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Isolation and Selection of *Bacillus pumilus* against Colistin-Resistant *Escherichia coli* F4

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Abstract

The emergence of antibiotic-resistant *Escherichia coli*, a major causative agent of diarrhea in piglets, presents significant challenges in the swine industry. *Bacillus pumilus*, widely recognized for its probiotic potential, has been utilized to promote growth performance, enhance immune responses, and inhibit various pathogens in livestock. This study aimed to isolate and characterize *B. pumilus* with probiotic characteristics from healthy pigs in Hanoi and Hung Yen. A total of three *B. pumilus* strains were isolated using a routine culture method, PCR, and MALDI-TOF mass spectrometry. The results revealed that all the isolated *B. pumilus* strains showed non-hemolytic activity on blood agar. The isolates also exhibited high tolerance in acidic (pH 3.0) and bile salt (0.3%) conditions, with survival rates ranging from 77.97% to 95.38% and 82.61% to 93.62%, respectively. Antimicrobial susceptibility tests revealed resistance to tetracycline and erythromycin, while all isolates were also strongly antagonistic to *E. coli* F4, with inhibition zones ranging from 16-19mm. Overall, the three *B. pumilus* isolates demonstrated promising probiotic and antibacterial characteristics, highlighting their potential as alternative agents to control *E. coli* F4 infections in piglets.

Keywords

B. pumilus, antibiotic alternatives, *Escherichia coli* F4, antibiotic resistance

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Introduction

Diarrhea remains one of the most challenging intestinal diseases, with a high mortality rate and great economic losses to the pig industry worldwide (Saha *et al.*, 2024). This disease is often triggered by abrupt dietary and environmental changes during the weaning

period, compounded by inadequate feed quality and poor hygiene. These stressors disrupt the gut microbiota balance, creating favorable conditions for the colonization of opportunistic pathogens (Upadhaya & Kim, 2021). Among these pathogens, Enterotoxigenic *E. coli* (ETEC) with typical serotypes such as F4 (F4), F5 (K99), F6 (987P), F41, and F18 are considered the primary etiological causes of diarrhea in piglets before and after weaning (Castro *et al.*, 2022). This serotype is capable of producing various intestinal toxins, causing illness in piglets with clinical symptoms such as diarrhea, fatigue, weakness, and dehydration, and ultimately death (Luppi *et al.*, 2016). Even in cases of recovery, piglets often suffer long-term consequences such as growth retardation and malnutrition (Luppi *et al.*, 2016). For many years, antibiotics have been widely used to prevent and treat *E. coli*-induced diarrhea in piglets. However, their extensive use has led to the emergence of multidrug-resistant *E. coli* strains, complicating therapeutic options (Hoang Minh Duc *et al.*, 2022). Furthermore, a national ban on the prophylactic use of antibiotics in livestock is set to be enforced in Vietnam in 2026 (Government, 2020). Therefore, finding antibiotic alternatives to control antibiotic-resistant *E. coli*, especially *E. coli* F4, is extremely necessary.

Probiotics are regarded as a promising alternative to antibiotics due to their numerous benefits, such as (i) inhibiting the growth of pathogenic bacteria, (ii) maintaining intestinal microflora balance, (iii) enhancing host immunity, and (iv) increasing nutrient absorption. These factors improve health, increase growth performance, enhance feed conversion efficiency, and reduce the incidence of diarrhea in piglets (Zhang *et al.*, 2023). Among the probiotic candidates, *Bacillus* spp. are one of the most extensively studied and are widely utilized in probiotic formulations for livestock (Gonzalez-Ronquillo *et al.*, 2022). Their notable advantage is the ability to form endospores, enabling survival under harsh environmental conditions such as high temperatures, strong acidity, ionizing radiation, and chemical disinfectants (Todorov *et al.*, 2022). *Bacillus* spp. used for probiotic purposes are commonly isolated from natural resources, including soil, water, and the gastrointestinal tract of animals (Abriouel *et al.*, 2011). *B. pumilus* has been reported to be one of the most common species for probiotic use, with many suitable characteristics (Dobrzyński *et al.*, 2023). For example, *B. pumilus* has been shown to produce antibacterial and antifungal substances against pathogens (Chu *et al.*, 2019; Morita *et al.*, 2019). In addition, *B. pumilus* was shown to be effective in promoting growth, enhancing immune responses, and inhibiting pathogenic bacteria (Zhang *et al.*, 2022; Zhang *et al.*, 2023). This study was conducted to isolate and select *B. pumilus* strains suitable for controlling antibiotic-resistant *E. coli* F4.

Materials and Methods

Baterial strains

E. coli (PETEC58) used in this study was previously isolated and characterized from piglets with diarrhea in Hung Yen and has been kept at -86˚C in our laboratory since 2022. This bacterial strain was found to carry fimbriae (F4) and enterotoxin (STa). In addition, the PETEC58 also harbors the *mcr-1* gene and is resistant to colistin. Therefore, PETEC58 was selected for evaluating the antibacterial activity of the isolated *B. pumilus* strains.

Sample collection

One-hundred fecal swab samples were collected from healthy pigs in Hanoi and Hung Yen. Samples were kept in an ice box and transported to the laboratory for isolation within 24h.

Isolation and identification of B. pumilus

The fecal swab samples were incubated at 80˚C in a water bath for 15 minutes. Then, each sample was streaked on Tryptone Soya Agar (TSA) and incubated at 37˚C for 24h. *B. pumilus* colonies on the TSA were whitish to cream colored, circular, raised, and undulated. The presumptive *B. pumilus* strains were further verified by sequencing 16S rRNA as described by Foysal & Lisa (2018). The PCR thermal cycling began with an initial denaturation at 94˚C

for 4min, followed by 35 cycles at 94˚C for 1min, 62˚C for 60s, and 62˚C for 90 s, and a final elongation at 72˚C for 10min. The PCR products were analyzed by 2% agarose electrophoresis and viewed under ultraviolet light using a BioRad Molecular Imager® GelDocTM XR (BioRad Laboratories, Hercules, CA, USA). PCR amplicons were then sequenced by the Sanger method using an Applied Biosystems 3500 genetic analyzer (ABI 3500, Applied Biosystems, Foster City, CA, USA). The identified *B. pumilus* strains were stored at -86˚C.

Hemolytic activity of the *B. pumilus* isolates

The safety assessment of the *B. pumilus* strains was performed by streaking the *B. pumilus* strains on blood agar, as described by Ritter *et al*. (2018). The agar plates were then incubated at 37˚C for 24h. After incubation, the hemolytic activity was indicated as clear zones around the colonies.

Stability of the *B. pumilus* isolates

The stability of the *B. pumilus* isolates was tested according to the previous methods described by Huligere *et al.* (2023). For the determination of bile salt tolerance, 100µL of *B. pumilus* broth was inoculated into 5mL of PBS supplemented with 0.3% bile salt and incubated at 37˚C for 3h. Similarly, to conduct the pH tolerance test, 100µL of bacterial broth was added to 5mL of PBS previously adjusted to pH = 3.0 and incubated at 37˚C for 3h. Viable counts of *B. pumilus* were determined before and after 3h of incubation on TSA by the plating method.

Antimicrobial susceptibility of the B. pumilus isolates

The antimicrobial resistance of the *B. pumilus* isolates was determined by the micro broth dilution method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2015). The antibiotics used in this study were ampicillin, meropenem, gentamicin, erythromycin, tetracycline, ciprofloxacin, clindamycin, trimethoprim/sulfamethoxazole, and chloramphenicol.

Antibacterial activity against *E. coli* F4

The antibacterial activity of *B. pumilus* against *E. coli* F4 was examined by the agar well diffusion method as described by Gupta *et al.* (2021) with slight modifications. The *B. pumilus* isolates were grown in BHI broth and then centrifuged at 6000 ×g for 20min at 4˚C. The cell-free supernatant (CFS) was collected and filtered through a 0.22µm filter to remove all cell debris. The *E. coli* F4 strain was inoculated into BHI broth at 37˚C overnight. After incubation, the bacterial culture was diluted to reach the desired concentration of 10^7 CFU mL^{-1}, mixed with BHI 0.8% agar, and poured onto the surface of the TSA plates. Wells with 5mm diameters were then made on each TSA plate. Next, CFS (50µL) was transferred to each agar well and incubated at 37˚C for 24h. The ability of *B. pumilus* to inhibit colitis *E. coli* F4 was indicated by clear zones around the wells.

Data analysis

Collected data were analyzed using One-way ANOVA (Minitab 2021 software).

Results and Discussion

Isolation and identification of *B. pumilus*

A total of three *B. pumilus* strains were isolated from the 100 fecal swab samples collected from healthy pigs in Hanoi and Hung Yen. The isolates exhibited typical colony morphology (whitish to cream colored, raised, and undulated) on TSA **(Figure 1A)**. Microscopic examination of the three isolates revealed they were Gram-positive, rod-shaped bacilli capable of forming endospores **(Figure 1B)**. PCR and sequencing analysis of the 16S rRNA gene were used to for the identification of *B. pumilus* **(Figure 2)**.

Table 1. Primer sequences used for the amplification of 16S rRNA universal sequence

Target gene	Primer	Primer sequence (5'-3')	Product size (bp)
16S rRNA	8F	AGAGTTTGATCCTGGCTCAG	1484 bp
	1429R	AGGAGGTGATCCAACCGCA	

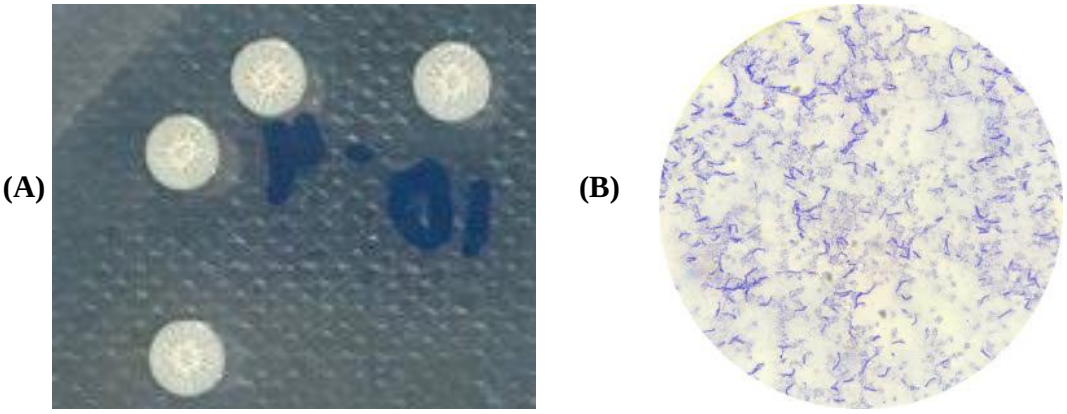
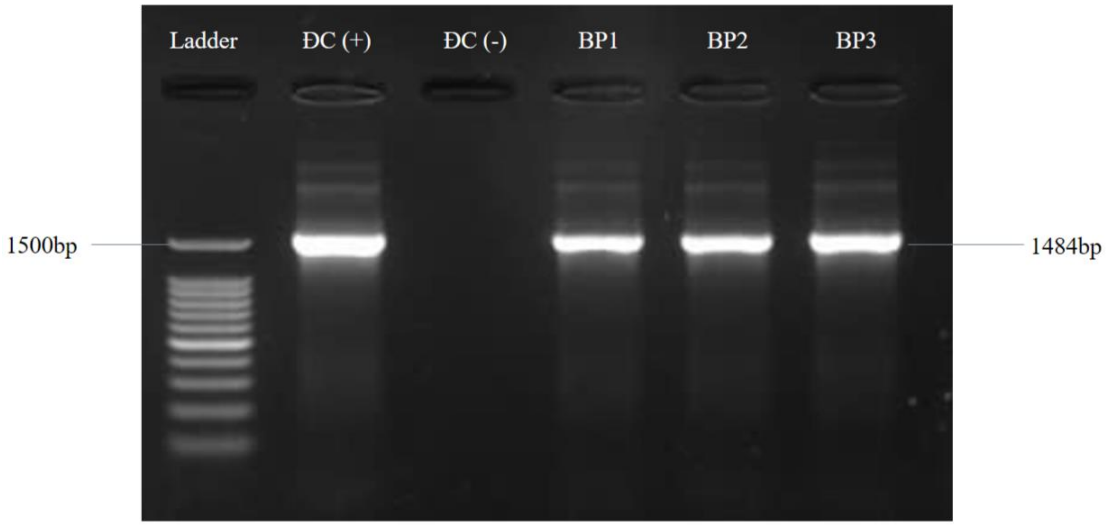


Figure 1. Colonies of *B. pumilus* on TSA (A), microscopic morphology of *B. pumilus* (B)



Note: Ladder: 100bp DNA standard ladder; ĐC(+): positive control ; ĐC(-): negative control; BP1, BP2, BP3: isolated Bacillus strains.

Figure 2. PCR detecting the 16S rRNA gene of Bacillus isolates

Previous studies have focused on isolating *B. pumilus* from soil, water, milk, ruminant feces, and aquatic products (Thy *et al.*, 2017; Nguyen Thi Hanh Chi *et al.*, 2021; Jiang *et al.*, 2023; Chaima *et al.*, 2024). Meanwhile, pig manure has been recognized to be a rich potential source of beneficial microorganisms (Balasingham *et al.*, 2017). However, there is limited information about the presence of *B. pumilus* in this sample type. To the best of our knowledge, this is the first report on the successful isolation of *B. pumilus* from pig manure. *B. pumilus* has been known as a probiotic strain that can grow in aerobic conditions, form spores, and is highly resistant to harsh environmental conditions such as radiation, disinfectants, and extreme temperatures. Due to their exceptional resilience, *B. pumilus* strains can survive under various biological product manufacturing processes, indicating their potential for applications in developing animal feed additives (Zhang *et al.*, 2022).

Hemolytic activity of the *B. pumilus* isolates

Assessment of safety is considered a crucial step in screening and selecting potential bacterial strains for probiotic production. Non-hemolysis is a key safety criterion for probiotic candidates. In this study, all three *B. pumilus* isolates showed non-hemolytic activity (γ– hemolysis), fulfilling the requirement for the production of probiotics (**Table 2** and **Figure 3)**. Similarly, a study by Dao Gia Bach *et al.* (2024) found that all the *Bacillus* isolates were not hemolytic. In contrast, Golnari *et al.* (2024) reported that five *Bacillus* isolates produced incomplete hemolysis, and another five exhibited complete hemolysis.

Table 2. Hemolytic activity of the *B. pumilus* isolates

Isolate ID	Hemolysis		
	α – hemolysis	β – hemolysis	γ– hemolysis
BP 1	-	-	+
BP 2	-	-	+
BP3	-	-	+

Note: (1) – Positive control, (2) – BP1, (3) – BP2, (4) – BP3

Figure 3. Hemolytic activity of the B. pumilus isolates

Stability of the *B. pumilus* isolates

Acid and bile salt tolerance are other important criteria for evaluating the survivability of probiotic strains in the digestive tract. There are no definitive criteria for assessing the pH and bile salt resistance of probiotic candidates. However, a pH of 3.0 and a bile salt concentration of 0.3% in the stomach and small intestine are commonly used as standard evaluation parameters (Halder *et al.*, 2017; Zommara *et al.*, 2023). The three-hour experimental period in this study was determined based on the food digestion process in the digestive tract (Huligere *et al.*, 2023).

In the present study, all the isolated *Bacillus pumilus* strains showed high stability in the pH 3.0 and 0.3% bile conditions after three hours of incubation (**Figure 4**), indicating their high survivability rates in the animal digestive tract. Probiotic characteristics are strain-specific rather than species-specific, so not all strains of the same species are equally resistant to acid and bile salt stress (Zommara *et al.*, 2023). The results of the pH test in **Figure 4** show that BP1 had the highest survival rate (95.38%), followed by BP2 (80.65%) and BP3 (77.97%). The survival rates of BP2, BP1, and BP3 in the 0.3% bile salts condition were 93.62%, 85.71%, and 82.61%, respectively. The findings in this study support previous studies that *Bacillus* species can exhibit high levels of stability in the gastrointestinal tract. In a study conducted by Le *et al*. (2023), *Bacillus* spp. strains isolated from shrimp mud and intestines were resistant to pH values ranging from 4 to 9. Nguyen *et al*. (2023) examined the stability of *Bacillus* spp. isolated from soil samples at chicken farms. The results revealed that the isolates had high survivability in pH values of 2 to 4 (90%-98%) and bile salt values of 0.5%-2% (80%-97%). Similar results were observed in a study conducted by Hyronimus *et al*. (2000). *B. laevolacticus* DSM 6475 and *B. racemicus* IAM 12395 strains were stable at a pH of 2.5. While *B. racemilacticus* and *B. coagulans* isolates exhibited great tolerance to bile salt values of over 0.3%. In another study, Bezpalk *et al.* (2023) found that 61.9% of isolated *Bacillus* strains had high stability in acidic and bile salt conditions, with survival rates ranging from 75.3% to 85.2% and 74.5% to 83.2%, respectively.

Antimicrobial susceptibility of the *B. pumilus* isolates

Antimicrobial resistance is another criterion that should be assessed in the selection of probiotic candidates (Hummel *et al.*, 2007). The results in **Table 3** of this study show that BP1 and BP2 were resistant to tetracycline and erythromycin, respectively. Meanwhile, BP3 exhibited resistance to both tetracycline and erythromycin. Antibiotic resistance may increase the survival rate of the *B. pumilus* isolates when antibiotics are used for the prevention and treatment of animal diseases. It cannot, however, rule out the possibility that they could spread genes that confer resistance to antibiotics to other bacterial species. The results of our study were somewhat similar to previous reports. According to a study conducted by Adamski *et al.* (2023), *B. pumilus* isolates were resistant to ampicillin (6.25%) and clindamycin (31.25%) but completely susceptible to erythromycin, gentamicin, trimethoprim/sulfamethoxazole, meropenem, and chloramphenicol. Galarza *et al*. (2015) reported that *Bacillus* spp. strains showed resistance to erythromycin and clindamycin but were sensitive to gentamicin, penicillin, and tetracycline.

Antibacterial activity against *E. coli* F4

The antibacterial activity of *B. pumilus* against *E. coli* F4 is presented in **Table 4** and **Figure 5**. All three isolates had strong antagonistic activity to *E. coli* F4 with clear zone diameters ranging from 16mm to 19mm.

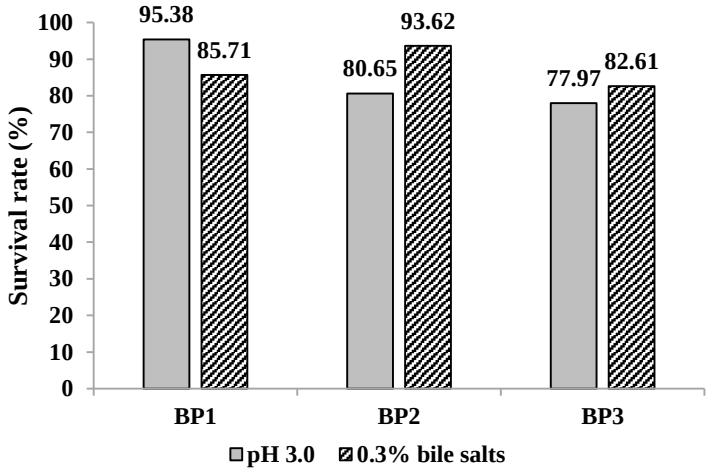


Figure 4. The stability of the *B. pumilus* isolates

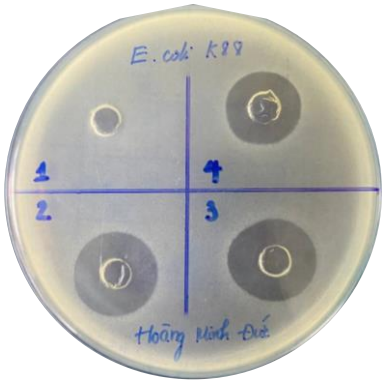
Table 3. Antimicrobial susceptibility of the *B. pumilus* isolates

Isolate ID	Resistance pattern	No. of antibiotics
BP 1	TET	1
BP 2	ERY	1
BP 3	TET -ERY	2

Note: TET: Tetracycline, ERY: Erythromycin.

Table 4. Antagonistic ability test of *B. pumilus* against *E. coli* F4

No.	Strain symbol	Inhibition zone size (mm)
1	BP 1	18
2	BP2	19
3	BP3	16



Note: (1) – Negative control, (2) – BP1, (3) – BP2, (4) – BP3

Figure 5. Antagonistic ability test of B. pumilus against E. coli F4

The antibacterial activity of *B. pumilus* has been demonstrated in previous studies. Nguyen Thi Hanh Chi *et al.* (2021) found that a *B. pumilus* isolate was antagonistic to *E. coli* with a diameter inhibitory zone of 13.17 mm. Similarly, Irkitova *et al.* (2021) evaluated the antibacterial activity of *B. pumilus* against *E. coli* using the agar well-diffusion method, reporting an inhibitory zone diameter of 5.3 mm. Probiotic strains of *Bacillus* spp.**,** in general, including *B. pumilus,* can produce several active substances, such as bacteriocin, bacitracin, gramicidin S, polymyxin, and tyrothricidin, which have antibacterial activity against pathogens such as *Salmonella, E. coli, Vibrio* sp., *Shigella* sp., *Staphylococcus aureus, Aeromonas* sp., and *Pseudomonas* sp. (Huynh Ngoc Thanh Tam & Huynh Van Thinh, 2020). Bacteriocins, in particular, are crucial because of their strong inhibitory action against a variety of bacteria, including antibiotic-resistant strains. However, each form of bacteriocin exclusively targets specific bacterial species, meaning that their activity is frequently particular (Darbandi *et al.*, 2022). In addition, *Bacillus* spp. can produce various extracellular enzymes, including glycoenzymes, proteases, and lipases, which exhibit antibacterial activity and contribute to immune regulation (Latorre *et al.*, 2016). The use of beneficial *Bacillus* spp. may strengthen the natural intestinal immune system (Latorre *et al.*, 2015). *Bacillus* spp. strains ferment carbohydrates to produce lactic acid, reducing the pH in the intestine and creating anaerobic, acidic conditions favorable for the growth of probiotics, while preventing the invasion and inhibiting the growth of aerobic and eosinophilic pathogenic bacteria (Tang *et al.*, 2024). In particular, *Bacillus* spp. can inhibit the adhesion of pathogenic bacteria, such as *Clostridium*, *E. coli*, and *Salmonella*, to the intestinal mucosa, thereby reducing the incidence of diarrhea and improving growth performance in piglets (Marubashi *et al.*, 2012).

Conclusions

Three *B. pumilus* strains were successfully recovered from healthy pigs. The isolates showed great antibacterial activity against *E. coli* F4. In addition, the isolated strains exhibited high pH and bile salt tolerance. However, further *in vivo* assays are needed to verify potential applications of these isolates in controlling *E. coli* F4 in animals.

References

Abriouel H., Franz C. M. A. P., Omar N. B. & Galvez A. (2011). Diversity and applications of *Bacillus* bacteriocins. FEMS Microbiology Reviews. 35(1): 201-232. DOI: 10.1111/j.1574-6976.2010.00244.x.

Adamski P., Byczkowska-Rostkowska Z., Gajewska J., Zakrzewski A. J. & Kłębukowska L. (2023). Prevalence and Antibiotic Resistance of Bacillus sp. Isolated from Raw Milk. Microorganisms. *11*(4): 1065. DOI: 10.3390/microorganisms11041065.

Balasingham K., Valli C., Radhakrishnan L. & Balasuramanyam D. (2017). Probiotic characterization of lactic acid bacteria isolated from swine intestine. Veterinary World. 10(7): 825-829. DOI: 10.14202/vetworld.2017.825-829.

Bezpalk O. O., Machuskyi O. V., Vygovska L. M. & Danchuk V. V. (2023). Determination of antagonistic

properties and biofilm formation in *Bacillus* app. and *Lactobacillus* spp. Scientific Reports of NULES of Ukraine. 4(104). DOI: 10.31548/dopovidi4(104).2023.007.

Castro J., Barros M. M., Araújo D., Campos A. M., Oliveira R., Silva S. & Almeida C. (2022). Swine enteric colibacillosis: Current treatment avenues and future directions. Frontiers in Veterinary Science. 9. DOI: 10.3389/fvets.2022.981207.

Chaima B., Boutlelis D. A., Touhami L. A., Ghemam Amara, D., Khemakhem M. M., Naccache C., Atanassova M., Zahnit, W., Ahmad S. F., & Messaoudi, M. (2024). Phenotypic characterization and molecular identification of *Bacillus pumilus* isolated from Algerian goat's milk and evaluation of its synbiotic effect with Hammada scoparia L. extract and ZnO-NPs. International Journal of Food Properties. 27(1): 1215-1229.

Chu J., Wang Y., Zhao B., Zhang X-M., Liu K., Mao L. & Kalamiyets E. (2019). Isolation and identification of new antibacterial compounds from Bacillus pumilus. Applied Microbiology and Biotechnology. 103(20): 8375-8381. DOI: 10.1007/s00253-019-10083-y.

CLSI (2015). M45. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Proposed Guideline. Guidelines CLSI. 35(17).

Dao Gia Bach, Doan Thi Thuy Linh, Luu Thi Thuy Ngan, & Le Thi Hoi (2024). Isolation, selection, and evaluation of characteristics of potential Bacillusstrains for probiotic products. Ministry of Science and Technology, Vietnam. 66(5): 29-33. DOI: 10.31276/VJST (in Vietnamese).

Darbandi A., Asadi A., Ari M. M., Ohadi, E., Talebi M., Halaj Zadeh M., Emamie A. D., Ghanavati R. & Kakanj M. (2022). Bacteriocins: Properties and potential use as antimicrobials. Journal of Clinical Laboratory Analysis. 36(1): e24093. DOI: 10.1002/jcla.24093.

Dobrzyński J., Jakubowska Z., Kulkova I., Kowalczyk P., & Kramkowski K. (2023). Biocontrol of fungal phytopathogens by *Bacillus pumilus*. Frontiers in Microbiology. 14(July): 1-12. DOI: 10.3389/fmicb.2023.1194606.

Foysal M. J. & Lisa A. K. (2018). Isolation and characterization of Bacillus sp. strain BC01 from soil displaying potent antagonistic activity against plant and fish pathogenic fungi and bacteria. Journal of Genetic Engineering and Biotechnology. 16(2): 387-392. DOI: 10.1016/j.jgeb.2018.01.005.

Galarza-Seeber R., Latorre J. D., Hernandez-Velasco X., Wolfenden A. D., Bielke L. R., Menconi A., Hargis B. M. & Tellez G. (2015). Isolation, screening and identification of Bacillus spp. as direct-fed microbial candidates for aflatoxin B1 biodegradation. Asian Pacific Journal of Tropical Biomedicine. 5(9): 702-706. DOI: 10.1016/j.apjtb.2015.07.014.

Government (2020). Decree No. 13/2020/ND-CP of the Government on the Detailed Guidelines for the Animal Husbandry Law. Retrieved from https://vanban.chinhphu.vn/default.aspx?pageid=27160&docid=198924 on January 16, 2024 (in Vietnamese).

Golnari M., Bahrami N., Milanian Z., Khorasgani M. R., Asadollahi M. A., Shafiei R. & Fatemi S. S. A. (2024). Isolation and characterization of novel Bacillus strains with superior probiotic potential: comparative analysis and safety evaluation. Scientific Reports. 14(1): 1457. DOI: 10.1038/s41598-024-51823-z.

Gonzalez-Ronquillo M., Villegas-Estrada D., Robles-Jimenez L. E., Garcia Herrera R. A., Villegas-Vázquez V. L. & Vargas-Bello-Pérez E. (2022). Effect of the inclusion of Bacillus spp. in growing–finishing pigs' diets: A meta-analysis. Animals. 12(17): 2269.

Gupta S., Mohanty U. & Majumdar R. K. (2021). Isolation and characterization of lactic acid bacteria from traditional fermented fish product Shidal of India with reference to their probiotic potential. LWT. 146: 111641. DOI: 10.1016/j.lwt.2021.111641.

Halder D., Mandal M., Chatterjee S. S., Pal N. K., & Mandal S. (2017). Indigenous probiotic Lactobacillus isolates presenting antibiotic like activity against human pathogenic bacteria. Biomedicines. 5(2): 31. DOI: 10.3390/biomedicines5020031.

Hoang Minh Duc, Cam Thi Thu Ha, Pham Hong Ngan, Hoang Minh Son, Le Van Hung, Tran Van Nen, Vu Thi Thu Tra, Vu Van Hoat & Le Van Tung (2022). Antibiotic resistance profile of *Escherichia coli* isolated from pork and chicken meat selling in retail market in Gia Lam district, Hanoi city. Journal of Veterinary Science and Technology. XXIX(6): 41-47 (in Vietnamese).

Huligere S. S., Chandana Kumari V. B., Alqadi T., Kumar, S., Cull C. A., Amachawadi R. G. & Ramu R. (2023). Isolation and characterization of lactic acid bacteria with potential probiotic activity and further investigation of their activity by α-amylase and α-glucosidase inhibitions of fermented batters. Frontiers in Microbiology. 13. DOI: 10.3389/fmicb.2022.1042263.

Hummel A. S., Hertel C., Holzapfel W. H. & Franz C. M. A. P. (2007). Antibiotic Resistances of Starter and Probiotic Strains of Lactic Acid Bacteria. Applied and Environmental Microbiology. 73(3): 730-739. https://doi.org/10.1128/AEM.02105-06.

Huynh Ngoc Thanh Tam & Huynh Van Thinh (2020 Characteristics of probiotic strains of *Bacillus* spp. from white leg shrimp (Litopenaeus vannamei) in Kien Giang province. CTU Journal of Innovation and Sustainable Development. 56: 44-52 (in Vietnamese).

Hyronimus B., Le Marrec C., Sassi A. H. & Deschamps A. (2000). *Acid and bile tolerance of spore-forming lactic acid bacteria*. International Journal of Food Microbioly. 61(2-3): 193-197. DOI: 10.1016/s0168-1605(00)00366-4.

Irkitova A. N., Malkova A. V. & Dudnik D. E. (2021). A natural bacterial strain *Bacillus pumilus* 16: Identification and antibiotic resistance evaluation. Acta Biologica Sibirica. 7: 391-406. DOI: 10.3897/ABS.7.E78412.

Jiang N., Hong B., Luo K., Li Y., Fu H. & Wang J. (2023). Isolation of Bacillus subtilis and *Bacillus pumilus* with Anti-Vibrio parahaemolyticus Activity and Identification of the Anti-Vibrio parahaemolyticus Substance. Microorganisms. 11(7): 1667. DOI: 10.3390/microorganisms11071667.

Latorre J. D., Hernandez-Velasco X., Kuttappan V. A., Wolfenden R. E., Vicente J. L., Wolfenden A. D., Bielke L. R., Prado-Rebolledo O. F., Morales E., Hargis B. M. & Tellez G. (2015). Selection of Bacillus spp. for cellulase and xylanase production as direct-fed microbials to reduce digesta viscosity and Clostridium perfringens proliferation using an in vitro digestive model in different poultry diets. Frontiers in Veterinary Science. 2(AUG). DOI: 10.3389/fvets.2015.00025.

Latorre J. D., Hernandez-Velasco X., Wolfenden R. E., Vicente, J. L., Wolfenden, A. D., Menconi, A., Bielke, L. R., Hargis, B. M., & Tellez, G. (2016). Evaluation and selection of Bacillus species based on enzyme production, antimicrobial activity, and biofilm synthesis as direct-fed microbial candidates for poultry. Frontiers in Veterinary Science. 3(OCT). DOI: 10.3389/fvets.2016.00095.

Lei J., Ran X., Guo M., Liu J., Yang F. & Chen D. (2023). Screening, Identification, and Probiotic Properties of *Bacillus pumilus* From Yak. Probiotics and Antimicrobial Proteins. 16: 1-10. DOI: 10.1007/s12602-023-10054-w.

Luppi A., Gibellini M., Gin T., Vangroenweghe F., Vandenbroucke V., Bauerfeind R., Bonilauri P., Labarque G. & Hidalgo Á. (2016). Prevalence of virulence factors in enterotoxigenic Escherichia coli isolated from pigs with post-weaning diarrhoea in Europe. Porcine Health Management. 2: 20. DOI: 10.1186/s40813-016-0039-9

Marubashi T., Gracia M. I., Vilà B., Bontempo V., Kritas S. K. & Piskoríková M. (2012). The efficacy of the probiotic feed additive Calsporin® (Bacillus subtilis C-3102) in weaned piglets: combined analysis of four different studies. Journal of Applied Animal Nutrition. 1: e2. DOI: 10.1017/jan.2012.1.

Morita T., Tanaka I., Ryuda N., Ikari M., Ueno D. & Someya T. (2019). Antifungal spectrum characterization and identification of strong volatile organic compounds produced by Bacillus pumilus TM-R. Heliyon. 5(6): e01817. DOI: 10.1016/j.heliyon.2019.e01817.

Nguyen Thi Hanh Chi, Van Khanh Ly, Dang Nguyen Hoang Minh, Vo Hong Nhinh & Nguyen Tuyet Giang (2021). Isolation and selection of *Bacillus* sp. strains for the enzyme production and resistance to *E. coli*. Journal of Animal Husbandry Sciences and Technics. 265: 93-99 (in Vietnamese).

Ritter A. C., Paula A., Correa F., Veras F. F. & Brandelli A. (2018). Characterization of *Bacillus subtilis* Available as Probiotics. Journal of Microbiology Research. 8(2): 23-32. DOI: 10.5923/j.microbiology.20180802.01.

Saha S., Namai F., Nishiyama K., Villena J. & Kitazawa H. (2024). Role of immunomodulatory probiotics in alleviating bacterial diarrhea in piglets: a systematic review. Journal of Animal Science and Biotechnology. 15(1): 112.

Tang X., Zeng Y., Xiong K. & Zhongj J. (2024). Bacillus spp. as potential probiotics: promoting piglet growth by improving intestinal health. Frontiers in Animal Nutrition and Metabolism. 11. DOI: 10.3389/fvets.2024.1429233.

Thy H. T. T, Tri N. N., Quy O. M., Fotedar R., Kannika K., Unajak S. & Areechon N. (2017). Effects of the dietary supplementation of mixed probiotic spores of Bacillus amyloliquefaciens 54A, and *Bacillus pumilus* 47B on growth, innate immunity and stress responses of striped catfish (*Pangasianodon hypophthalmus*). Fish and Shellfish Immunology. 60: 391-399. DOI: 10.1016/j.fsi.2016.11.016.

Todorov S. D., Ivanova I. V., Popov I., Weeks R. & Chikindas M. L. (2022). *Bacillus* spore-forming probiotics: benefits with concerns? Critical Reviews in Microbiology. 48(4): 513-530. DOI: 10.1080/1040841X.2021.1983517.

Upadhaya S. D. & Kim I. H. (2021). The impact of weaning stress on gut health and the mechanistic aspects of several feed additives contributing to improved gut health function in weanling piglets - A review. In Animals. 11(8): 2418. DOI: 10.3390/ani11082418.

Zhang N., Ding J., Li J., Wang L. & Wei Y. (2023). Effects of *Bacillus pumilus* on growth performance, serum indexes and muscle development-related gene expression of weanling Jintang black goat. Beneficial Microbes. 14: 1-9. DOI: 10.1163/18762891-20220093.

Zhang Q., Lin Y., Shen G., Zhang H. & Lyu S. (2022). Siderophores of *Bacillus pumilus* promote 2-keto-L-gulonic acid production in a vitamin C microbial fermentation system. Journal of Basic Microbiology, 62(7): 833-842. DOI: 10.1002/jobm.202200237.

Zommara M., El-Ghaish S., Haertle T., Chobert J.-M. & Ghanimah M. (2023). Probiotic and technological characterization of selected Lactobacillus strains isolated from different egyptian cheeses. BMC Microbiology. 23(1): 160.