defense against *E. coli*. Autoaggregation is a common potential linked to cell adhesion characteristics, which are associated to their survival in GIT. These characteristics include competitive advantages for preserving bacteria in the host GIT [35].

Moreover, coaggregation between the LAB and pathogenic bacteria creates barriers, preventing adhesion of harmful bacteria to intestinal epithelial cells and urinary tract. In addition, coaggregation sets probiotics in a closer integration with pathogenic bacteria, increasing effectiveness of the antibacterial compounds produced by LAB [36]. Aggregation mechanism involves a complex interplay of various factors in cell-surface characteristics (hydrophobicity), cell-structure (protein, exopolysaccharides and carbohydrates) and enzymes. For example, Isenring et al. reported that glucose activated aggregation of *Lactiplantibacillus plantarum*, suggesting catabolite repression via the canonical regulatory carbon catabolite protein A. However, the authors highlighted that other regulators might be involved, resulting in unclear descriptions for the exact regulation mechanism [37]. Therefore, factors linked to the aggregation phenotype of *L. farciminis* HN11 need further investigations. A high coaggregation activity suggests that elimination of the biofilm formation by pathogen bacteria is involved in antibacterial mechanisms by *L. farciminis* HN11.

3.6. Antioxidant activity

To investigate antioxidant activity of *L. farciminis* HN11, the bacterial ABTS free radical scavenging capacity was assessed. Results showed that the CFS of *L. farciminis* HN11 included a high ABTS free radical scavenging ability (78.36% ±1.62). Furthermore, intact cells demonstrated a lower ABTS free radical scavenging ability (18.00% ±3.33). The bacterial ABTS free radical scavenging was lower than that of 100 µg ml^{-1} ascorbic acid, but much higher than that of 10 µg ml^{-1} ascorbic acid (Fig. 3B). Thus, *L. farciminis* HN11 expressed high antioxidant activities. Antioxidant activity of LAB induces production of lipoteichoic acid, exopolysaccharides and cell surface proteins. The *L. farciminis, L. casein, L. rhamnosus, L. plantarum, L. reuteri, L. acidophilus* and *L. fermenti* showed high antioxidant activities against free radicals such as hydroxyl, superoxide and DDPH [38]. The CFS of *L. farciminis* HN11 contained high ABTS free radical scavenging abilities of 78.36% ±1.62, promising good antioxidant capacities of this strain.

3.7. Antibiotic resistance

Results of assessing *L. farciminis* HN11 antibiotic resistance showed that this strain was resistant to several antibiotics such as kanamycin, sulfapiridine, oxytetracyclin hydrochloride, sulfathiazole, chlortetracyclin hydrochloride and sulfamethoxazole at a concentration of 500 µg ml^{-1}. Moreover, this strain was sensitive to two antibiotics, ampicillin and chloramphenicol, at a concentration of 25 µg ml^{-1} (Table 2). Lactobacilli are generally sensitive to penicillin that targets the cell wall. Moreover, antibiotics such as tetracycline, chloramphenicol and lincomycin inhibit growth of lactobacilli [39].

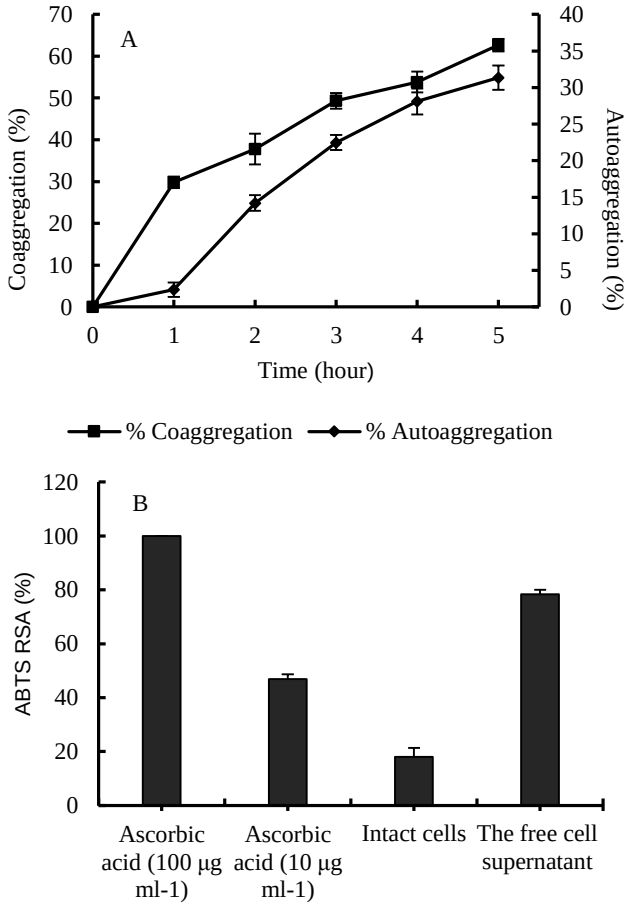

Figure 3. Autoaggregation and coaggregation abilities (A) and antioxidant activity (B) of *Lactobacillus farciminis* HN11. Data are presented as mean ±SD of three experimental replicates

A study by Jawan et al. showed that *L. farciminis* TY1 was resistant to antibiotics such as erythromycin, amikacin, nalidixic acid, streptomycin, norfloxacin and colistin sulphate, while it was sensitive to β-lactams (penicillin G and ampicillin), chloramphenicol and ceftriaxone [19]. Similarity, *L. farciminis* HN11 was sensitive to ampicillin and chloramphenicol.

3.8. Hemolytic and amino-acid decarboxylase activities

The *L. farciminis* HN11 did not show hemolytic activity on 5% rabbit blood agar. The bacterial negative hemolytic activity is considered as an advantage for using the bacterial strain as a probiotic strain (Fig. 4A). In this study, AADC activity of *L. farciminis* HN11, specifically its tyrosine decarboxylase activity, was assessed. Results shown revealed no purple rings around the culture well of *L. farciminis* HN11, indicating that this strain was negative for AADC activity (Fig. 4B).

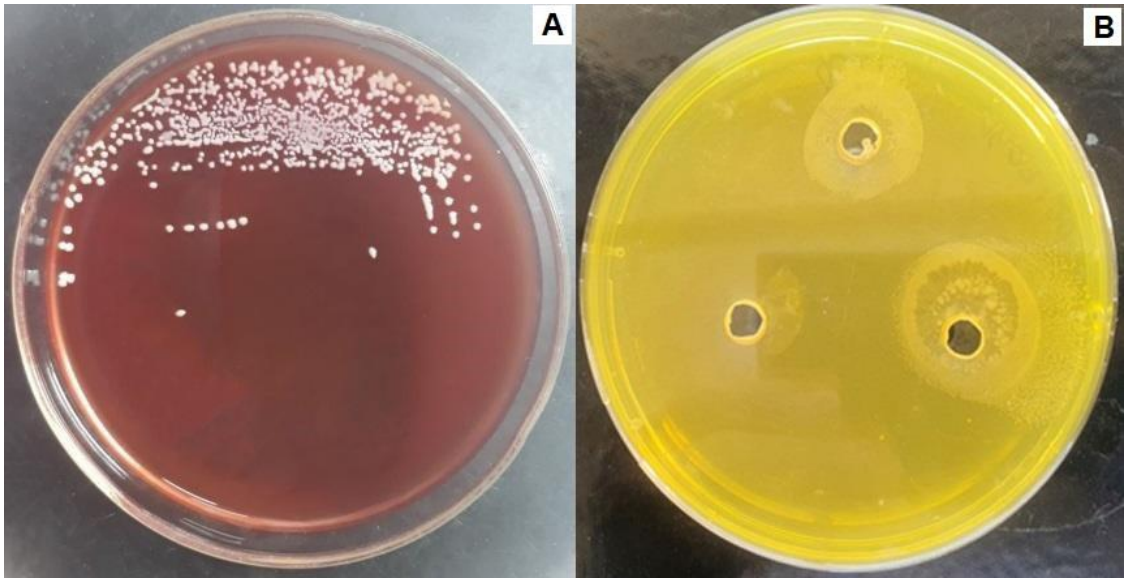

Figure 4. Hemolytic (A) and amino-acid decarboxylase activities (B) of *Lactobacillus farciminis* HN11. Hemolytic activity was assessed on blood agar plate and amino-acid decarboxylase activity was assessed in MRS broth containing 0.1% (w v^{-1}) of L-tyrosine

Table 2. Antibiotic resistance ability of *Lactobacillus farciminis* HN11

Antibiotics	Concentration (µg ml^{-1})				
	500	250	100	50	25
Sulfathiazole	+	+	+	+	+
Sulfapiridine	+	+	+	+	+
Oxytetracyclin hydrochloride	+	+	+	+	+
Sulfamethoxazole	+	+	+	+	+
Chlotetracyclin hydrochloride	+	+	+	+	+
Kanmycin	+	+	+	+	+
Ampicilin	-	-	-	-	-
Chloranphenicol	-	-	-	-	-

+: growth; -: No growth

Absence of the hemolytic activity is considered a safety prerequisite for the microbial strain selection. Hemolysis is a well-known feature of the pathogenic microorganisms. The *L. farciminis* HN11 was negative for hemolysis on blood agar plate assay, indicating a non-virulent species in nature. This result is similar to a previous result, showing that all assessed *Lactobacillus* strains isolated from cow milk did not show β-hemolytic activity [40]. Biological amines (BAs) are organic compounds formed by decarboxylation of AAs such as tyrosine, histidine, lysine putrescine and ornithine, producing tyramine, histamine, cadaverine and putrescine, respectively [18]. Due to the bacterial activity, BAs naturally present in fermented foods such as dairy, meat and processed fish products, posing risks of food poisoning. Tyramine and histamine have been demonstrated to cause severe allergic reactions or tissue damages if their concentrations exceed in food products [41]. The *L. farciminis* HN11 was not able to form BAs that is valuable for controlling BAs in fermented products. Yalcinkaya and Kilic have reported similar results, showing that *L. farciminis* Z4A could not decarboxylate tyrosine [26].

4. Conclusion

The present study have shown that *L. farciminis* HN11 expresses antibacterial activities against several foodborne pathogens such as *E. coli* and *Vibrio* spp., growing at a wide range of NaCl concentrations of 5-20% (w v^{-1}). The strain includes good characteristics of general probiotic bacteria such as tolerance to 0.3-1% (w v^{-1}) bile salts as well as autoaggregation, coaggregation and antioxidant activities. Organic acid production and prevention of biofilm formation may be key factors involving in antibacterial mechanisms by

the strain. Furthermore, *L. farciminis* HN11 is resistance to kanamycin, sulfapiridine, oxytetracyclin hydrochloride, sulfathiazole, chlortetracyclin hydrochloride and sulfamethoxazole and sensitive to ampicillin and chloramphenicol. In addition, this strain does not contain hemolytic or AA dercarboxylase activities. Thus, this strain includes great potentials to be developed as a probiotic strain in fermented foods to enhance antioxidant characteristics and decrease harmful foodborne bacteria. Addressing variable resistances of this bacterial strain in acidic environments, its encapsulation with acid-resistance materials is recommended to protect the strain against acid contents of the stomach (pH~2). Moreover, modification of the culture media may be carried out to enhance its acidic resistance ability.

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6. Author contribution

Conceptualization, N.D.H.; methodology, T.T.P.T. and L.T.K.T; investigation: N.Q.H.V, N.Q.L. and L.C.T; writing the original draft, T.T.P.T. and N.T.C.A.; writing the review and editing, N.D.H. and S.M.P.; funding acquisition N.D.H.; resources, T.D.B., T.T.P.L. and S.M.P.

7. Conflict of Interest

The authors report no conflicts of interest.

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ارزیابی فعالیت ضداکسایشی و ضدباکتریایی *لاکتوباسیلوس فارسیمینیس* HN11 به‌عنوان زیست‌یار انتخابی

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چکیده

سابقه و هدف: *لاکتوباسیلوس فارسیمینیس* گونه‌ای باکتریایی هموفرمنتاتیو اجباری در غذاهای تخمیر شده می‌باشد. اگرچه سایر سوش‌ها مانند *لاکتوباسیلوس پلانتاروم*، *لاکتوباسیلوس اسیدوفیلوس*، *لاکتوباسیلوس کازئی* و *لاکتوباسیلوس رامنوسوس* در جنس لاکتوباسیلوس به‌خوبی شناخته شده می‌باشند، ویژگی‌های زیست‌یاری *لاکتوباسیلوس فارسیمینیس* نیازمند بررسی می‌باشد. از این‌رو، هدف مطالعه حاضر بررسی ویژگی‌های زیست‌یاری و فعالیت ضدباکتریایی *لاکتوباسیلوس فارسیمینیس* HN11 جدا شده از سس‌های سولانوم ماکروکارپون شهر هیو در کشور ویتنام بوده است.

مواد و روش ها: *لاکتوباسیلوس فارسیمینیس* HN11 در محیط‌های کشت روگوسا و شارپ کشت داده شد و فعالیت ضدباکتریایی سوسپانسیون فاقد سلول در برابر *اشرشیا کلی*، *ویبریو پاراهمولیتیکوس* K5 ، *ویبریو پاراهمولیتیکوس* ***KS-02*** ، *ویبریو آلژیولیتیکوس* ***KS-08***، *ویبریو آلژیولیتیکوس* A1_1 و *استافیلوکوکوس اورئوس*، با استفاده از روش انتشار چاهک آگار مورد بررسی قرار گرفت. ویژگی های گوناگون زیست‌یاری جدایه شامل خواص ضدباکتریایی و ضداکسایشی، انبوهش خودکار و انبوهش باهم بررسی شد. سپس، به منظور ارزیابی ایمنی زیستی فعالیت همولیتیک و آمینواسید دکربوکسیلاز مورد بررسی قرار گرفت. توانایی سویه در پایداری در برابرسدیم کلرید و نمک‌های صفراوی نیز بررسی شد.

یافته‌ها و نتیجه‌گیری: *لاکتوباسیلوس فارسیمینیس* HN11 مقاومت قابل توجهی در برابر NaCl و نمک‌های صفراوی از خود نشان داد. توانایی انبوهش باهم بالایی در برابرگونه‌های *ویبریو* و *اشرشیا کلی* را بروز داد. بررسی خاصیت ضداکسایشی نشان دادکه *لاکتوباسیلوس فارسیمینیس* HN11 فعالیت ضداکسایشی بالایی دارد. اینسوش فعالیت‌های همولیتیک وآمینواسید دکربوکسیلاز نداشت. علاوه بر این، آمپی سیلین و کلرامفنیکل رشد *لاکتوباسیلوس فارسیمینیس* HN11 را مهار کردند. این مطالعه با بررسی ویژگی‌های *لاکتوباسیلوس فارسیمینیس* HN11 توانایی بالای آن به‌عنوان یک زیست‌یار در غذاهای تخمیر شده، افزایش آنتی‌اکسیدان و کاهش باکتری‌های غذازاد[1] مضر را نشان داد. اگرچه، به‌منظور محافظت بهتر آن در برابر اسید زیاد معده، ریزپوشانی سویه در موادی مقاوم به اسید توصیه می‌شود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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- باکتری‌های لاکتیک اسید
- زیست‌یار

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[1] foodborne