AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Probiotic properties and antibacterial activity against aquatic pathogens of non-starch polysaccharide degrading *Bacillus velezensis* newly isolated from gut of the termite *Termes comis*

Kittipong Chanworawit, Putsawee Tomtong, Poramet Chuglum, Pinsurang Deevong*

Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

Article Info

Article history:
Received 12 April 2025
Revised 29 June 2025
Accepted 12 July 2025
Available online 31 July 2025

Keywords:
Aquatic animal pathogens,
Bacillus,
Non-starch polysaccharides,
Probiotic,
Termite guts

Abstract

Importance of the work: Termite gut bacteria harboring non-starch polysaccharide (NSP) degradation and probiotic properties are beneficial as a microbial fermentation additive for plant-based animal feed.

Objectives: To evaluate the probiotic properties and antimicrobial activity against aquatic animal pathogens of NSP-degrading *Bacillus* sp. isolated from the guts of the termite *Termes comis*.

Materials and Methods: NSP-degrading bacteria were isolated and screened from the guts of termites. The selected bacteria were identified molecularly and assayed for NSP-degrading activity, as well as being evaluated for their antimicrobial ability and probiotic properties (stress tolerance, antioxidant activity, bacterial safety profile and cell surface characteristics).

Results: Among the 49 bacterial isolates obtained from the termite gut samples, the isolates Tc10, Tc19 and Tc44 were selected based on the presence of NSP-degrading enzymes (cellulase, pectinase and xylanase). Based on 16S rRNA gene sequence analysis, these three isolates were closely related to *Paenibacillus lutimineralis* (Tc10), *Paenibacillus alvei* (Tc19) and *Bacillus velezensis* (Tc44). Among them, only *B. velezensis* Tc44 could survive in gastrointestinal tract conditions of pH 2.5 and 0.3% bile salt and exhibit broad-spectrum inhibition against all five tested aquatic pathogenic bacteria. Furthermore, it had the highest antioxidant activity and presented NSP-degrading enzyme activity against all tested types, as well as being harmless based on hemolytic activity, biogenic amine production and antibiotic susceptibility. In addition, Tc44 had the highest adhesion capability to Caco-2 and HT-29 cells, with anti-adhesion ability against all the tested pathogens, based on competition, inhibition and displacement assays.

Main finding: Scientific knowledge was documented of NSP-degrading *Bacillus*-based probiotics obtained from gut samples of the soil-feeding *T. comis*, which could be beneficial for nutritive improvement in animal feed and the prevention of aquatic animal disease in aquaculture.

* Corresponding author.
E-mail address: fsciprd@ku.ac.th (P. Deevong)

https://doi.org/10.34044/j.anres.2025.59.4.14

Introduction

Non-starch polysaccharides (NSPs) are polymeric carbohydrates that are different from common starch and difficult to degrade (Tomtong and Deevong, 2024). NSPs, such as cellulose, hemicellulose and pectin, are the main components of plant cell walls (Sinha et al., 2011). Many plant-based raw materials containing NSPs are most commonly used as main ingredients for animal feed, including aquafeed (Arriaga et al., 2021). Due to the lack of NSP-degrading enzymes in monogastric animals (including fish), the undigested NSPs can remain along the gastrointestinal tract (GIT) leading to a major increase in digestion and absorption inhibition of feed nutrients and a reduction in the growth performance of aquatic animals, even causing metabolic damage, resulting in metabolic disorders (Amirkolaie et al., 2005). NSP hydrolysis by microbial enzymes is one alternative way to minimize the anti-nutritive impacts of NSPs and to improve their utilization. In addition, NSP hydrolysis products may act as prebiotics (Sargautiene et al., 2018).

Termites (Isoptera) are terrestrial insects that feed on lignocellulosic and other compounds in plants. Although the feeding habits of different termite species vary, they are preferred as promising sources of plant cell wall-degrading microorganisms in their guts and some microbial species are able to digest NSPs such as cellulose, hemicellulose and lignin (Auer et al., 2017). Generally, the termite guts are considered an extreme environment with a diverse microbial community and the presence of various carbohydrate-degrading microorganisms. Many bacterial species obtained from termite guts have the potential to produce hydrolytic enzymes (Nualkul et al., 2022) and studies have revealed that some NSP degrading bacteria isolated from termite guts are taxonomically classified as species in the *Bacillus* group such as *Bacillus subtilis, Bacillus thuringiensis, Bacillus siamensis* and *Bacillus amyloliquefaciens* (Chanworawit et al., 2023; Tomtong and Deevong, 2024).

Probiotics are live and beneficial microorganisms that inhabit animal gastrointestinal tracts and have important roles for improving animals and human health and are found in a variety of microorganisms, including the genera *Bacillus, Lactobacillus, Bifidobacterium, Enterococcus, Streptococcus* and some yeast species (Mu et al., 2018). Endospore-forming *Bacillus* spp. have high stability in extreme-environment conditions such as heat, acidity and alkalinity (Elshaghabee et al., 2017). Commercial *Bacillus* probiotics in use include *B. coagulans, B. subtilis, B. amyloliquefaciens, B. pumilus, B. licheniformis, B. polyfermenticus, B. clausii* and some non-pathogenic *B. cereus* strains (Duc et al., 2004). These probiotic strains have antipathogenic and antioxidant activity, immunomodulatory properties, can produce vitamins and tolerate stressful conditions. In addition to their probiotic properties, *Bacillus* spp. are used as fermentation starters to improve the nutrient value of plant-based animal feeds, based on the digestibility of microbial extracellular enzymes such as protease, lipase, amylase, cellulase, pectinase, xylanase and chitinase (Molva et al., 2009). The important pathogens in aquaculture including the genera *Aeromonas, Pseudomonas, Vibrio, Streptococcus, Flavobacterium, Edwardsiella* and *Lactococcus* have been associated with health problems (Jlidi et al., 2022). Many studies have shown that probiotic *Bacillus* spp. can produce antimicrobial substances which offer inhibition to pathogens and prevent their intestinal adhesion and lead to better health properties (Li et al., 2020).

Therefore, the objectives of the current study were to evaluate the probiotic properties and antimicrobial activity against aquatic animal pathogens of NSP-degrading *Bacillus* sp. newly isolated from the guts of the soil-feeding higher termite *Termes comis*. This *in vitro* study aims to provide a scientific foundation for potential probiotic bacteria from the termite guts useful for future research in feed microbiology and biotechnology.

Materials and Methods

Bacterial isolation from guts of T. comis

Samples of the soil-feeding higher termite, *T. comis*, were collected from the Sakaerat Environmental Research Station, Nakhon Ratchasima province, Thailand (14°30’34.3”N 101°55’50.9”E). Following collection, 30 termites on an ice-cold plate were washed three times with phosphate buffered saline (PBS) solution (pH 7.4). The termite guts were removed and the gut content was squeezed using a sterile pestle and mixed by vortexing. The gut homogenate was heated at 80°C for 30 min and then cooled in an ice bath. The suspension was serially diluted and spread on nutrient agar (NA) pH 7.0, followed by incubation at 37°C for 24 hr, after which the bacterial colonies were collected. Morphologically different colonies were purified using the cross-streak method on an NA plate and characterized based on Gram and endospore staining.

Screening of non-starch polysaccharide-degrading bacteria

Gram-positive bacilli were selected and tested for the ability to produce NSP-degrading enzymes (cellulase, pectinase and xylanase). According to the methods described by Tomtong and Deevong (2024), the bacterial isolates were point-inoculated on NA supplemented with 1% of different substrates: carboxymethyl cellulose (CMC; Sigma-Aldrich, Finland), citrus pectin (Sigma-Aldrich, Denmark) and beech-wood xylan (Megazyme, Ireland). After incubation at 37°C for 24 hr, the rate of enzyme production was measured based on the ratio of the hydrolytic zone diameter to bacterial colony diameter, with all diameters measured in millimeters. The isolates with broad-spectrum NSP-degrading activities were selected for subsequent experiments.

Identification of potential non-starch polysaccharide degrading bacteria

Three selected bacteria were identified based on sequence analysis of the 16S rRNA gene. Bacterial genomic DNA of the selected isolates were extracted using a Genomic DNA Purification Kit (Thermo Scientific, USA) according to the manufacturer's instruction. The 16S rRNA gene (~ 1.5 kb) was amplified using polymerase chain reaction (PCR) with the primer pair, 616V (forward primer: 5' AGAGTTGATYMTGGCTC 3') and 1492R (reverse primer: 5' TACGGYTACCTTGTTACGACTT 3'), according to Loy et al. (2005). The PCR conditions were described by Chanworawit et al. (2023). The PCR reactions were carried out in a BIONEER MyGenie32 Thermal Block (Bioneer, Republic of Korea) and amplicons were analyzed based on 1% agarose gel electrophoresis to confirm their fragment size. The PCR products were purified using the TIANquick Midi Purification Kit (Tiangen Biotech (Beijing) Co., Ltd., China) according to the manufacturer's protocol. DNA sequencing was conducted by Macrogen, Inc. (Republic of Korea). The 16S rRNA nucleotide sequences were identified using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and a phylogenetic tree was constructed using the MEGA version X after multiple sequence alignments, applying the maximum-likelihood method with 1,000 bootstrap replicates.

Tolerance in acidity and bile salt conditions

For preparation of bacterial inoculum, the isolates were cultivated in nutrient broth (NB) at 37°C for 18 hr. Then, the culture turbidity was adjusted to McFarland No. 0.5 (1.5×10^8 cells/mL). The bacterial inoculum (1% v/v) was inoculated into NB pH 2.5 and NB supplemented with 0.3% (w/v) bile bovine (Sigma-Aldrich, USA). After incubation at 37°C for 3 hr, the suspension was serially diluted in PBS solution (pH 7.4), then spread on NA and incubated at 37°C for 24 hr. The bacterial colonies were counted and calculated for bacterial viability using Equation 1:

$$\text{Survival rate (\%)} = \frac{\text{Bacterial number (log CFU/mL) at 3 hours}}{\text{Bacterial number (log CFU/mL) at the initial time}} \times 100 \quad (1)$$

where numbers are expressed as log colony forming units (CFU)/milliliter.

Assay of antimicrobial activity against aquatic animal pathogens

Bacterial isolates were tested for their ability to inhibit the growth of aquatic-animal pathogenic bacteria (*Aeromonas hydrophila* ATCC 7966*, Aeromonas schubertii* ATCC 43700*, Streptococcus agalactiae* ATCC 27956*, Vibrio parahaemolyticus* ATCC 17802 and *Lactococcus garvieae* ATCC 49156) using the drop assay method (Tomtong and Deevong, 2024). The bacterial isolates and pathogens were cultivated in trypticase soy broth at 37°C for 18 hr, then the turbidity was adjusted to McFarland No.0.5 (1.5×10^8 cells/mL). Culture suspension of each pathogen was swabbed on trypticase soy agar (TSA) using a sterile cotton swab; then, 5 µl of the test bacterial suspension was dropped on the agar surface. After incubation at 37°C for 24 hr, the rate of pathogen inhibition was measured based on the ratio of the inhibition zone diameter to the tested bacterial colony diameter, with the diameters measured in millimeters.

Determination of antioxidant activity

The scavenging of hydroxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was determined in this step. The hydroxyl radical scavenging assay was adapted from the method of Sroka and Cisowski (2003). The reaction mixture containing 2 mL of phosphate buffer pH 7.4, 1 mL of each 1,10-phenanthroline (0.75 mM), $FeSO_4$ (0.75 mM) and bacterial suspension (1.5×10^8 cells/mL) was added into each tube. Then, 1 mL of 0.01% (v/v) hydrogen peroxide (H_2O_2) was added to start the reaction. After incubation at 37°C for 1 hr, the absorbance of mixture was measured at 536 nm and calculated for hydroxyl scavenging rate using Equation 2:

$$\text{Hydroxyl scavenging rate (\%)} = [(As - Ac) / (Ab - Ac)] \times 100 \quad (2)$$

where As is the absorbance of bacterial suspension at 536 nm, Ac is the absorbance of the control including 1,10-phenanthroline, $FeSO_4$ and H_2O_2 and Ab is the absorbance of the blank including 1,10-phenanthroline and $FeSO_4$.

The method for scavenging the DPPH free radicals was modified from Rahman et al. (2015). The reaction mixture containing 1 mL of DPPH (0.05 mM; Sigma-Aldrich, USA) and 1 mL of bacterial suspension (1.5×10^8 cells/mL) was added into each tube. After incubation in the dark at 37°C for 1 hr, the absorbance of mixture was measured at 517 nm and the DPPH scavenging rate was determined using Equation 3:

$$\text{DPPH scavenging rate (\%)} = [(Ac - As) / Ac] \times 100 \quad (3)$$

where As is the absorbance of bacterial suspension at 517 nm and Ac is the absorbance of the control including DPPH and distilled water.

Assay of non-starch polysaccharide degrading enzyme activity

Enzyme activity was determined using the 3,5-dinitrosalicylic acid (DNS) method (Miller 1959). To prepare the bacterial inoculum, the isolates were grown in NB at 37°C for 18 hr and further diluted to an optical density at 600 nm value of 1.0. Each bacterial inoculum (0.5 mL) was inoculated into a 250 mL Erlenmeyer flask containing 50 mL of enzyme production medium, modified from Sreena and Sebastian (2018). Then 1 L of the modified medium, consisting of yeast extract 5 g, $(NH_4)_2SO_4$ 4.5 g, $CaCl_2$ $2H_2O$ 0.1 g, $MgSO_4$ $7H_2O$ 0.1 g, NaCl 0.1 g, KH_2PO_4 0.7 g, $MnSO_4 \times 4H_2O$ 0.01 g and $FeSO_4 \times 7H_2O$ 0.01 g, supplemented with 1% (w/v) of different substrates (CMC, citrus pectin and beech-wood xylan). After incubation by shaking at 150 rpm and 37°C, the enzyme activity was evaluated every 24 hr. The reaction mixture of 1 mL of 1% (w/v) of the different substrates in 50 mM phosphate buffer (pH 7.0) and 1 mL of crude protein was incubated at 37°C for 30 min. After incubation, 3 mL of DNS reagent was added to the reaction mixture and boiled at 100°C for 10 min. After cooling, the absorbance at 540 nm was measured. The standard substances for analyzing NSP-degrading activity were glucose, galacturonic acid and xylose. One unit of enzyme activity was defined as the amount of enzyme which liberated 1 µmol of reducing sugar per minute.

Evaluation of safety profile based on hemolysis, biogenic amine production and antibiotic susceptibility

The bacterial culture was streaked on blood agar containing 5% (v/v) sheep blood to evaluate hemolytic activity. The hemolytic zone was observed after incubation at 37°C for 24 hr. *Staphylococcus aureus* ATCC 25923 was used as a positive control. To test for the biogenic amine production, the bacterial culture was inoculated on Moeller decarboxylase broth pH 6.0 (Bover-Cid and Holzapfel, 1999) composed of tryptone 5 g/L, yeast extract 5 g/L, meat extract 5 g/L, NaCl 5 g/L, $CaCO_3$ 1 g/L, pyridoxal-5-phosphate 0.05 g/L, bromocresol purple 0.01 g/L and cresol red 0.005 g/L, supplemented with 1% (w/v) of the different amino acids (histidine, tyrosine, arginine and lysine). After incubation at 37°C for 24 hr, the change in medium color was observed. The pH change is dependent on the production of the more alkaline biogenic amine from the amino acids initially included in the medium. Antibiotic susceptibility of each bacterial isolate was tested on Muller Hinton agar plates using a disc diffusion test (Duche et al., 2023). The antibiotic susceptibility pattern was assessed using ampicillin (10 µg/disc), vancomycin (30 µg/disc), streptomycin (10 µg/disc), kanamycin (10 µg/disc), chloramphenicol (30 µg/disc), erythromycin (15 µg/disc), ciprofloxacin (5 µg/disc), penicillin (6 µg/disc) and tetracycline (30 µg/disc). The diameter was measured in millimeters of each zone of inhibition. The results were compared with the interpretative zone diameters described in Clinical and Laboratory Standards Institute (2020), where sensitive (S) = ≥ 21 mm diameter, intermediate (I) = 16–20 mm diameter and resistant (R) = ≤ 15 mm diameter.

Assay of adhesion and anti-adhesion ability to human colon adenocarcinoma cells

The method for assay of adhesion and anti-adhesion ability to human colon adenocarcinoma cells was modified from Tomtong and Deevong (2024). Human colon adenocarcinoma cell lines (Caco-2 and HT-29 cells) were cultured in Dulbecco's modified eagle medium (Himedia) supplemented with 2 mM glutamine and 10% (v/v) fetal bovine serum in 6-well plates at 37°C in 5% CO_2 for 72 hr. Before use, the cell monolayer was washed three times with sterile PBS pH 7.4. The bacterial culture (1.5×10^8 cells/mL) was added into each well to evaluate bacterial adhesion to cells. After incubation at 37°C in 5% CO_2 for 1 hr, each well was washed three times with sterile PBS to remove non-adherent bacteria. After that, the adherent bacteria were released by adding 0.01% (v/v) Triton X-100 in PBS and the suspension was serially diluted in PBS and spread on NA. After incubation at 37°C for 24 hr, bacterial colonies were counted and the adhesion index was calculated using Equation 4:

$$\text{Adhesion index (\%)} = \frac{\text{Number of adherent bacteria}}{\text{Number of initial bacteria}} \times 100 \quad (4)$$

Prior to obtaining images of bacterial adhesion to cells, the Caco-2 and HT-29 cells were fixed in 2.5% (v/v) glutaraldehyde and then observed at 10,000 × magnification using a scanning electron microscope (SEM) in the Scientific Equipment Center, Faculty of Science, Kasetsart University, Bangkok, Thailand.

To evaluate the anti-adhesion ability of selected isolates to Caco-2 and HT-29 cells against aquatic animal pathogens, three procedures comprising competition, inhibition and displacement assays were conducted as described by Wang et al. (2021) with minor modification. The five species of aquatic animal pathogens used in this study consisted of *Aeromonas hydrophila* ATCC 7966, *Aeromonas schubertii* ATCC 43700, *Streptococcus agalactiae* ATCC 27956, *Vibrio parahaemolyticus* ATCC 17802 and *Lactococcus garvieae* ATCC 49156. Competitive tests were performed by adding culture of the test bacteria (1.5×10^8 cells/mL) and pathogen suspension (1.5×10^8 cells/mL) to the monolayer cells. After incubation at 37°C in 5% CO_2 for 1 hr, the wells were washed three times with sterile PBS to remove non-adherent bacteria. After that, the adherent bacteria were released by adding 0.01% (v/v) Triton X-100 in PBS. For the inhibition test, the culture of test bacteria (1.5×10^8 cells/mL) was added to the monolayer cells, then incubated at 37°C in 5% CO_2 for 1 hr. After washing, pathogen suspension (1.5×10^8 cells/mL) was added to the monolayer cells. After further incubation at 37°C in 5% CO_2 for 1 hr, the wells were washed three times with sterile PBS to remove non-adherent bacteria and the adherent bacteria were released. In the displacement test, pathogen suspension (1.5×10^8 cells/mL) was added to the monolayer cells and incubated at 37°C in 5% CO_2 for 1 hr. After washing, the culture of test bacteria (1.5×10^8 cells/mL) was added to the monolayer cells. After incubation at 37°C in 5% CO_2 for 1 hr, the wells were washed three times with sterile PBS to remove non-adherent bacteria and the adherent bacteria were released. To evaluate the anti-adhesion ability, the suspension was serially diluted in PBS and then spread on the appropriate medium including TSA (for *Streptococcus agalactiae* and *Lactococcus garvieae*), McConkey agar (for *Aeromonas hydrophila* and *Aeromonas schubertii)* and thiosulfate citrate bile salts sucrose agar (for *Vibrio parahaemolyticus*). After incubation at 37°C for 24 hr, the bacterial colonies were counted and their anti-adhesion ability was calculated using Equation 5:

$$\text{Anti-adhesion ability (\%)} = \frac{\text{Pathogen numbers (CFU/mL) with treatment bacteria}}{\text{Pathogen numbers (CFU/mL) without treatment bacteria}} \times 100 \quad (5)$$

Animal use protocol

The animal use protocol in the current research was approved by the Kasetsart University Institutional Animal Care and Use Committee, Bangkok, Thailand (Approval ID. ACKU68-SCI-009) and was in accordance with the Guidelines of Animal Care and Use under the Ethical Review Board of the Office of National Research Council of Thailand for conducting scientific research.

Statistical analysis

All tests were performed at least in triplicate. The results were expressed as mean ± SD values. The data were analyzed using one-way analysis of variance (ANOVA) in the SPSS version 25.0 software and the different superscripts indicate significant difference ($p < 0.05$).

Results

Isolation and screening of non-starch polysaccharide degrading bacteria

Aerobic bacteria were isolated from the guts of *T. comis* (soil-feeding higher termites) collected from the Sakaerat Environmental Research Station, Nakhon Ratchasima province, Thailand. After morphological study, 49 Gram-positive endospore-forming bacilli were selected and labelled as isolates Tc01 – Tc49. Then, all isolates were tested for their ability to produce three NSP-degrading enzymes (cellulase, pectinase and xylanase). Of the 49 isolates, 21 produced at least one enzyme (Fig. 1). There were more isolates with the ability to produce cellulase (17 isolates with production rates in the range 1.17 – 4.67) than pectinase (10 isolates with production rates in the range 1.83 – 8.94) and xylanase (10 isolates with production rates in the range 1.71 – 4.62). Among them, three isolates (Tc10, Tc19 and Tc44) produced all three enzymes; the ratios of enzyme production are provided in Table 1. The isolates Tc13 (4.67 ± 0.76), Tc05 (8.94 ± 1.83) and Tc02 (4.62 ± 0.33) had the highest production levels of cellulase, pectinase and xylanase, respectively. The bacteria were preserved in glycerol stock solution (20% v/v) at -80°C. The three bacteria (isolates Tc10, Tc19 and Tc44) with the ability to produce all three enzymes were used for subsequent experiments.

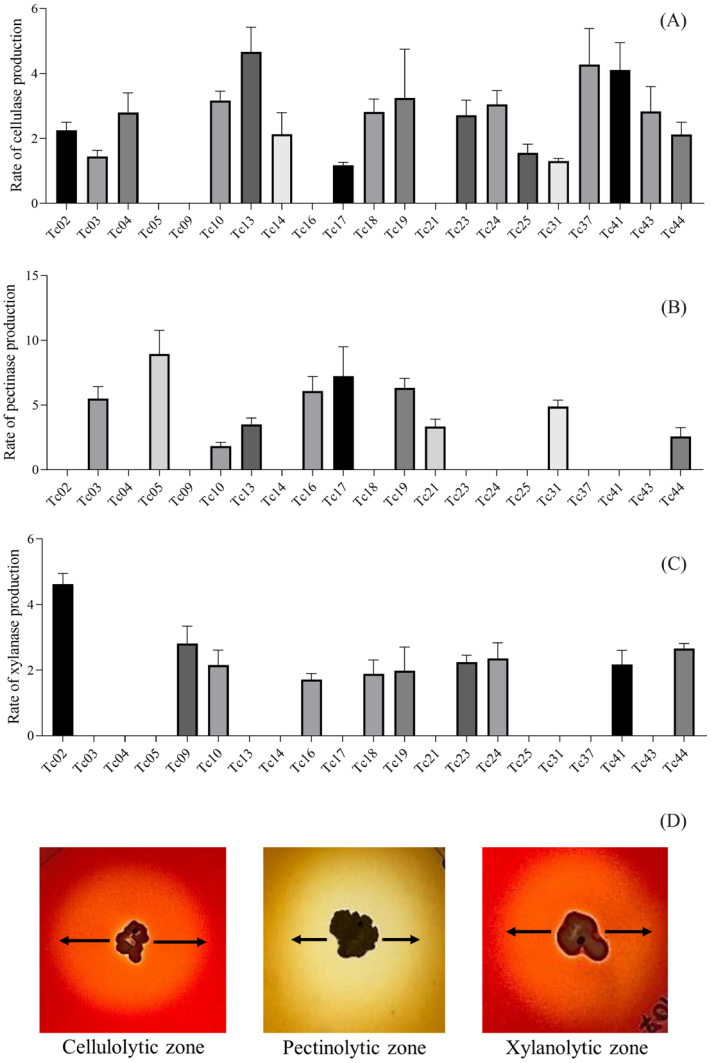

Fig. 1 Production rates of non-starch polysaccharide-degrading enzymes of isolated bacteria: (A) cellulase; (B) pectinase; (C) xylanase; (D) hydrolytic zones indicated by arrows, where error bars indicate ± SD

Table 1 NSP-hydrolytic enzyme production and probiotic characteristics of selected isolates

	Tc10	Tc19	Tc44
16S rRNA identification	*Paenibacillus lutimineralis*	*Paenibacillus alvei*	*Bacillus velezensis*
Rate of NSP-hydrolytic enzyme production			
Cellulase	3.17 ± 0.29[a]	3.25 ± 1.50[a]	2.12 ± 0.38[a]
Xylanase	2.16 ± 0.45[a]	1.98 ± 0.73[a]	2.66 ± 0.15[a]
Pectinase	1.83 ± 0.29[a]	6.33 ± 0.73[b]	2.58 ± 0.68[a]
Survival rate (%) under stress			
pH 2.5	-	-	100.39 ± 2.58
0.3% Bile salt	-	-	70.24 ± 3.60
Rate of pathogen inhibition			
Aeromonas hydrophila ATCC 7966	-	-	1.40 ± 0.08
Aeromonas schubertii ATCC 43700	-	-	1.22 ± 0.02
Streptococcus agalactiae ATCC 27956	-	-	1.78 ± 0.17
Vibrio parahaemolyticus ATCC 17802	1.03 ± 0.06[a]	1.21 ± 0.25[a]	2.60 ± 0.49[b]
Lactococcus garvieae ATCC 49156	1.32 ± 0.16[a]	1.09 ± 0.03[a]	1.91 ± 0.03[b]
Relative scavenging activity (%)			
Hydroxyl radical	16.67 ± 4.73[a]	25.00 ± 6.01[a]	60.61 ± 6.94[b]
DPPH radical	7.99 ± 2.69[a]	24.12 ± 3.65[b]	56.76 ± 2.27[c]

DPPH = 2,2-diphenyl-1-picrylhydrazyl; –= not detectable.

Data are the mean ± SD (n = 3); different lowercase superscripts indicate significant differences ($p < 0.05$) within each trait

Identification of potential non-starch polysaccharide degrading bacteria

The selected isolates Tc10, Tc19 and Tc44 were identified based on nucleotide sequence analysis of the 16S rRNA gene compared with the GenBank database. Based on these results, the isolates Tc10, Tc19 and Tc44 were closely related to *Paenibacillus lutimineralis* strain MBLB1234 (98.0% nucleotide identity), *Paenibacillus alvei* strain CCM2B (99.5% nucleotide identity) and *Bacillus velezensis* strain GYL4 (100.0% nucleotide identity), respectively. The phylogenetic tree based on similarity of the 16S rRNA gene sequences constructed using the MEGA X software are shown in Fig. 2. The bacteria *Escherichia coli* strain ATCC 11775 (X80725.1) was used as an outgroup in the analysis.

Tolerance in acidity and bile salt conditions

Tolerance in acidity and bile salt of the bacterial isolates was evaluated and the results are shown in Table 1. Among the three selected isolates, only Tc44 survived in both conditions of pH 2.5 (100.39% survival) and 0.3% bile salt (70.24% survival) after 3 hr of incubation. The isolates Tc10 and Tc19 could not survive in the acidic and bile salt conditions.

Assay of antimicrobial activity against aquatic animal pathogens

The results are shown in Table 1. Among the three isolates, Tc44 showed a broad spectrum inhibition against all five pathogens (including *Aeromonas hydrophila* ATCC 7966, *Aeromonas schubertii* ATCC 43700, *Streptococcus agalactiae* ATCC 27956, *Vibrio parahaemolyticus* ATCC 17802 and *Lactococcus garvieae* ATCC 49156). In addition, this isolate showed the highest antimicrobial activity against *Vibrio parahaemolyticus* with the inhibition rate of 2.60 ± 0.49. Among these three isolates, only Tc44 inhibited *Aeromonas hydrophila* ATCC 7966 (1.40 ± 0.08), *Aeromonas schubertii* ATCC 43700 (1.22 ± 0.02) and *Streptococcus agalactiae* ATCC 27956 (1.78 ± 0.17). However, all isolates inhibited *Vibrio parahaemolyticus* ATCC 17802 and *Lactococcus garvieae* ATCC 49156, with inhibition rates in the approximate range 1.03 – 2.60.

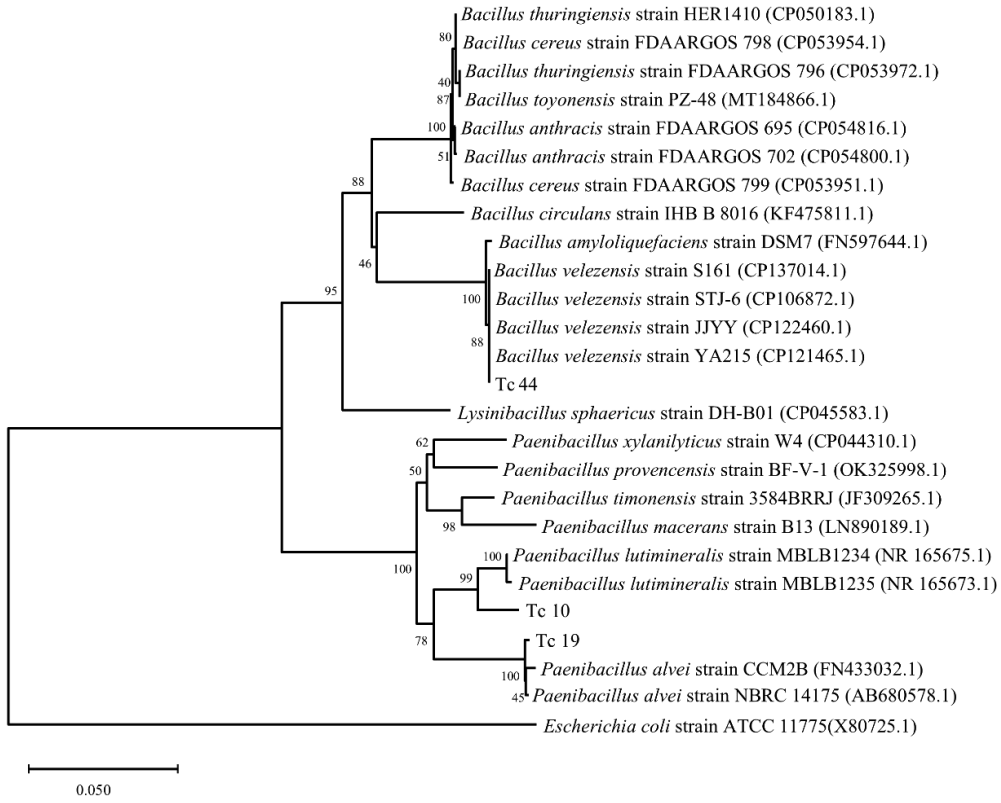

Fig. 2 Phylogenetic tree of 16S rRNA gene sequences of three selected isolates, constructed using the MEGA X software after multiple sequence alignments, where numbers at nodes indicate bootstrap level percentage based on maximum likelihood method analysis of 1,000 resampled datasets, while evolutionary distances were computed using Kimura two-parameter method. *Escherichia coli* strain ATCC 11775 (X80725.1) was used as an outgroup in the analysis.

Determination of antioxidant activity

Antioxidant activity levels were assessed based on hydroxyl and DPPH free radical scavenging, with the results summarized in Table 1. All three isolates had hydroxyl and DPPH radical scavenging activity levels in the ranges 16.67 – 60.61% and 7.99 – 56.76%, respectively. The bacterial isolate Tc44 exhibited the highest antioxidant activity (60.61 ± 6.94% hydroxyl radical scavenging activity and 56.76 ± 2.27% DPPH radical scavenging activity).

Non-starch polysaccharide degrading enzymes activity assay

The enzyme activity levels of cellulase, pectinase and xylanase produced by the isolate Tc44 were determined using the DNS method. The time-course enzyme activities of Tc44 are shown in Fig. 3. Based on these results, *Bacillus velezensis* Tc44 showed the highest value of cellulase, pectinase and xylanase activities at different incubation periods (0.19 ± 0.04 U/mL at 120 hr, 0.42 ± 0.04 U/mL at 48 hr, and 0.93 ± 0.01 U/mL at 24 hr, respectively). The enzyme activity levels of *Bacillus velezensis* Tc44 decreased after the maximum incubation time for enzyme production.

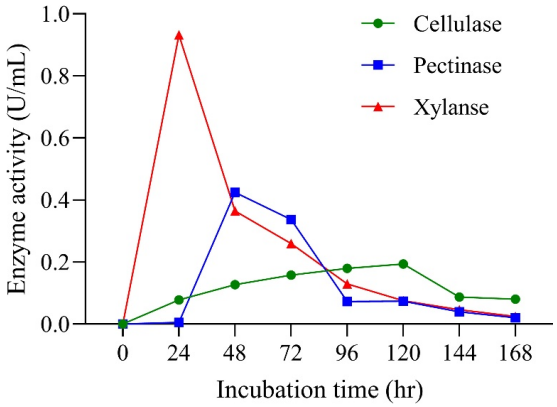

Fig. 3 Incubation time profiles of non-starch polysaccharide-degrading enzyme activity of cellulase, pectinase and xylanase of *Bacillus velezensis* Tc44

Evaluation of safety profile based on hemolysis, biogenic amine production and antibiotic susceptibility

In the test for hemolytic activity, the isolate Tc44 exhibited gamma-hemolytic in sheep blood agar after incubation for 24 hr. The detection of the major biogenic amines (histamine, tyramine, cadaverine and putrescine) using Moeller decarboxylase medium showed that *Bacillus velezensis* Tc44 did not produce all tested biogenic amines from precursor amino acids. In the test for antibiotic susceptibility (inhibition zone diameter measured in millimeters) using nine antibiotic agents, the isolate Tc44 was sensitive (S) to almost all antibiotics—ampicillin (44.5), chloramphenicol (38.0), ciprofloxacin (36.0), tetracycline (35.0), vancomycin (33.5), streptomycin (32.0), kanamycin (31.5) and penicillin (29.0)—and intermediate (I) to erythromycin (16.3). Ampicillin was the most potent antibiotic, whereas erythromycin displayed a weak effect on isolate Tc44.

Assay of adhesion and anti-adhesion ability to human colon adenocarcinoma cells

Adhesion ability to human colon adenocarcinoma cells, Caco-2 and HT-29, was assayed for the bacterial isolate Tc44 and its anti-adhesion ability to bacterial pathogens was evaluated. The results are shown in Fig. 4. The percentages of adhesion varied among bacteria, in the ranges 11.67 – 26.85% to Caco-2 cells and 13.06 – 42.08% to HT-29 cells. Notably, *Bacillus velezensis* Tc44 had significantly higher adhesion capability than the pathogens. The adhesion images of Tc44 to human colon adenocarcinoma cells are shown in Fig. 5.

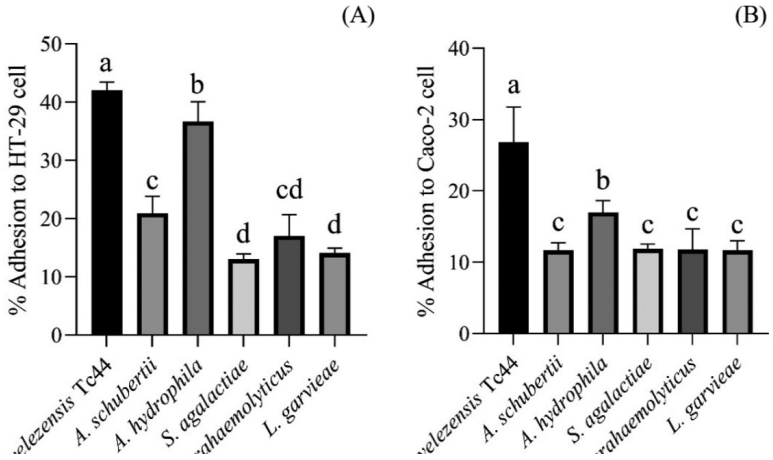

Fig. 4 Adhesion ability to human colon adenocarcinoma cells of *Bacillus velezensis* Tc44 and aquatic pathogenic bacteria: (A) HT-29; (B) Caco-2, where each value represents mean + SD of triplicate determinations, and different superscripts indicate significant differences ($p < 0.05$)

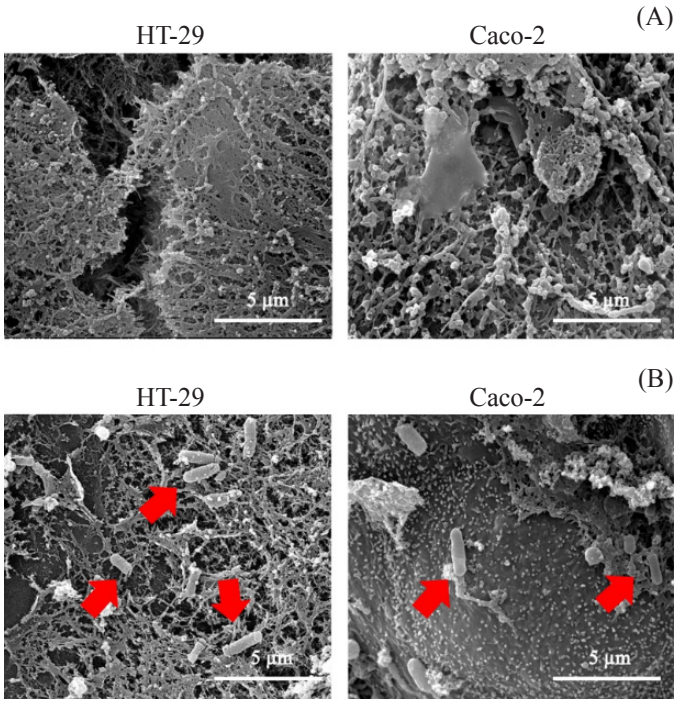

Fig. 5 Bacterial adhesion (indicated by red arrows) to human colon adenocarcinoma cell lines (HT-29 and Caco-2) visualized using scanning electron microscope (SEM) at 10,000× magnification: (A) control; (B) *Bacillus velezensis* Tc44

The investigated anti-adhesion effects of *Bacillus velezensis* Tc44 against pathogens based on competition, inhibition and displacement assays are shown in Fig. 6. The five aquatic animal pathogens used in this study were *Aeromonas hydrophila* ATCC 7966, *Aeromonas schubertii* ATCC 43700, *Streptococcus agalactiae* ATCC 27956, *Vibrio parahaemolyticus* ATCC 17802 and *Lactococcus garvieae* ATCC 49156. In the competition assay, *Bacillus velezensis* Tc44 had the most competitive adhesion of *Vibrio parahaemolyticus* (80.42% to Caco-2 cells and 64.58% to HT-29 cells), while the other pathogens had slightly lower competitive effects in the ranges 43.84 – 72.95% to Caco-2 cells and 9.52 – 52.77% to HT-29 cells. Based on the results of the inhibition assay, all tests indicated good inhibition effects against the adhesive pathogens, except for *Aeromonas hydrophila* to HT-29 cells that had poor inhibition (19.43%). In addition, based on the displacement assay results, *Bacillus velezensis* Tc44 had the most effective displacement ability against *Vibrio parahaemolyticus* (72.08% to Caco-2 cells and 72.45% to HT-29 cells) and the lowest displacement ability against *Aeromonas hydrophila* (24.41% to Caco-2 cells and 13.53% to HT-29 cells), while the other pathogens had ranges of 32.37 – 57.51% to Caco-2 cells and 28.85 – 55.65% to HT-29 cells.

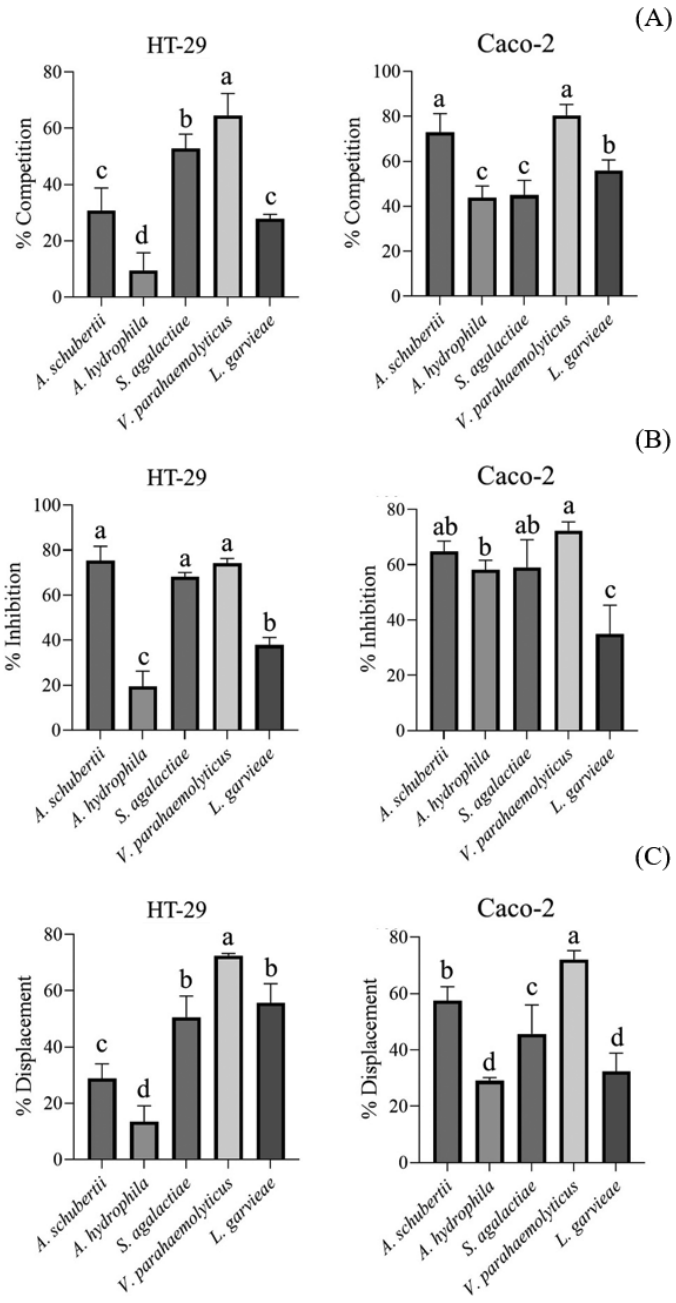

Fig. 6 Anti-adhesion ability to human adenocarcinoma cells (HT-29 and Caco-2) of *Bacillus velezensis* Tc44 against five aquatic pathogenic bacteria, based on assays of: (A) competition; (B) inhibition; (C) displacement, where different lowercase superscripts indicate significant differences ($p < 0.05$)

Discussion

The current findings have provided useful information on the non-starch polysaccharide (NSP)-degrading ability, probiotic characteristics and antimicrobial activity against aquatic animal pathogens of bacteria isolated from the guts of the soil-feeding termite *T. comis* collected from the forest in the Sakaerat Environmental Research Station, Nakhon Ratchasima province, Thailand. These newly isolated bacteria could degrade NSPs (cellulose, pectin and xylan), which are the main component of the plant structure and important as animal feed. Based on the current results, *Bacillus velezensis* Tc44 was preferred as a potential probiotic according to its evaluated probiotic properties. Other studies have reported numerous *Bacillus* species with hydrolytic enzyme-producing ability based on their potential probiotic characteristics. For example, Anyairo et al. (2024) reported that the probiotics *Bacillus tequilensis* K15.4 and *Bacillus siamensis* K29.2 were capable of producing protease and NSP degrading enzymes (cellulase, xylanase and β-mannanase) and inhibiting the growth of fish pathogens, which would support reducing the risk of disease in aquaculture. Boonmee et al. (2024) reported that microbial hydrolysis of fermented soybean meal by *Bacillus subtilis* Hs-2 improved the nutritive value, reduced the anti-nutritional factor and enhanced the digestibility of soybean meal for use as raw material in Nile Tilapia (*Oreochromis niloticus*) diet.

In the current study, according to the DNS assay conditions used to evaluate the NSP-degrading enzyme activity, *Bacillus velezensis* Tc44 produced the highest values of cellulase, pectinase and xylanase activities at different incubation periods of 120 hr (0.19 ± 0.04 U/mL), 48 hr (0.42 ± 0.04 U/mL) and 24 hr (0.93 ± 0.01 U/mL), respectively. The impact of temperature, pH and substrate concentration on enzyme activity should be considered for optimization in future research. The results of the current study indicated that the maximum cellulase activity of *B. velezensis* Tc44 was higher than that of bacterial strains reported in other studies, such as *B. velezensis* CC1-1 with 0.03 U/mL at 20 hr of incubation (Narkthewan and Makkapan, 2019) and *Bacillus licheniformis* PANG L with 0.044 ± 0.004 U/mL (Shyaula et al., 2023). However, the cellulase activity of *B. velezensis* Tc44 was lower than that of *Bacillus velezensis* Z2.6 at 3.02 U/mL (Cai et al., 2024). The maximum value for the pectinase activity of Tc44 was higher than for the strains *Bacillus subtilis* PKC2, *Bacillus licheniformis* PKC4, *Bacillus sonorensis* ADCN and *Paenibacillus lactis* PKC5 that had ranges of 0.08 – 0.13 U/mL at 48 hr of incubation (Sheladiya et al., 2022). Furthermore, *B. velezensis* Tc44 had higher xylanase activity than *Bacillus* sp. GA1(6) that was reported at 0.82 ± 0.16 U/mL, but was lower than *Bacillus* sp. GA2(1) that was reported at 1.58 ± 0.25 U/mL at 18 hr of incubation (Chantorn et al., 2016).

Recently, many strains of NSP-degrading *Bacillus* spp. have been isolated from various sources and used for various applications. For example, *Bacillus amyloliquefaciens* OKB3 from soil samples showed high cellulase production to degrade cellulose (Bhatt et al., 2024); the cellulase and xylanase produced by *Bacillus safensis* NPUST1 isolated from the guts of Nile Tilapia improved the growth performance and innate immunity against the aquatic pathogen *Streptococcus iniae* in Nile Tilapia (*Oreochromis niloticus*) according to Wu et al. (2021); and *Bacillus subtilis* 15A-B92 isolated from vegetables harboring 14.41-kDa pectinase clarified orange and apple juice samples, justifying its application in the food industry (Alqahtani et al., 2022). In recent years, there have been several studies using *Bacillus* spp. as microbial probiotics in aquaculture. For example, Neissi et al. (2024) selected the acid- and bile-resistant *Bacillus subtilis* MS. 45 to improve growth performance of rainbow trout (*Oncorhynchus mykiss*); Sam-on et al. (2023) reported that *Bacillus velezensis* FS26 was preferred as a potential bacterial probiotic in aquaculture with a high antagonistic effect on *Aeromonas* spp. and *Vibrio* spp.; Huang et al. (2023) reported that the probiotic *Bacillus licheniformis* strain VLPPro® SB538 improved water quality by reducing the ammonia, nitrate and nitrite levels and enhancing growth performance and intestine morphology of grouper (*Epinephelus* spp.); and Soto-Marfileño et al. (2024) reported that the *Bacillus pumilus* strain sonora had probiotic potential in shrimp due to its antagonistic activity against pathogens in shrimp aquaculture.

The gut morphology has been described of the soil-feeding higher termites (Termitidae) such as *T. comis* (Termitinae) and *Cubitermes* spp. (Cubitermitinae) by Ngugi (2008) and Thongaram et al. (2003). These researchers reported that the intestinal tract of these termites comprises different gut compartments: the crop; the midgut, with the mixed segment; and the hindgut, containing five proctodeal segments (P1-P5). Furthermore, in the worker termite of *Cubitermes* spp., all gut regions had reported nearly neutral to alkaline pH values (6.0−11.9), except for P5 (the rectum) that was more acidic (pH 4.8). They considered that physicochemical conditions could lead to a variety of potential properties of the termite gut bacteria. In practice, microbial tolerance to stressful conditions is tested for screening the potential of a probiotic candidate. In the current study, among all three selected isolates,

only *Bacillus velezensis* Tc44 could survive in a gastrointestinal tract environment with conditions of high levels of acidity (pH 2.5) and bile salts (0.3%). Additionally, Tc44 could grow in acidic conditions, enhancing its potential value and scientific interest. Similarity, *Bacillus velezensis* D-18 was reported by Monzón-Atienza et al. (2021) to survive after 1.5 hr in bile salts and acidic conditions. Furthermore, in the current study, *Bacillus velezensis* Tc44 had high relative scavenging activity against H_2O_2 and DPPH radicals (antioxidant rates of 60.61 ± 6.94% and 56.76 ± 2.27%, respectively), which is a good characteristics for a probiotic. Kadaikunnan et al. (2015) reported that *Bacillus amyloliquefaciens* VJ-1 belonging to the *Bacillus subtilis* group (the same group as Tc44) had strong H_2O_2 and DPPH scavenging activities, with antioxidant rates of 56.84% and 67.12%, respectively. Anti-microbial activity is one of the most common probiotic functions. Thus, the current study investigated the antimicrobial activity of the tested isolates against aquatic pathogens because aquaculture is important for global nutrition and food security. Based on this testing, *Bacillus velezensis* Tc44 had broad-spectrum antimicrobial activities against five bacterial species of aquatic animal pathogens: *Aeromonas hydrophila* ATCC 7966, *Aeromonas schubertii* ATCC 43700, *Streptococcus agalactiae* ATCC 27956, *Vibrio parahaemolyticus* ATCC 17802 and *Lactococcus garvieae* ATCC 49156. Liu et al. (2024) reported that *Bacillus velezensis* CYS06 produced strong inhibition against the common bacterial pathogen *Aeromonas hydrophila* strain GYK1 in freshwater fish. In addition, Ke et al. (2022) reported that *Bacillus* sp. NY5 isolated from the guts of healthy Tilapia (*Oreochromis niloticus*) inhibited the growth of the pathogenic *Streptococcus agalactiae* WC1535. In addition, Gao et al. (2017) reported that four *Bacillus* strains (*B. velezensis* V4, *B. methylotrophicus* L7, *B. pumilus* H2 and *B. safensis* H2-2) had antimicrobial effects on various *Vibrio* strains, while *B. pumilus* H2 had a broad-spectrum impact on pathogenic activity against 29 *Vibrio* strains.

In vitro probiotic studies have long been used for the evaluation of probiotic characteristics, potential and safety profile. In the current study, the gamma-hemolytic *Bacillus velezensis* Tc44 remained remarkably susceptible to most antibiotics (ampicillin, vancomycin, streptomycin, kanamycin, chloramphenicol, ciprofloxacin, penicillin and tetracycline) and was not able to produce biogenic amines (histamine, tyramine, cadaverine and putrescine). These results were consistent with the study by Chen et al. (2024), who reported that *Bacillus velezensis* TS5 had gamma hemolytic activity and was susceptible to most antibiotics. Similarity, Chang and Chang (2012) reported that members of the *Bacillus subtilis* group did not produce biogenic amine. Safety evaluations, based on hemolysis, biogenic amine production and antibiotic susceptibility, are essential for real-world applications; the evaluated safety profile of *Bacillus velezensis* Tc44 indicated its preliminary safety for use in feed biotechnology. However, additional tests should be carried out, regarding pathogenicity, other toxigenicity and genetic stability and transferability to provide more robust evidence for the safety and functionality of the selected bacteria.

The adhesion ability to gastrointestinal epithelial cells and an anti-adhesion effect to pathogenic bacteria are considered important characteristics for potential probiotics. The current study evaluated the ability of the tested probiotics to adhere to human adenocarcinoma cells (Caco-2 and HT-29 cells). Based on these results, *Bacillus velezensis* Tc44 had stronger adhesion ability to HT-29 (42.08%) than to Caco-2 (26.85%). Comparable results have been reported elsewhere such as *Bacillus siamensis* TpNSP_72 having adherence levels of 42% and 49% to Caco-2 and HT-29 cells, respectively (Tomtong and Deevong 2024). In addition, one of the essential characteristics of probiotics is their ability to prevent pathogen colonization on intestinal epithelial surfaces. Based on the results of the present study, *Bacillus velezensis* Tc44 reduced the bacterial numbers of all five tested species of aquatic animal pathogens from intestinal cells (Caco-2 and HT-29) with various anti-adhesion percentages. Ye et al. (2013) reported that *Bacillus subtilis* B2 (belonging to the same *Bacillus* group as Tc44) suppressed the adhesion of the enterotoxigenic *Escherichia coli* strain K88 to the surface of Caco-2 cells in the ranges 58 – 72%, based on the adhesive experiments involving competition, exclusion and displacement assays.

In summary, the results of the current study suggest that the NSP-hydrolytic *Bacillus velezensis* Tc44 isolated from the guts of the soil-feeding *T. comis* had a wide range of antagonistic activities against important aquatic animal pathogens and had potential probiotic characteristics. New bacterial isolates obtained from termite guts should be useful for future applications in microbiology and biotechnology, especially for reducing NSPs and improving nutritional value in plant-based animal feed and preventing the spread and infection of aquatic animal disease.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This research and innovation activity was partially funded by the National Research Council of Thailand (NRCT) and supported in part by the Graduate Program Scholarship from the Graduate School, Kasetsart University, Bangkok, Thailand. We thank Assoc. Professor Dr. Kiattawee Choowongkomon for providing HT-29 and Caco-2 cells and Mr. Pachara Wangsoontorn for valued assistance.

References

Alqahtani, Y.S., More, S.S., Shaikh, I.A., More, V.S., Niyonzima, F.N., Muddapur, U.M., Khan, A.A. 2022. Production and purification of pectinase from *Bacillus subtilis* 15A-B92 and its biotechnological applications. Mol. 27: 4195. doi.org/10.3390/molecules27134195

Amirkolaie, A.K., Leenhouwers, J.I., Verreth, J.A., Schrama, J.W. 2005. Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia (*Oreochromis niloticus* L.). Aquac. Res. 36: 1157−1166. doi.org/10.1111/j.1365-2109.2005.01330.x

Anyairo, C.S., Unban, K., Wongputtisin, P., Rojtinnakorn, J., Shetty, K., Khanongnuch, C. 2024. *Bacillus* spp. isolated from miang as potential probiotics in Nile tilapia culture-*in vitro* research. Microorganisms. 12: 1687. doi.org/10.3390/microorganisms12081687

Arriaga, D., Hernández, C., Martínez, E., Ibarra, L., Lizárraga, E., Leyva, N., Chávez, M. 2021. Fish meal replacement by soybean products in aquaculture feeds for white snook, *Centropomus viridis*: Effect on growth, diet digestibility, and digestive capacity. Aquac. 530: 735823. doi.org/10.1016/j.aquaculture.2020.735823

Auer, L., Lazuka, A., Dussès, D., Miambi, E., Donohue, M., Raquet, G. 2017. Uncovering the potential of termite gut microbiome for lignocellulose bioconversion in anaerobic batch bioreactors. Front. Microbiol. 8: 2623. doi.org/10.3389/fmicb.2017.02623

Bhatt, B., Bhatt, K., Lal, S., Bhatt, V. 2024. Production of a novel cellulase by *Bacillus amyloliquefaciens* OKB3 isolated from soil: Purification and characterization. Int. J. Biol. Macromol. 282: 137454. doi.org/10.1016/j.ijbiomac.2024.137454

Boonmee, T., Deevong, P., Rinthong, P., Yuangsoi, B. 2024. Improvement of nutritive value of soybean meal by microbial hydrolysis with *Bacillus subtilis* Hs-2 for use as raw material in Nile tilapia (*Oreochromis niloticus*) diet. Aquac. 35: 101943. doi.org/10.1016/j.aqrep.2024.101943

Bover-Cid, S., Holzapfel, W.H. 1999. Improved screening procedure for biogenic amine production by lactic acid bacteria. Int. J. Food Microbiol. 53: 33-41. doi.org/10.1016/S0168-1605(99)00152-X

Cai, Z., Wang, Y., You, Y., et al. 2024. Introduction of cellulolytic bacterium *Bacillus velezensis* Z2.6 and its cellulase production optimization. Microorganisms. 12: 989. doi.org/10.3390/microorganisms12050979

Chang, M., Chang, H. 2012. Development of a screening method for biogenic amine producing *Bacillus* spp. Int. J. Food Microbiol. 153: 269−274. doi.org/10.1016/j.ijfoodmicro.2011.11.008

Chantorn, S., Piyapittayanun, C., Khumphai, P., Pannanusorn, S., Phannachet, K., Apiraksakorn, J. 2016. Suitable conditions for xylanases activities from *Bacillus* sp. GA2 (1) and *Bacillus* sp. GA1 (6) and their properties for agricultural residues hydrolysis. Songklanakarin J. Sci. Technol. 38: 177−182. doi.org/10.14456/sjst-psu.2016.24

Chanworawit, K., Wangsoonthorn, P., Deevong, P. 2023. Characterization of chitinolytic bacteria newly isolated from the termite *Microcerotermes* sp. and their biocontrol potential against plant pathogenic fungi. Biosci. Biotechnol. Biochem. 87: 1077−1091. doi.org/10.1093/bbb/zbad080

Chen, B., Zeng, Y., Wang, J., et al. 2024. Targeted screening of fiber degrading bacteria with probiotic function in herbivore feces. Probiotics Antimicrob. Proteins. 17: 1473–1497. doi.org/10.1007/s12602-024-10215-5

Clinical and Laboratory Standards Institute. 2020. Performance standards for anti-microbial susceptibility testing. 30th ed. CLSI Supplement M100. Clinical and Laboratory Standards Institute. Wayne, Pennsylvania, USA.

Duc, H., Hong, H.A., Barbosa, T.M., Henriques, A.O., Cutting, S.M. 2004. Characterization of *Bacillus* probiotics available for human use. Appl. Environ. Microbiol. 70: 2161−2171. doi.org/10.1128/AEM.70.4.2161-2171.2004

Duche, R.T., Singh, A., Wandhare, A.G., Sangwan, V., Sihag, M.K., Nwagu, T.N.T., Panwar, H., Ezeogu, L.I. 2023. Antibiotic resistance in potential probiotic lactic acid bacteria of fermented foods and human origin from Nigeria. BMC Microbiol. 23: 142. doi.org/10.1186/s12866-023-02883-0

Elshaghabee, F., Rokana, N., Gulhane, R.D., Sharma, C., Panwar, H. 2017. *Bacillus* as potential probiotics: Status, concerns, and future perspectives. Front. Microbiol. 8: 1490. doi.org/10.3389/fmicb.2017.01490

Gao, X.Y., Liu, Y., Miao, L.L., Li, E.W., Hou, T.T., Liu, Z.P. 2017. Mechanism of anti-*Vibrio* activity of marine probiotic strain *Bacillus pumilus* H2, and characterization of the active substance. AMB Express. 7: 23. doi.org/10.1186/s13568-017-0323-3

Huang, J., Amenyogbe, E., Wen, Z., Ou, G., Li, Y., Jiang, X., Xie, R., Chen, G. 2023. Effect of *Bacillus licheniformis* probiotic on the culture of hybrid grouper (♀ E*pinephelus fuscoguttatus* × ♂ *Epinephelus polyphekadion*). Aquac. Res. 33: 101798. doi.org/10.1016/j.aqrep.2023.101798

Jlidi, M., Akremi, I., Ibrahim, A.H., Brabra, W., Ali, M.B. 2022. Probiotic properties of *Bacillus* strains isolated from the gastrointestinal tract against pathogenic vibriosis. Front. Microbiol. 9. doi.org/10.3389/fmars.2022.884244

Kadaikunnan, S., Rejiniemon, T., Khaled, J.M., Alharbi, N.S., Mothana, R. 2015. *In-vitro* antibacterial, antifungal, antioxidant and functional properties of *Bacillus amyloliquefaciens*. Ann. Clin. Microbiol. Antimicrob. 14: 9. doi.org/10.1186/s12941-015-0069-1

Ke, X., Liu, Z., Zhang, M., et al. 2022. A *Bacillus cereus* NY5 strain from tilapia intestine antagonizes pathogenic *Streptococcus agalactiae* growth and adhesion *in vitro* and *in vivo*. Aquac. 561: 738729. doi.org/10.1016/j.aquaculture.2022.738729

Li, X., Gao, X., Zhang, S., Jiang, Z., Yang, H., Liu, X., Jiang, Q., Zhang, X. 2020. Characterization of a *Bacillus velezensis* with antibacterial activity and inhibitory effect on common aquatic pathogens. Aquac. 523: 735165. doi.org/10.1016/j.aquaculture.2020.735165

Liu, L., Jiang, D., Ren, Y., Shi, C., Wang, Y., Yin, J., Wang, Q., Zhang, D. 2024. The *Bacillus velezensis* CYS06 strain exhibits promising applications in fighting grass carp bacterial diseases. Fishes. 9: 7. doi.org/10.3390/fishes9010007

Loy, A., Schulz, C., Lücker, S., Schöpfer-Wendels, A., Stoecker, K., Baranyi, C., Lehner, A., Wagner, M. 2005. 16S rRNA gene-based oligonucleotide microarray for environmental monitoring of the betaproteobacterial order "*Rhodocyclales*". Appl. Environ. Microbiol. 71: 1373−1386. doi.org/10.1128/AEM.71.3.1373-1386.2005

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426-428. doi.org/10.1021/ac60147a030

Molva, C., Sudagidan, M., Okuklu, B. 2009. Extracellular enzyme production and enterotoxigenic gene profiles of *Bacillus cereus* and *Bacillus thuringiensis* strains isolated from cheese in Turkey. Food Control. 20: 829-834. doi.org/10.1016/j.foodcont.2008.10.016

Monzón-Atienza, L., Bravo, J., Torrecillas, S., et al. 2021. Isolation and characterization of a *Bacillus velezensis* D-18 strain, as a potential probiotic in European seabass aquaculture. Probiotics Antimicrob. Proteins. 13: 1404−1412. doi.org/10.1007/s12602-021-09782-8

Mu, Q., Tavella, V.J., Luo, X.M. 2018. Role of *Lactobacillus reuteri* in human health and diseases. Front. Microbiol. 9: 757. doi.org/10.3389/fmicb.2018.00757

Narkthewan, P., Makkapan, W. 2019. Cellulase activity of *Bacillus velezensis* isolated from soil in a dairy farm. IOP Conf. Ser.: Earth Environ. Sci. 346: 012040. doi.org/10.1088/1755-1315/346/1/012040

Neissi, A., Majidi Zahed, H., Roshan, R. 2024. Probiotic performance of *Bacillus subtilis* MS. 45 improves aquaculture of rainbow trout *Oncorhynchus mykiss* during acute hypoxia stress. Sci. Rep. 14: 3720. doi.org/10.1038/s41598-024-54380-7

Ngugi, D. 2008. Transformation and mineralization of nitrogenous soil components in the gut of soil-feeding termites. Ph.D. thesis, Faculty of Biology, Philipps University. Marburg, Germany.

Nualkul, M., Yuangsoi, B., Hongoh, Y., Yamada, A., Deevong, P. 2022. Improving the nutritional value and bioactivity of soybean meal in solid-state fermentation using *Bacillus* strains newly isolated from the gut of the termite *Termes propinquus*. FEMS Microbiol. Lett. 369: fnac044. doi.org/10.1093/femsle/fnac044

Rahman, M.M., Islam, M.B., Biswas, M., Alam, A.H.M. 2015. *In vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. BMC Res. Notes. 8: 621. doi.org/10.1186/s13104-015-1618-6

Sam-on, M.F.S., Mustafa, S., Mohd Hashim, A., Yusof, M.T., Zulkifly, S., Malek, A.Z.A., Roslan, M.A.H., Mohd Asrore, M.S. 2023. Mining the genome of *Bacillus velezensis* FS26 for probiotic markers and secondary metabolites with antimicrobial properties against aquaculture pathogens. Microb. Pathog. 181: 106161. doi.org/10.1016/j.micpath.2023.106161

Sargautiene, V., Nakurte, I., Nikolajeva, V. 2018. Broad prebiotic potential of non-starch polysaccharides from Oats (*Avena sativa* L.): An *in vitro* study. Pol. J. Microbiol. 67: 307−313. doi.org/10.21307/pjm-2018-036

Sheladiya, P., Kapadia, C., Prajapati, V., Ali, H., Malek, R., Marraiki, N., Zaghloul, N.S.S., Sayyed, R.Z. 2022. Production, statistical optimization, and functional characterization of alkali stable pectate lyase of *Paenibacillus lactis* PKC5 for use in juice clarification. Sci. Rep. 12: 7564. doi.org/10.1038/s41598-022-11022-0

Shyaula, M., Regmi, S., Khadka, D., et al. 2023. Characterization of thermostable cellulase from *Bacillus licheniformis* PANG L isolated from the Himalayan soil. Int. J. Microbiol. 2023: 1−12. doi.org/10.1155/2023/3615757

Sinha, A.K., Kumar, V., Makkar, H.P., De Boeck, G., Becker, K.J.F.C. 2011. Non-starch polysaccharides and their role in fish nutrition–A review. Food Chem. 127: 1409−1426. doi.org/10.1016/j.foodchem.2011.02.042

Soto-Marfileño, K.A., Molina Garza, Z.J., Flores, R.G., Molina-Garza, V.M., Ibarra-Gámez, J.C., Gil, B.G., Galaviz-Silva, L. 2024. Genomic characterization of *Bacillus pumilus* sonora, a strain with inhibitory activity against *Vibrio parahaemolyticus*-AHPND and probiotic candidate for shrimp aquaculture. Microorganisms 12: 1623. doi.org/10.3390/microorganisms12081623

Sreena, C.P., Sebastian, D. 2018. Augmented cellulase production by *Bacillus subtilis* strain MUS1 using different statistical experimental designs. J. Genet. Eng. Biotechnol. 16: 9−16. doi.org/10.1016/j.jgeb.2017.12.005

Sroka, Z., Cisowski, W. 2003. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem. Toxicol. 41: 753-758. doi.org/10.1016/s0278-6915(02)00329-0

Thongaram, T., Kosono, S., Ohkuma, M., et al. 2003. Gut of higher termites as a niche for alkaliphiles as shown by culture-based and culture-independent studies. Microbes environ. 18: 152−159. doi.org/10.1264/jsme2.18.152

Tomtong, P., Deevong, P. 2024. Probiotic potentials of non-starch polysaccharide hydrolyzing *Bacillus* strains newly isolated from guts of the termite *Termes propinquus*: A source of probiotic bacteria. Korean J. Microbiol. 60: 68-82. doi.org/10.7845/kjm.2024.4014

Wang, T., Sun, H., Chen, J., et al. 2021. Anti-adhesion effects of *Lactobacillus* strains on Caco-2 cells against *Escherichia coli* and their application in ameliorating the symptoms of dextran sulfate sodium-induced colitis in mice. Probiotics Antimicrob. Proteins. 13: 1632−1643. doi.org/10.1007/s12602-021-09774-8

Wu, P.S., Liu, C.H., Hu, S.Y. 2021. Probiotic *Bacillus safensis* NPUST1 administration improves growth performance, gut microbiota, and innate immunity against *Streptococcus iniae* in Nile tilapia (*Oreochromis niloticus*). Microorganisms. 9: 2494. doi.org/10.3390/microorganisms9122494

Ye, X., Li, P., Yu, Q., Yang, Q. 2013. *Bacillus subtilis* inhibition of enterotoxic *Escherichia coli*-induced activation of MAPK signaling pathways in Caco-2 cells. Ann. Microbiol. 63: 577-581. doi.org/10.1007/s13213-012-0506-8