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Evaluation of probiotic properties of *Bacillus aryabhattai* HY1 isolated from Vietnamese pickled mustard greens

Minh Tan Pham✉, Phung Thao Uyen Nguyen, Tran Hai Yen Nguyen, Khanh Linh Ma & Thi Da Chau

Probiotics are live microorganisms, when consumed in certain numbers confer health benefits on the host beyond inherent basic nutrition. This study was conducted to characterize the probiotic properties of bacteria isolated from pickled Vietnamese cabbage. Identification by 16S rRNA gene sequence analysis revealed that the isolate was *Bacillus aryabhattai*. The ability of *B. aryabhattai* HY1 to resist acidic condition, 0.3% (*w/v*) bile salts, sensitivity to Amoxicillin (25–40 µg) and Ampicillin (30–40 µg). In addition, the isolate was able to show inhibition towards pathogenic bacteria including *Escherichia coli* (EC), *Staphylococcus aureus* (SA), *Salmonella typhimurium* (ST), *Pseudomonas aeruginosa* (PA). The combination of 2% glucose with *B. aryabhattai* HY1 increased the production of acetic acid and butyric acid. These findings help to explain the health advantage and antimicrobial properties of *B. aryabhattai* HY1.

Keywords *B. aryabhattai*, *Brassica juncea*, Lactic acid bacteria, Probiotics, SCFAs

Vietnam has produced a variety of traditional fermented foods for a very long time. These products include a variety of microorganisms with advantageous food processing technology, preservation properties, and organoleptic abilities, among other functional characteristics. The beneficial indigenous bacteria included in traditional fermented meals are abundant[1]. A well-liked fermented food is Vietnamese mustard greens pickles, sometimes referred to as vegetable fermented. During pickling, the glucosinolates of *Brassica juncea* are hydrolysed to the glucosinolate hydrolysis products modulating enzyme activity and preventing certain cancers[2]. High antioxidant capacities of phenolic compounds in mustard greens can inhibit oxidative damage diseases, such as stroke and cancers[3,4]. A microbial community involved in traditional Vietnamese fermented pickles including lactic acid bacteria and *Bacillus* strains such as *B. subtilis*, *B. amyloliquefaciens, B. licheniformis, B. circulans, B, pumilus,* and *B. brevis*[5,6]. Our previous study demonstrated that *B. amyloliquefaciens* EH-9 strain shows a positive effect on the GABA production, which can further improve COL1 production in the skin[7]. *Bacillus* spp. act as probiotics have exhibited probiotic capabilities with a variety of advantages mediated by several complex mechanisms.

Probiotics are defined as living microorganisms which upon ingestion in adequate amounts, exert beneficial physiological effects on their host[8]. The human gut microbiota, being represented principally by the two major phyla Firmicutes and Bacteroidetes[9]. Less well known than the lactobacilli and bifidobacteria are certain species of the spore-forming probiotic *Bacillus* genus, which can be considered as probiotic applications[10]. The members of genus *Bacillus* are a Gram-positive, rod shaped, spore-forming, aerobic or facultative anaerobic bacterium[11]. *Bacillus* spp. inhabits the gastrointestinal tract (GIT) due to its highly resistant to agents such as heat, radiation, desiccation, digestive enzymes, and gastric acidity conditions, with 10^3–10^8 CFU g^{-1} *Bacillus* spp. spores from human feces[12]. Beneficial health action refers to probiotic ingestion including proven biological activity in the treatment of gastrointestinal diseases and cancer prevention[13]. Mounting evidence suggests that among the probiotics, *Bacillus* spp. has been used for enzyme production in varying food stuffs as well as vitamins and carotenoids for human consumption[11]. *Bacillus aryabhattai* (*B. aryabhattai*) has recently attracted interest because of its probiotic potential and application in a variety of fermented foods, drinks, and dairy products[14,15]. It can lessen the prevalence of dangerous germs that lead to illnesses or diseases that can affect humans[16–18]. Probiotic products predominantly feature several *Bacillus* species such as *B. subtilis, B. licheniformis*, and *B. indicus*[19]. However, the potential for *B. aryabhattai* to act as a probiotic has received limited research attention.

Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam. ✉email: phamminhtan@tdtu.edu.vn

The gut microbiota significantly influences many physiological functions, including glucose metabolism, early dissociation and dihydroxylation of bile acid and biosynthesis of essential vitamins and amino acids[20]. Short chain fatty acids (SCFAs) are major homofermentative products of gut microbiota from dietary fiber and have been recognized as important mediators in regulating immunomodulatory functions[8,21]. SCFAs such as butyric acid and acetic acid, have demonstrated the capacity to mitigate both metabolic dysfunctions and inflammation[22]. Various prebiotic fibers yield varying quantities and compositions of primary SCFAs, specifically acetic, propionic, and butyric acids[23]. Butyric acid is a vital energy source for the colonic epithelium and show growth inhibition and apoptosis on two human colorectal tumour cell lines, one adenoma (S/RG/C2) and one carcinoma (HT29) in vitro[24]. Acetic acid has been reported to show some health benefits, potentially contributing to antihypertensive, anti-hyperglycemic, and anti-tumor effects[25]. The purpose of this study is to isolate *B. aryabhattai* HY1 strain capable of producing butyric and acetic acids from pickled *Brassica juncea* and to evaluate its probiotic potential through assessments of acid and bile tolerance, antibiotic susceptibility, and antibacterial activity against pathogenic bacteria.

Materials and methods

Isolated bacteria and identification

The pickled mustard greens were homogenized in sterile distilled water using a grinder. The homogenate was serially diluted and plated on De Man-Rogosa-Sharpe (MRS, Hi-Media, Mumbai, India) agar, then incubated at 37 °C for 48 h. Distinct colonies were picked and purified by streaking on fresh MRS agar. The sequence analysis of 16S rRNA genes was used to identify the bacteria[26]. In addition to sequencing of Polymerase chain reaction (PCR) products, 16S rRNA genes were amplified with 16S rRNA primers 27F and 534R (Fig.S1)[27]. The 16S rRNA gene sequences were analyzed using the basic local alignment search tool (BLASTn, National Library of Medicine 8600 Rockville Pike, Bethesda, MD, USA).

Glucose fermentation of *B. aryabhattai* HY1

To induce fermentation, *B. aryabhattai* HY1 [10^7 colony-forming unit (CFU)/mL] was incubated in rich media [10 g/L yeast extract (Himedia), 5 g/L TSB, 2.5 g/L K_2HPO_4 and 1.5 g/L KH_2PO_4], with or without 20 g/L (2%) glucose at 37 °C for 24h. Rich media only or rich media plus 20 g/L glucose without bacteria were served as a control. Phenol red [0.001% (w/v), Sigma] was served as a pH indicator. A color change from red–orange to yellow indicated acid production.

Acid tolerance

A good potential probiotic needs to be stable at a low pH and in the presence of high bile salts in the stomach and digestive tract[28]. Following the method of Lee et al., bacterial cells were harvested, washed, and resuspended to 10^7 CFU/mL in sterile PBS. Aliquots were inoculated into MRS broth adjusted to pH 2, 3, 4, 5, and 6 (using 1 M HCl)[29]. A control was only MRS broth. Then, samples were incubated at 37 °C for 3 h. Surviving bacteria were counted by spread plating on MRS agar and incubating for 24 h at 37 °C. The relative survival of the bacteria was calculated with the formula below

$$Viability\,(\%) = \frac{N_1}{N_0} \times 100;\ \mathrm{N1} = \mathrm{Log\,CFU\ at\ test\ pH}\ 2, 3, 4, 5, 6\ \mathrm{and\ N0} = \mathrm{Log\,CFU\ at\ control.}$$

Bile salt tolerance

Bile tolerance was performed using the method of Lee et al. An aliquot of overnight culture (10^7 CFU/mL) was inoculated to MRS broth containing 0.3, 0.5, 1.0 and 2.0% bile salts. Cultures were incubated at 37 °C for 3 h. MRS without bile acids was used as the control. Surviving bacteria were counted by spread plating on MRS agar and incubating for 24 h at 37 °C. The relative survival of the bacteria was calculated with the formula below:

$$Viability\ (\%) = \frac{L_1}{L_0} \times 100;\ \mathrm{L1} = \mathrm{Log\,CFU\ in\ in\ bile\ salt\ broth\ and\ L0} = \mathrm{Log\,CFU\ in\ control\ broth.}$$

Antibacterial activity against pathogenic bacteria

Antibacterial activity was evaluated against *Escherichia coli* (EC), *Staphylococcus aureus* (SA), *Salmonella typhimurium* (ST), *Pseudomonas aeruginosa* (PA) using method modified from Rokana, et al. (2017). The pathogens were obtained from the stock culture of Microbiology Laboratory, Ton Duc Thang University, Vietnam. Aliquots of the actively growing pathogenic strains (10^7 CFU/mL) were seeded in sterilized molten MRS agar, and dispensed into plates. Wells (5 mm diameter) were punched in the agar plates using a sterile borer. Cell-free supernatant of overnight HY1 cultures was filter-sterilized and 100 µL was dispensed into wells. The plates were pre-incubated at 7 °C to allow the supernatants diffuse into the agar following incubated at 37 °C for 24 h. The inhibition zones was measured using a caliper.

Antibiotic susceptibility

To evaluate the antibiotic susceptibility of the *B. aryabhattai* HY1, the fresh culture of the bacteria was streaked densely on Mueller–Hinton agar by a sterile cotton swab. Ampicillin, amoxicillin, tetracycline, gentamicin, levofloxacin, penicillin, streptomycin (10–40 µg/disc) were loaded on the plates[30]. The zone diameters were measured after incubation at 37 °C for 48 h. Results were interpreted as resistant (R), intermediate (I) or sensitive (S) according to CLSI guidelines[31].

Detection of short-chain fatty acid (SCFAs) by gas chromatography-flame ionization detector (GC-FID)

B. aryabhattai HY1 (10^7 CFU/mL) was cultured in MRS broth in the presence or absence of 2% glucose at 37 °C for 24 h. The culture supernatants were filtered through a 0.22 µm microfiltration membrane. Samples were added with the concentrated HCl (100 µL) and extracted with 5 ml diethyl ether for 20 min by gently rolling. After centrifugation (3,500 rpm, 5 min), the supernatant was transferred to a Pyrex extraction tube, neutralized with NaOH (500 µL, 1 mM). The aqueous phase was moved to an autosampler vial, and added the concentrated HCl (100 µL). The analysis of the SCFAs in culture media was conducted using an Agilent 7890A GC-FID equipped with an Agilent J&W GC Column DB-FFAP (30 m × 0.25 mm × 0.5 µm). The Agilent OpenLab ChemStation (version B.04.03) was used for data collection. The injection temperature was 300 °C. Samples were injected in pulsed split injection mode in 1 µL aliquots. The initial oven temperature was 100 °C, with a hold time of 0 min. The oven temperature increased from 100 °C to 250 °C at 20 °C /min, with a final hold time of 0 min. Helium was the carrier gas, with a flow rate of 1.7682 mL/min. The total run time of the method was 10 min.

Statistical analysis

GraphPad Prism 5 (GraphPad Software, Inc., San Diego, USA) was utilized for data analysis by unpaired t-test. The statistical significance was considered as p-value < 0.05 (*), < 0.01 (**), and < 0.001 (***). Results are expressed as mean ± standard deviation (SD) from at least three independent experiments.

Results

Identification and phylogenetic analyses of the test bacteria

The colony of the bacteria isolated from Vietnam pickled mustard greens on MRS agar was 3 mm in diameter, yellowish-cream in color, pigment-free, with round to irregular shapes and whole to undulate edges (Fig. 1a). The strain are Gram-positive and short rod-shaped cells (Fig. 1b). The overlapping parts of the 16S sequences from our strain and strains of different *Bacillus* species in BLAST searches in GenBank were 99.93% identical. The sequence were deposited in GenBank under accession number PQ653493 (Fig. S2). The isolated bacteria were conspecific, but could not be unequivocally determined as *B. aryabhattai* with BLAST search in GenBank, but only by conducting a phylogenetic analysis (Fig. S2) with a representative own sequence (PQ653493) and carefully selected sequences of reliably identified species from taxonomic papers. Our strain nested in a clade composed exclusively of *B. aryabhattai* with strong support. *Priestia* (formerly known as *Bacillus*) is a new

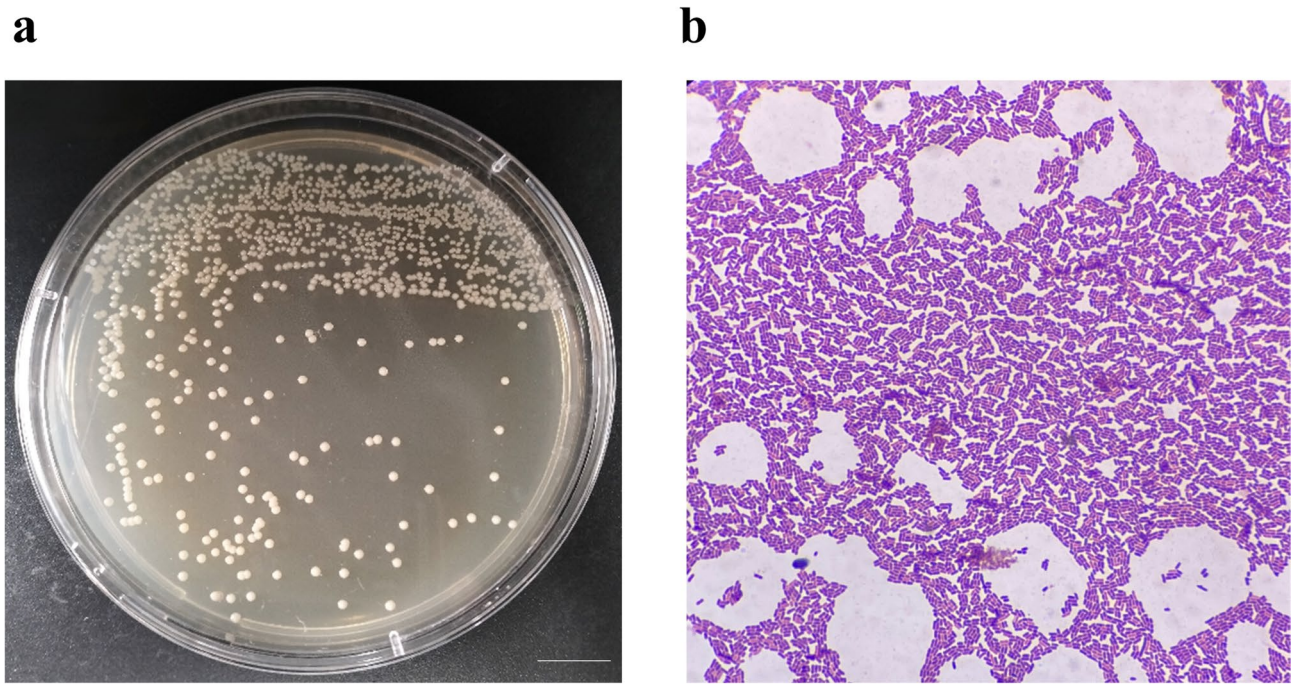

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Fig. 1. *B. aryabhattai* HY1 isolated from Vietnamese pickle mustard green; (**a**). Colony characteristics, (**b**). Shape of isolated bacterial cell after Gram staining.

species of the family Bacillaceae of the order Bacillales. *B. aryabhattai* cells are characterized by their highly variable shape and size (2.5–4 µm long and 0.8–1 µm wide)[32–34].

Glucose fermentation of *B. aryabhattai* HY1

To examine the fermentative capabilities of the isolate, *B. aryabhattai* HY1 was cultured in MRS medium with or without 2% glucose for 24 h. MRS media with glucose only or bacteria only served as controls. As shown in Fig. 2a, b, yellowish media and a decrease in OD_{562} (0.099 ± 0.001) were detected in the culture of *B. aryabhattai* HY1 in MRS medium without glucose. Medium exhibited a more yellowish colour and showed a considerable decline in OD_{562} value upon the addition of 2% glucose (0.094 ± 0.003) compared to controls. The data further confirmed that glucose can trigger and enhance the fermentation in *B. aryabhattai* HY1.

Determination of acid tolerance

A probiotic strain possesses the ability to withstand unfavourable conditions of the human body such as low pH and bile salt; gastric candidate probiotic is potential in the prevention and treatment of gastric disorders and dysbiosis[35]. The resistance of the *B. aryabhattai* HY1 to acidic conditions was accessed at pH 2–6 for 3h. Viability was 16 ± 1% at pH 2 and 53 ± 3% at pH 3 (Fig. 3). Survival increased at higher pH values, indicating that the strain can recover from acid stress and resume growth.

Determination of bile tolerance

The viability of bacteria upon bile secretion at different incubation times simulating the physiological aspects of human digestive system[36]. In vitro experiments have been conducted to investigate high tolerance of *B. aryabhattai* HY1 to bile salts. Results are shown on Fig. 4, the strain maintained 93.30 ± 2.30% viability in 0.3% bile salt after 3 h. At higher concentrations such as 0.5% and 1% bile, survival rate (%) was reduced to 49.71 ± 1.72 and 24.31 ± 3.40, respectively. Even at 2% bile, viable counts remained at 0.27×10^8 CFU/mL (Table S1 and Fig. S3).

Antibiotic resistance

Several *Bacillus* strains have been employed for centuries as biomass for animal feed, feed additives, manufacture of traditional fermented dishes in Asia, and plant production products[37,38]. From the perspective of antibiotic use, some probiotic strains with intrinsic antibiotic resistance could be useful for restoring lost gut microbial diversity after antibiotic treatment and provide colonization resistance against the proliferation of disease-causing organisms. Antibiotic sensitivity test showed that the *B. aryabhattai* HY1 was highly susceptible to

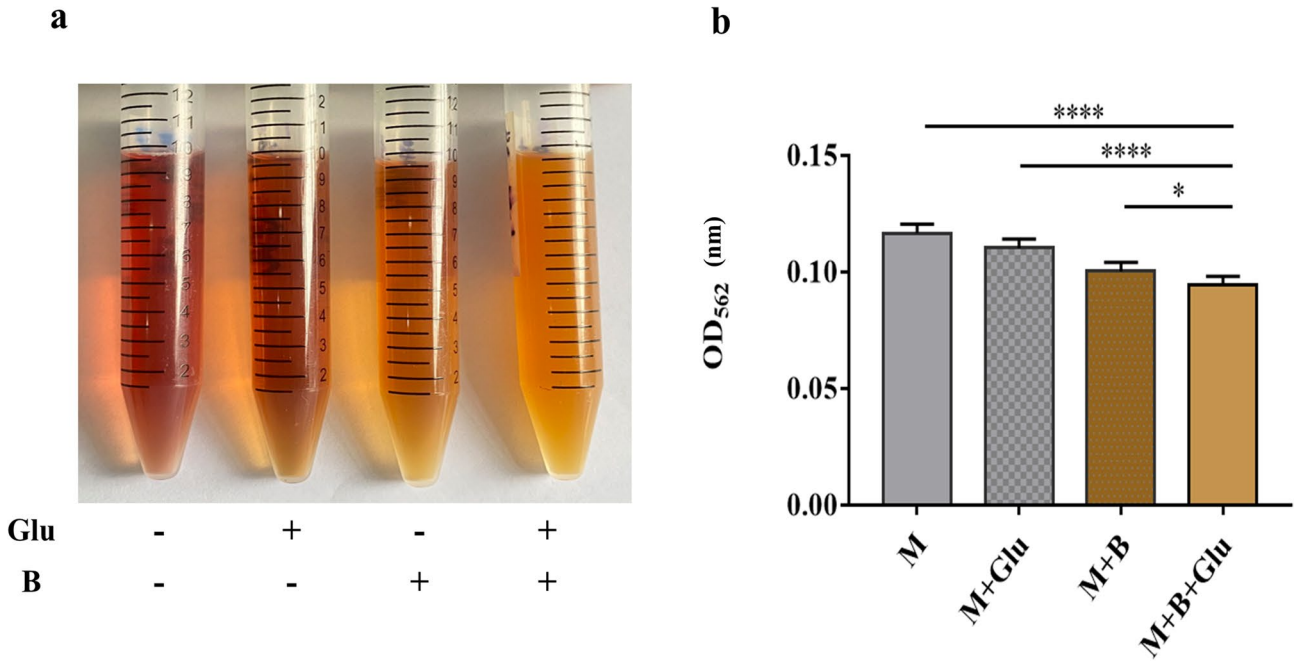


Fig. 2. Glucose fermentation by *B. aryabhattai* HY1. (**a**) *B. aryabhattai* (B) was cultured in MRS media (M) with or without the addition of 2% glucose (Glu) for 24 h. MRS media added with 2% glucose alone were included as a control. Bacterial fermentation was indicated by the colour change of phenol red from red to yellow. (**b**) Media of culture with bacteria and/or glucose were measured by OD_{562}. The reduction of OD_{562} was detected when fermentation happened. After 24 h, the OD_{562} value of *B. aryabhattai* (B) with glucose (Glu) was significantly reduced (0.094 ± 0.003, $n = 3$) compared to that (0.108 ± 0.001, $n = 3$) of MRS medium (M) and glucose (Glu). Data are the mean ± SD of experiments performed in triplicate. $^{*}p < 0.05$. $^{****}p < 0.0001$ (two-tailed t -test).

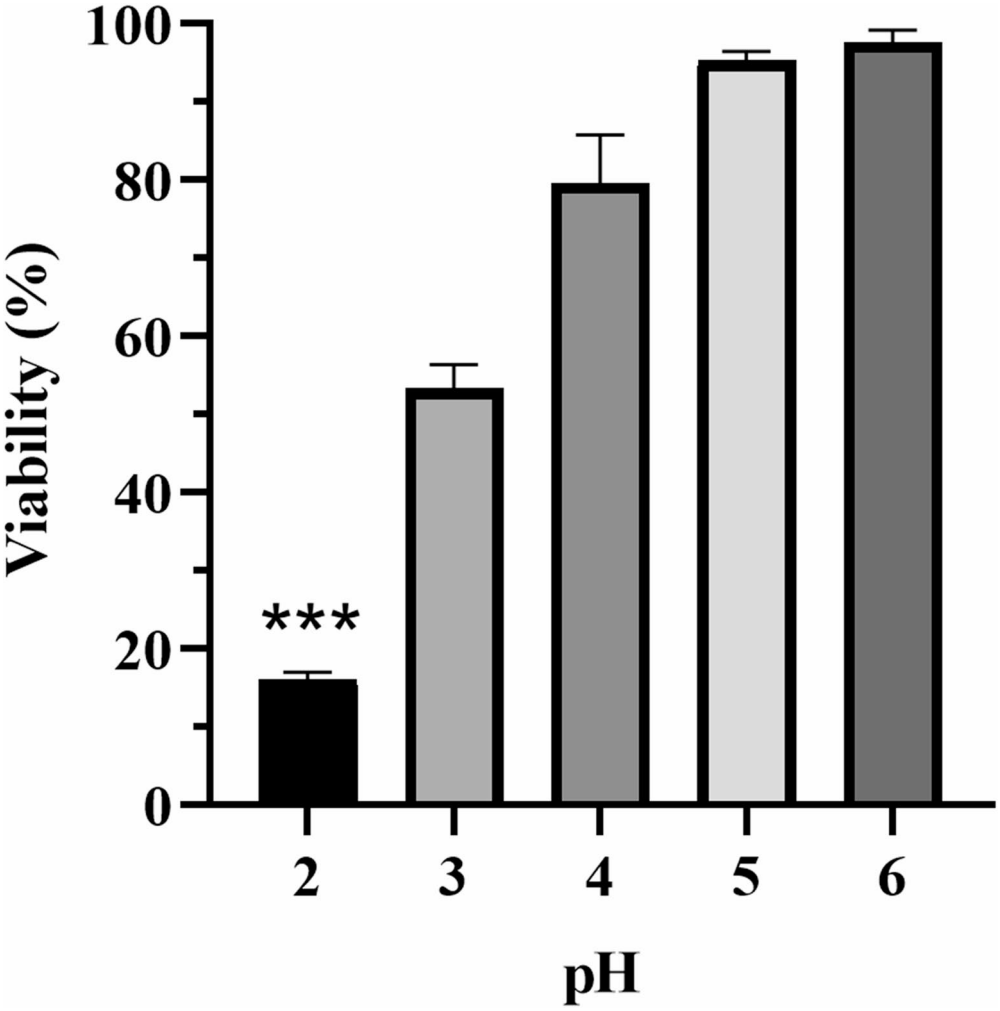


Fig. 3. Acid tolerance of *B. aryabhattai* HY1. Data are the mean ± SD of experiments performed in triplicate. $^{***}p < 0.001$ (two-tailed t -test).

30–40 µg ampicillin (AMP) and 25–40 µg amoxicillin (AMC) (Table 1), showing large inhibition zone diameters (IZDs) (Fig. S4). The strain exhibited intermediate susceptibility to 20 µg amoxicillin (AMC) (Table S2), but resistant to tetracycline (TCY), gentamicin (GEN), levofloxacin (LVX), and penicillin (PEN).

Antibacterial activity

Bacillus strains from traditional fermented food, which have also been shown to possess characteristics such as anti-oxidant, antimicrobial, immunomodulation[11]. *B. aryabhattai* HY1 produced inhibition zones against *E. coli* (14.63 ± 0.32 mm), *S. aureus* (6.17 ± 0.58 mm), *S. typhimurium* (16.83 ± 0.35 mm), *P. aeruginosa* (11.47 ± 0.55 mm) (Fig. 5, Table S3).

SCFAs was produced by glucose fermentation of *B. aryabhattai* HY1

SCFA production during glucose fermentation was analyzed by GC-FID. *B. aryabhattai* HY1 produced acetic and butyric acids in MRS medium with or without added glucose (Fig S5). In the absence of glucose, cultures yielded 96.80 mg/L acetic acid and 57.91 mg/L butyric acid (Table 2). However, with 2% glucose supplementation, production increased to 354.71 mg/L acetic acid and 249.20 mg/L butyric acid, illustrating the prebiotic property of glucose for *B. aryabhattai* HY1. No SCFAs were detected in control media without bacteria. The acetic acid and butyric acid were detected in MRS medium with *B. aryabhattai* HY1 because the presence of dextrose in MRS which serves as a potential carbon source. This finding indicates that *B. aryabhattai* HY1 can ferment glucose to generate acetic acid and butyric acid.

Discussion

The isolated bacterium (GenBank accession number PQ653493) was identified as *B. aryabhattai* HY1 by BLAST search in GenBank and a phylogenetic analysis (Fig. S2). This species has been recognized as a potential probiotic bacterium exhibiting multiple beneficial effects. Previous studies have reported that *B. aryabhattai* demonstrates bacteriocin-producing activity against *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Salmonella enterica* serovar

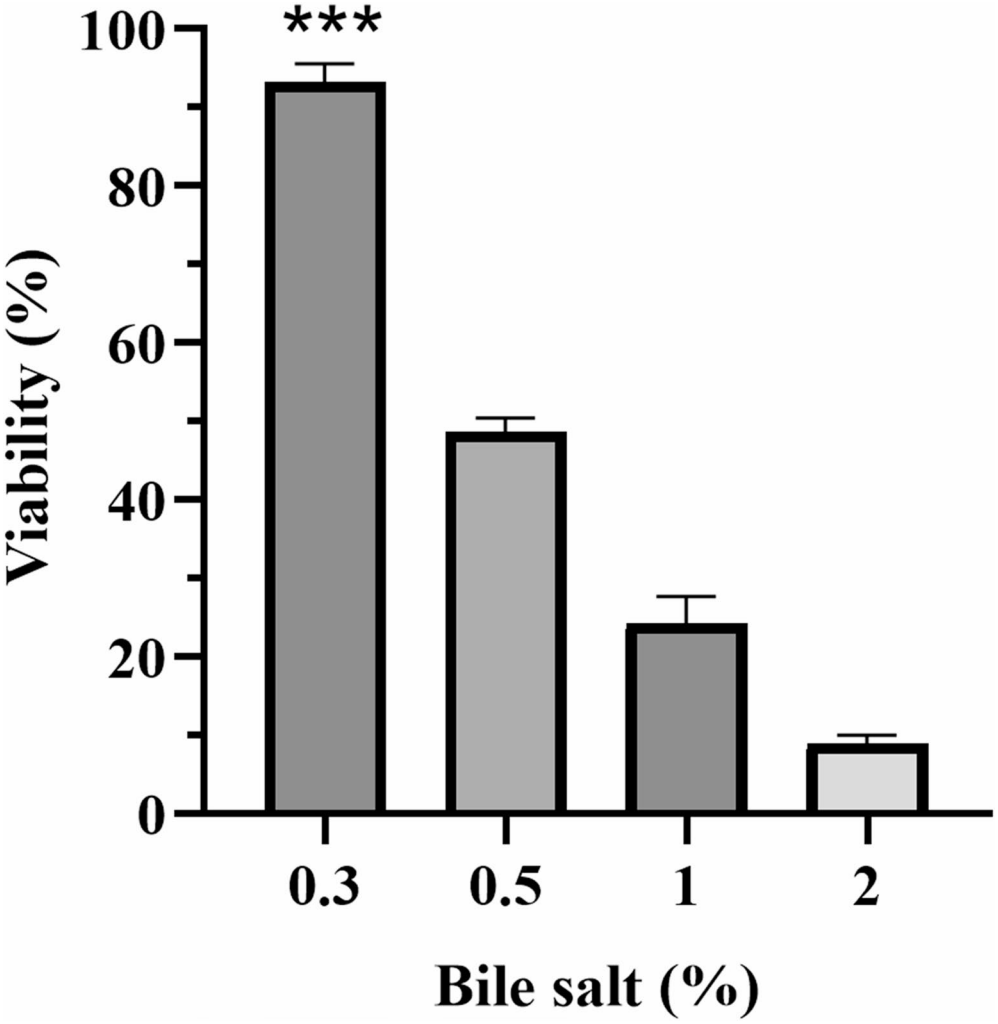


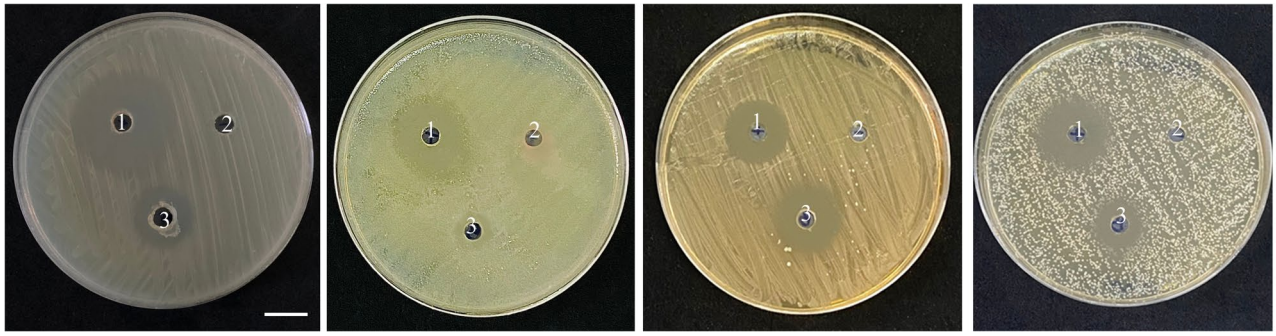
Fig. 4. Bile salts tolerance of *B. aryabhattai* HY1. Data are the mean ± SD of experiments performed in triplicate. $^{***}p < 0.001$ (two-tailed t -test).

Antibiotics	Concentration (µg/mL)						
	10	15	20	25	30	35	40
Ampicillin (AMP)	R	R	R	R	S	S	S
Amoxicillin (AMC)	R	R	I	S	S	S	S
Tetracycline (TCY)	R	R	R	R	R	R	R
Gentamicin (GEN)	R	R	R	R	R	R	R
Levofloxacin (LVX)	R	R	R	R	R	R	R
Penicillin (PEN)	R	R	R	R	R	R	R

Table 1. Antibiotic susceptibility of *B. aryabhattai* HY1. Inhibition zones, < 10 mm (Resistant, R), 10–15 mm (Intermediate, I), > 15 mm (Susceptible, S).

Typhimurium TISTR 29215[14], as well as the ability to boosted innate immunity and antioxidant defences[17], and reduce inflammatory responses in turbot juveniles[39]. However, no studies have investigated the probiotic attributes of *B. aryabhattai* isolated from pickled *Brassica juncea*.

The antagonistic effect of various *Bacillus* species against foodborne pathogens, attributed to the secretion of antimicrobial peptides, small extracellular effector molecules, and their ability to form biofilm, sporulate anaerobically, and adhere to host tissues[40–42]. A probiotic *B. aryabhattai* strain isolated from fish gut was also shown to inhibit *Streptococcus mutans*, a pathogen responsible for oral diseases[43]. Rajabi et al. demonstrated that *B. aryabhattai* effectively inhibited the growth of *Listeria monocytogenes*, the causative agent of listeriosis[44]. Collectively, these findings highlight *B. aryabhattai* as a promising probiotic candidate with broad antimicrobial



Escherichia coli *Staphylococcus aureus* *Salmonella typhimurium* *Pseudomonas aeruginosa*

Fig. 5. Antipathogen activity of *B. aryabhattai* HY1. The inhibition zone diameter of *B. aryabhattai* HY1 against *E. coli, S. aureus, S. typhimurium, P. aeruginosa*. Positive control: Amoxicillin (1); Negative control: distilled water (2), *B. aryabhattai* HY1 (3).

Samples	Concentration (mg/L)	
	Acetic acid	Butyric acid
M	< LOQ	< LOQ
M + G	< LOQ	< LOQ
M + B	96.80	57.91
M + G + B	354.71	249.20

Table 2. Acetic acid and Butyric acid were determined by GC-FID method. LOQ, Limit of quantitation, LOQ = 10 mg/L. Bacillus aryabhattai (B) was cultured in MRS media (M) with or without the addition of 2% glucose (G) for 24h. MRS media (M) with only 2% glucose (G) as a control.

potential and possible applications in food safety, aquaculture, and human health. Similar antibacterial effects were reported for *Lactobacillus paracasei* against *E. coli* and *S. aureus*, highlighting the relevance of these inhibitory patterns among probiotic candidates[45]. Nevertheless, the probiotic properties of *B. aryabhattai* isolated from fermented vegetables, particularly Vietnamese pickled mustard greens, remain largely unexplored.

The antibiotic susceptibility profile of *Bacillus aryabhattai* HY1 is consistent with previous findings reported for other *B. aryabhattai* strains. For instance, *B. aryabhattai* AB211 was resistant to tetracycline, rifamycin, troleandomycin, lincomycin, nalidixic acid, and aztreonam[46]. Similarly, strains isolated from rhizospheric soil displayed resistance to some tested antibiotics, including ampicillin (10 µg) and Penicillin (10 µg)[47]. *B. aryabhattai* LAD harbors 31 antibiotic resistance genes, suggesting a strong adaptive capability in antibiotic-rich environments[48]. In this study, *B. aryabhattai* HY1 exhibited susceptibility to amoxicillin (25–40 µg) and ampicillin (30–40 µg). In accordance with the European Food Safety Authority (EFSA) and the Food and Agriculture Organization of the United Nations (FAO)/the World Health Organization (WHO) guidelines for probiotic safety assessment, particular attention should be given to the potential for horizontal gene transfer (HGT) of antibiotic resistance genes (ARGs) among gut microbiota[49,50]. Although the antibiotic resistance observed in *B. aryabhattai* HY1 may reflect intrinsic resistome of bacterial pathogens, the possibility of mobile genetic elements mediating HGT cannot be excluded. Therefore, future work should include whole-genome sequencing and molecular screening to identify and characterize potential ARGs and their genetic context. Such analyses will provide a comprehensive safety evaluation and ensure compliance with international regulatory frameworks for probiotic application.

Probiotics administered orally must endure the harsh conditions of the gastrointestinal tract, including exposure to gastric acid and bile salts[51]. Acid and bile tolerance are therefore crucial determinants of probiotic viability and functionality within the host[52]. In this study, *B. aryabhattai* HY1 exhibited substantial viability at pH 2 and pH 3, indicating strong acid resistance. The gastric pH in humans fluctuates between 1.5 during fasting and 4–6 after food intake, which corresponds to the optimal activity range of gastric lipase and pepsin[53]. A minimum tolerance threshold of pH 3.0 is essential for probiotic survival[54]. The comparable growth observed at pH 5 and pH 6 in *B. aryabhattai* HY1 aligns with the known pH growth range (4.5–7.0) for *Bacillus* species[55]. The passage time for gastric digestion can be from 1 to 4 h depending on the individual and diet[56]. This could be by a networked cellular enzymes from acidophilic microorganisms are functional at much lower pH than the cytoplasmic pH[57].

Bile tolerance is another essential criterion for probiotic efficacy. Consistent with previous findings that probiotic *Bacillus* species can withstand a wide range of bile concentrations[58,59]. This resilience may be attributed to bile salt hydrolase (BSH) activity, an enzyme involved in bile detoxification[60]. Studies recently showed that BSH activity and bile salt resistance capability *B. cereus* ATCC 14570[61] and *B. mojavensis* LY-06[62]. Collectively,

these data indicate that *B. aryabhattai* HY1 fulfills a key prerequisite for probiotic functionality by surviving in acid- and bile-rich environments.

The persistence of *B. aryabhattai* in fermented Assam tea leaves, showed great probiotic potential conferring health benefits on the host when adequate amounts are administered[63]. Acetate-producing *B. coagulans* has been shown to restore intestinal barrier function via Ffar2 regulates the NF-κB–MLCK–MLC pathway to improve tight junctions in *Apostichopus japonicus*[64]. *B. subtilis* strains have been associated with increased butyrate production in broiler gut microbiome and improved intestinal histomorphology[65]. Also, the supplementation of *B. subtilis* on in vitro ruminal fermentation have shown its ability to shift volatile fatty acid (VFA) profiles, including increases in butyrate under certain conditions[66]. It has been shown that *B. clausii* able to secrete acetic acid and butyric acids, whereas *B. coagulans* only produce acetic acid[67]. Here, we demonstrated, for the first time, that *B. aryabhattai* HY1 produced butyric acid and acetic acid. Taking together, dual SCFA production of *B. aryabhattai* HY1 strengthening the novelty of this bacteria as a direct SCFA-contributing probiotic.

SCFAs such as butyric and acetic acids, actively regulate anti-inflammatory signaling pathways and maintain gut immune homeostasis via SCFA receptors such as GPR43 and GPR109A[68,69]. Butyric acid serves as an energy source for probiotics and promotes mucin synthesis, thereby enhancing epithelial barrier integrity and preventing pathogen adherence[70]. These acids penetrate target cell membranes of pathogens in their undissociated hydrophobic state, causing cytoplasmic acidification and subsequent cell death[18]. Glucose levels in the small human intestine typically range from 50 to 500 mM, peaking above 300 mM after eating[71]. During digestion, complex carbohydrates are hydrolyzed into simple sugars such as glucose, fructose, and galactose, which can serve as substrates for fermentation and SCFAs production by probiotics[72,73]. Our results showed that *B. aryabhattai* HY1 can produce ample amounts of butyric acid and acetic acid, which inhibit pathogenic bacteria. However, as these findings are based on in vitro analyses, further in vivo investigations are necessary to validate its functional properties, safety, and efficacy within host systems.

Conclusion

In this study, *B. aryabhattai* HY1 was isolated from Vietnamese pickled mustard greens (*Brassica juncea*). The strain exhibited strong tolerance to simulated gastrointestinal conditions, maintaining high viability under acidic (pH 3) and bile salt (0.3%) environments. *B. aryabhattai* HY1 showed susceptibility to ampicillin (30–40 µg/mL) and amoxicillin (25–40 µg/mL), as well as inhibitory activity against common pathogenic bacteria including *E. coli*, *S. aureus*, *S. typhimurium*, and *P. aeruginosa*. Moreover, glucose fermentation by *B. aryabhattai* HY1 led to the production of acetic and butyric acids, which can support gut barrier integrity, mucosal immunity, and host metabolic health. Based on these findings, *B. aryabhattai* HY1 holds promise for potential incorporation into functional food products, dietary supplements, or fermented food formulations. Comprehensive in vivo studies and genomic safety evaluations are necessary to confirm the probiotic efficacy, stability, and safety.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions

T.H.Y.N. and M.K.L. were in charge of experiments; P.T.U.N. analyzed data; M.T.P. wrote, edited, and reviewed manuscript; C.T.D. designed and interpreted study. All authors approved the final version of the manuscript. M.T.P. is the guarantor of this work.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to M.T.P.