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EVALUATION OF ANTIBIOTIC SENSITIVITY OF POTENTIAL PROBIOTIC *BACILLUS* STRAINS ISOLATED FROM CHICKEN AND PIG FECES

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Abstract. Nowadays, the misuse and overuse of antibiotics in livestock production leads to the appearance of antibiotic resistant strains that promotes the use of probiotics in feed to minimise/replace antibiotic consumption. Among them, Bacilli are common supplements for feed effectiveness and livestock health benefits. However, there is a growing concern about the development of antibiotic resistance and the transfer of antibiotic resistance genes among bacteria. Thus, the European Food Safety Authority (EFSA) has suggested that products containing *Bacillus* strains intended to use as feed additive must be examined for susceptibility to antimicrobials of human and veterinary importance. In this study, the antibiotic susceptibility of potential probiotic *Bacillus* strains was investigated. A total of 59 *Bacillus* species were isolated from 10 samples of chicken and pig feces. Then, the *in vitro* probiotic characteristics including low pH tolerance, catalase activity, protease, amylase and cellulase enzymes production were tested to select appropriate isolates for further studies. Thirty-three promising probiotic strains were assessed for their antibiotic susceptibility against 13 antibiotics by disc diffusion method. In most cases, isolated *Bacillus* strains were susceptible to tested antibiotics. The obtained results demonstrated the potential application of *Bacillus* spp. as feed supplements in animal production.

Keywords: Bacillus, probiotic, antibiotic susceptibility, livestock.

Classification numbers: 1.4.8, 3.7.2

1. INTRODUCTION

Antibiotic residue in meat as well as the development of antibiotic resistant strains are important issues of food safety and public health. The reason for these problems is the misuse

and overuse of antibiotics in livestock production. Hence, the use of probiotics in feed to minimise/replace antibiotic consumption has been promoted. Probiotics are defined as live microorganisms that, when administered in adequate amounts, that can confer a health benefit on the host [1]. Among them, Bacilli are common supplements for feed effectiveness and livestock health benefits. *Bacillus* is Gram positive, aerobic or facultative anaerobic, endospore forming bacteria. Their spores are heat-stable, capable of surviving at the low pH of the gastric barrier and products made on them can be stored at room temperature for long term without the loss of viability compared to those containing non-spore forming bacterium. Up to the present, more than 100 species and subspecies of the genus *Bacillus* have been used as probiotic supplements in feed, of which the most common are *B. coagulans, B. clausii, B. cereus, B. subtilis* and *B. licheniformis* [2 - 4].

However, there is a growing concern about for the development of antibiotic resistance and the transfer of antibiotic resistance genes among bacteria [5]. Thus, in recent guidelines provided by authorities approving the use of antimicrobials, it is clearly stated that the presence of antimicrobial resistance in probiotic bacterial strains is not allowed although probiotic bacterial strains used in livestock production have been found to contain tetracycline resistance genes. There have also been reports of the presence and expression of resistance genes located on plasmids and transposons in *Lactobacillus* spp. and *Bacillus* spp. used as probiotics in foods [2].

According to the European Food Safety Authority (EFSA), products containing *Bacillus* strains intended to use as feed additive must be examined for susceptibility to antimicrobials of human and veterinary importance [6]. Strains with MICs above cut-off values for one or more antimicrobials require further investigation to determine whether the strains exhibit intrinsic or acquired resistance. Recently, Uddin *et al*. (2015) evaluated the antimicrobial resistance of seven probiotic products commonly used in Vietnam aquaculture. The results showed that 9/60 (15 %) isolates of *Bacillus* spp. were fully susceptible to all antimicrobials tested; 12/60 (20 %) isolates were resistant to more than three antimicrobials. Resistance among the *Bacillus* spp. was in particular seen in ampicillin, chloramphenicol, clindamycin, erythromycin, and penicillin [2]. Therefore, the antibiotic susceptibility is an important characteristic when selecting probiotic strains for the production of probiotic product.

The objective of this study was to investigate the antibiotic susceptibility of *Bacillus* spp. isolated, with the aim of identifying potential probiotic strains for use in livestock production. We, therefore (1) isolated *Bacillus* spp. from chicken and pig feces, these isolates were then sequentially (2) screened for acid tolerance and for enzymatic activities are related to feeding digestion, e.g., cellulase, protease, and amylase. Isolates with a profile of high enzymatic activities were further (3) evaluated for antibiotic susceptibility.

2. MATERIALS AND METHODS

2.1. Isolation of *Bacillus* strains from feces

The fresh feces samples, which were obtained from 10 locations in Ha Noi and Quang Ninh (Table 2.1), were dried at 40 ^{o}C for 20 mins, then suspended in sterile saline and heated at 80 ^{o}C for 30 min to eliminate vegetative cells. The samples were serially diluted ten-fold with 0.85 % saline, and plated onto MPA (Himedia, India) and Nutrient agar (NA, Himedia, India) and incubated at 37 ^{o}C for 24 h. After incubation, the colonies specified for *Bacillus* were selected and streaked onto new plates to obtain a single colony. The colonies and microscopic morphologies were observed. Gram staining was performed according to Patel et al. (2009) [7].

All the isolates were maintained in Nutrient Broth (NB, Himedia, India) medium containing 20% glycerol at −20 °C. Gram positive isolates were subjected to catalase test.

Table 2.1. List of pig chicken and pig feces collected

No	Strain code	Location	GPS
1	CTY	Tien Yen chicken feces – Tien Yen, Quang Ninh	21°32.437 N; 107°42.401 S
2	CHL	Ho Lai chicken feces – Tien Yen, Quang Ninh	21°32.437 N; 107°42.401 S
3	CQN	Bac Giang chicken feces – Tien Yen, Quang Ninh	21°19.30 N; 107°24.25
4	P1QN	Pig feces – 10 kg/pig - Tien Yen, Quang Ninh	21°19.30 N; 107°24.25 E
5	P2QN	Pig feces – 50 kg/pig - Tien Yen, Quang Ninh	21°19.30 N;107°24.25 E
6	P3QN	Pig feces – 90 kg/pig - Tien Yen, Quang Ninh	21°19.30 N; 107°24.25 E
7	P4QN	Pig feces – Tien Yen farm – 1.5 months, 20 kg – Quang Ninh	21°19.41 N; 107°24.16 E
8	P5QN	Pig feces – Tien Yen farm – 3.0 months, 60 kg – Quang Ninh	21°19.41 N; 107°24.16 E
9	P6QN	Pig feces – Tien Yen farm – 1 year, 130 kg – Quang Ninh	21°19.41 N; 107°24.16 E
10	PHN	Pig feces - Cau Rau, Phu Cat, Quoc Oai, Ha Noi	20°58.29 N; 105°32.51 E

2.2. Catalase test

The catalase activity of isolated Gram positive bacteria was detected by dropping 3 % hydrogen peroxide solution on the colonies [7]. The positive ones were subsequently tested for acid tolerance.

2.3. Acid tolerance

Gram positive and catalase positive isolates were screened for acid tolerance. The isolates were incubated overnight at 37 °C in NB control medium (pH 7.0) and NB acid medium (pH 2.5, adjusted with 0.1 M HCl). Cell viability was assessed through suspension turbidity, compared to control sample of the medium only. Turbid suspension was noted as growing bacteria, i.e. acid tolerant. Aliquots were taken after incubation and plated directly onto NA plates. These plates were incubated at 37 °C for 24 h to obtain acid-tolerance strains for further studies.

2.4. Enzyme production

To investigate the production of various extracellular enzymes in each *Bacillus* strain, 5 µL of overnight culture (representing approximately 10^8 CFU/mL) was dropped on different media. For the screening amylase activity, isolates were grown on 0.5 % starch agar plates and

incubated at 37 ^{o}C for 24 h. After incubation the plates were flooded with iodine-potassium iodide (lugol) solution for amylase activity. Screening for protease activity was performed by growing the isolates on 0.1 % casein agar plates at 37 ^{o}C for 24 h. For visualization of the clear zones of activity around the growing culture the plates were flooded with 10 % TCA. The selected isolates were grown for 24 hours in a medium containing 1.0 % carboxymethyl cellulose (CMC). Hydrolysis of CMC by cellulase enzyme was verified by staining the plates with lugol to intensify the zones [8]. After incubation, all plates were evaluated and the diameters of the zones of clearance were measured. The relative enzyme activity (REA) was determined using the following formula: REA = diameter of zone of clearance divided by the diameter of the bacterial colony in millimeters. Based on REA test organisms were categorized into excellent (REA > 5.0), good (REA > 2.0–5.0), or poor (REA < 2.0) [9]. Strains that produce at least one enzyme with high activity and represent for sample collection location will be chosen for antibiotic sensitivity.

2.5. Antibiotic susceptibility

The antibiotic susceptibility of the selected *Bacillus* strains was tested using a disk diffusion method according to Clinical and Laboratory Standard Institute (CLSI) performance standards for antimicrobial susceptibility testing [10]. Thirteen kinds of antibiotics (Nam Khoa Biotech, Viet Nam) were used: Gentamicin (GEN, 10 µg), Tetracycline (TE, 30 µg), Kanamycin (K, 30 µg), Rifampicin (RA, 30 µg), Erythromycin (E, 15 µg), Clindamycin (CM, 2 µg), Amoxicillin (AMX, 10 µg), Ampicillin (AMP, 10 µg), Ciprofloxacin (CIP, 5 µg), Streptomycin (S, 10 µg), Chloramphenicol (C, 30 µg), Cefoxitin (FOX, 30 µg), and Amikacin (AK, 30 µg). *Bacillus* cultures, adjusted to approximately 1×10^6 CFU/mL, (optical density of 0.1 at 600 nm, then diluted 100 folds), were spread onto Muller Hilton agar plates (Himedia, India). After that, antibiotic discs were loaded onto the agar. All plates were incubated at 37 ^{o}C for 24 h. The inhibition zones were measured (including 6 mm diameter of the disc) and interpreted referring to CLSI, which contains measurement ranges and their equivalent qualitative categories of susceptible (S), intermediately susceptible (I), or resistant (R) (Table 2.2). All experiments were done in triplicate.

Table 2.2. Susceptibility breakpoints (mm diameter) for the probiotics used in this study.

Antibiotic type	Disk content (µg)	S	I	R
Gentamicin (GEN)	10	≥ 15	13-14	≤ 12
Tetracycline (TE)	30	≥ 19	15-18	≤ 14
Kanamycin (K)	30	≥ 18	14-17	≤ 13
Rifampicin (RI)	30	≥ 26	-	<23
Erythromycin (E)	15	≥ 23	14-22	≤ 13
Clindamycin (CM)	2	≥ 21	15-20	≤ 14
Amoxicillin (AMX)	10	≥ 21	-	≤ 14
Ampicillin (AMP)	10	≥29	-	≤28
Ciprofloxacin (CIP)	5	≥ 21	16-20	≤15
Streptomycin (S)	10	≥ 14	-	<14
Cloramphenicol (C)	30	≥ 18	13-17	≤ 12
Cefoxitin (FOX)	30	≥ 22	-	≤ 21
Amikacin (AK)	30	≥ 17	15-16	≤ 14

The breakpoints used for the above antibiotics were for *Staphylococcus* spp. according to zone diameters of antimicrobial agents in accordance with CLSI guidelines 2011 [10]. It has been reported that the breakpoints of antibiotics for *Staphylococcus* spp. can be used to test *Bacillus* spp. (non-*Bacillus anthracis*).

3. RESULTS AND DISCUSSION

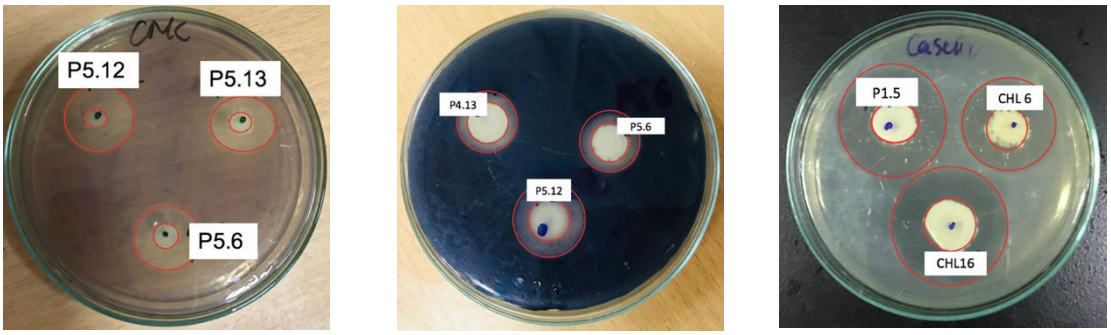
3.1. Isolation of *Bacillus* sp. from chicken and pig feces

In our study, spore-forming bacteria were selected by heat treatment, then treated samples were appropriately diluted, subsequently plated and incubated aerobically. A total of 141 colonies were isolated from 10 chicken and pig feces samples on two mediums MPA and NA. Among them, 59 of isolates were found to be Gram positive and catalase positive. As reported in literature, the members of *Bacillus* genus are Gram-positive, aerobic or facultative anaerobic, catalase positive, rod-shaped endospore forming bacteria including more than 60 species with quite different phenotypes. Studies on morphological and gram staining showed that 59 isolates were believed to belong to *Bacillus* species.

In other publications [11], *Bacillus* strains have been isolated from different sources such as soil, fecal, gastrointestinal tracts to use as probiotics for livestock production. 110 spore-forming bacterial strains were isolated from chicken gastrointestinal tracts of 11 cage-free chickens collected from various regions in Viet Nam. Seven non-pigmented strains belonging to *B. subtilis* and *B. licheniformis* species were selected for further studies with high sporulation efficiency (> 90 %) and spore ability survived to heat treatment at 80 °C for 20 min [11]. Similarly, as shown in our study, presumptive *Bacillus* isolated from livestock and poultry production had the potential to be used in feed production. Screening for other probiotic potential properties was conducted.

Bacillus species are efficient producers of catalase which can reduce the harmful effects of active oxygen molecules or free radicals, which are generated during the metabolic process and injurious to the host health [7]. Therefore, these potent probiotic strains can act as a good antioxidant if they inhabit in the gut. In addition, the survival of bacteria in gastric juice depends on their ability to tolerate low pH which is an important probiotic characteristic. In our study, the isolates exhibited a survival rate of 88.13 % (52/59 strains) after 24 h of incubation at pH 2.5.

3.2. Enzyme production of selected *Bacillus* sp. isolates



(A) **(B)** **(C)**

Figure 3.1. Representative examples of microbial enzyme activity.
An area of clearance around a bacterial colony can be observed, representing enzyme production of (A) cellulase (B) amylase, (C) protease. Circles indicate the bacterial colony and the outer limit of the zone of clearance.

The different enzymatic activities of 52 presumptive *Bacillus* isolates are presented in Table 3.1 and examples of plates are illustrated in Figure 3.1.

Table 3.1. Relative enzyme activity values produced by *Bacillus* spp. strains.

No	*Bacillus* isolates	Amylase	Celullase	Protease	No	*Bacillus* isolates	Amylase	Celullase	Protease
1	CTY4	0.0	0.0	1.2	27	P2QN1	0.0	0.0	2.0
2	CTY5	0.0	0.0	0.0	28	P2QN7	1.7	2.0	1.1
3	CTY6	0.0	**5.0**	1.3	29	P2QN9	0.0	0.0	1.2
4	CTY7	1.3	1.7	2.2	30	P2QN11	0.0	0.0	0.0
5	CTY14	0.0	0.0	1.6	31	P3QN2	0.0	0.0	1.9
6	CTY16	0.0	0.0	1.3	32	P3QN4	**5.0**	0.0	2.0
7	CTY18	1.0	0.0	1.1	33	P3QN6	2.6	1.5	1.9
8	CTY28	0.0	0.0	1.8	34	P3QN10	1.5	1.9	1.6
9	CHL2	0.0	1.7	1.9	35	P3QN12	0.0	0.0	2.3
10	CHL6	0.0	0.0	2.0	36	P3QN14	0.0	0.0	1.8
11	CHL9	0.0	0.0	1.2	37	P4QN2	1.5	2.5	2.5
12	CHL13	**5.0**	0.0	1.3	38	P4QN4	0.0	0.0	1.8
13	CHL14	0.0	0.0	1.7	39	P4QN5	0.0	0.0	2.1
14	CHL15	1.2	1.6	1.7	40	P4QN11	1.5	1.7	1.7
15	CHL16	1.2	2.4	1.6	41	P4QN13	1.2	1.5	1.2
16	CQN2	0.0	0.0	1.9	42	P5QN4	1.7	2.4	1.8
17	CQN5	1.2	1.5	1.8	43	P5QN6	1.7	2.6	1.7
18	CQN6	1.2	1.8	1.7	44	P5QN7	1.5	1.4	1.9
19	CQN9	0.0	0.0	1.6	45	P5QN10	0.0	0.0	0.0
20	CQN10	0.0	1.4	1.9	46	P5QN11	2.1	1.4	1.8
21	CQN12	0.0	0.0	2.1	47	P5QN12	1.3	2.0	1.8
22	P1QN1	0.0	0.0	1.8	48	P5QN13	1.5	1.6	1.8
23	P1QN3	1.4	1.4	1.8	49	P6QN2	1.7	1.7	1.6
24	P1QN5	0.0	0.0	1.7	50	PHN2	0.0	0.0	0.0
25	P1QN8	0.0	0.0	2.0	51	PHN3	0.0	0.0	0.0
26	P1QN11	0.0	0.0	1.6	52	PHN4	1.6	2.3	2.2

Differences in enzyme production activity, as well as REA values, were found in our study. 5 out of 52 (9.6 %) screened *Bacillus* isolates could not produce amylase, cellulase and protease enzymes. Meanwhile, there were 19 strains (36.5 %) capable of producing all of three tested

enzymes (Table 3.1). Four of 52 isolates showed good amylase productivity (REA > 2.0 - 5.0), including CHL13, P3QN4, P3QN6, and P5QN11. In the case of cellulase activity, strains CTY6, CHL16, P2QN7, P4QN2, P5QN4, P5QN6, P5QN12, and PHN4 were classified as good because they surpassed the cellulase enzyme activity values of all other screened strains. Protease activity was detected for the majority of tested strains, with the REA values ranging from 1.1 to 2.5. 33 isolates that were able to produce at least one enzyme with high activity and represent for sample collection location were selected for antibiotics susceptibility.

Pig and chicken feed consists of ground grains (mostly soybean and corn), mineral and vitamin supplements with different percentages. Microbial enzymes are used to assist in nutrient digestion and feed utilization. *Bacillus* species can synthesize extracellular enzymes directly in the digestive tract of the host organism such as proteases, cellulase, α-amylases, α-galactosidases, phytases, and xylanases [9]. Ghani *et al*. (2013) isolated 35 bacterial strains belonging to the genus *Bacillus* from soil. Of these, five strains were identified as *B. licheniformis*, which showed significant capability of producing important extracellular hydrolytic enzymes including α-amylase, glucoamylase, protease, pectinase, and cellulase in varying titers [8]. Abdel-Moneim *et al*. (2019) demonstrated that dietary supplementation with *B. subtilis* spores, which secretes protease, amylase and lipase enzymes, improved growth performance, blood metabolites, antioxidative status, and digestive enzyme activities in quail [12].

3.3. Antibiotic susceptibility of promising probiotic strain

The susceptibility of 33 presumptive *Bacillus* isolates was tested for 13 different antibiotics. Those antibiotics are commonly used in livestock for preventing and treating gastrointestinal diseases. They are listed in Circular 06-2016/TT/BNNPTNT issued by the Ministry of Agriculture and Rural Development (MARD) and the list of veterinary drugs licensed for circulation in Viet Nam (December 31, 2020), published on the website of the Department of Veterinary Medicine, MARD. The results of the antimicrobial tests are presented in Table 3.2. The isolates were susceptible to all the antibiotics tested to various degrees. All *Bacillus* strains were fully sensitive to gentamycin, ciprofloxacin, kanamycin, and streptomycin. The majority of the isolates were susceptible to chloramphenicol (97.0 %), tetracycline (84.8 %), amikacin (93.9 %), and clindamycin (66.7 %). In addition, the isolates were resistant to erythromycin (51.5 %), ampicillin (90.9 %), cefoxitin (75.8 %), amoxicillin (72.7 %), and rifampicin (84.8 %).

Multidrug resistance is a serious and emerging issue combined with the inevitable risk of drug-resistant gene transfer to commensals or pathogens of the gut, making the antibiotic susceptibility of probiotics an important factor of interest [5]. The results obtained were in good agreement with previous studies. *Bacillus* sp. isolates tested were found to be susceptible to chloramphenicol, erythromycin, ciprofloxacin, streptomycin, and gentamycin [13]. Mingmongkolchai *et al*. (2017) evaluated the safety of seven spore-forming *Bacillus* isolates in order to use as feed additive. The isolate CM40 was susceptible to 10 antibiotics, including six key antibiotics (chloramphenicol, erythromycin, gentamicin, tetracycline, streptomycin, and kanamycin) [14]. Ampicillin and amoxicillin are used for treating bacterial infections. However, some probiotic *Bacillus* spp. used extensively for pest control were found to be resistant to the β-lactams (amoxicillin and ampicillin) [15]. Resistance to antibiotics is acquired by a change in the gene makeup of bacterium, which can occur by either a gene mutation or the transfer of antibiotic resistance genes between bacteria in the environment.

Table 3.2. Inhibition zone diameter (mm) of antibiotics against *Bacillus* sp.

No	Species	**GEN**	**TE**	**K**	**RA**	**E**	**CM**	**AMX**	**AMP**	**CIP**	**S**	**C**	**FOX**	**AK**
1	CTY6	21.0*	21.7	17.7	17.0	23.0	21.0	12.3	9.3	26.7	20.0	22.5	10.0	21.0
2	CTY7	27.3	20.0	30.7	25.3	24.7	27.0	29.0	27.7	36.7	28.7	29.0	26.0	26.3
3	CTY14	22.0	19.3	19.7	18.0	21.0	20.0	10.7	13.0	26.3	19.7	20.7	9.7	23.0
4	CTY16	21.5	21.3	17.7	15.7	20.3	17.7	10.3	8.5	24.3	18.0	20.0	9.0	19.7
5	CTY18	19.0	20.7	20.0	17.3	20.3	17.7	11.0	10.7	23.3	23.0	21.0	9.7	15.7
6	CTY28	25.7	28.5	21.5	19.7	25.0	18.5	9.7	13.7	24.5	18.0	23.7	11.0	26.0
7	CHL2	20.5	22.7	19.5	20.3	27.0	23.7	17.0	17.0	28.0	19.0	21.0	14.7	18.7
8	CHL6	22.0	23.7	20.0	17.0	25.3	23.3	13.0	11.0	30.7	20.0	22.7	12.3	24.0
9	CHL13	20.3	22.0	20.0	17.0	20.3	20.3	10.3	9.0	26.3	18.0	20.3	8.7	20.7
10	CHL15	19.7	20.5	19.0	17.7	22.3	19.0	11.3	10.0	27.0	20.3	20.0	10.0	20.7
11	CHL16	27.3	16.7	26.0	20.3	22.7	20.0	23.3	24.0	32.0	21.0	24.0	26.3	27.7
12	CQN5	24.0	22.0	21.7	17.0	19.7	21.0	12.7	10.0	25.7	20.0	24.0	12.3	23.7
13	CQN6	31.3	26.7	34.7	25.7	31.0	28.0	27.0	29.0	39.7	27.3	32.0	18.7	28.7
14	CQN10	23.0	22.3	22.0	18.7	23.0	22.3	13.3	11.0	29.3	20.7	24.0	11.0	15.3
15	P1QN3	24.3	20.0	22.0	22.3	22.7	20.7	17.3	16.7	31.0	27.7	24.0	15.3	24.7
16	P2QN1	21.3	21.5	19.7	17.0	20.7	22.3	12.0	10.5	28.0	18.7	21.3	9.3	21.3
17	P2QN7	19.0	20.0	19.3	16.3	20.3	19.0	11.7	8.3	24.3	20.7	20.7	11.3	20.7
18	P3QN6	20.3	22.3	19.7	18.0	22.0	22.0	12.0	8.3	26.7	19.7	20.0	9.3	20.7
19	P3QN4	20.7	22.3	19.0	17.0	18.3	21.7	10.5	10.0	24.7	19.0	20.0	9.0	21.3
20	P3QN10	23.7	22.0	19.3	15.7	25.7	23.7	12.0	10.0	26.0	19.7	19.7	10.7	15.7
21	P3QN12	22.5	21.0	19.0	16.7	19.3	19.0	11.0	10.5	27.7	18.7	17.0	9.3	18.7
22	P4QN2	23.3	18.7	26.7	25.3	27.7	23.7	25.0	27.7	32.3	28.3	28.3	23.0	30.7
23	P4QN5	22.0	21.3	19.3	19.0	21.3	20.7	11.0	9.3	26.7	25.3	22.3	11.7	19.7
24	P4QN11	22.0	21.5	22.5	23.0	23.3	20.3	16.3	14.3	28.0	20.0	24.3	15.7	21.7
25	P4QN13	28.0	21.7	28.0	27.3	30.0	23.0	28.0	28.3	36.0	28.3	28.3	26.0	27.0
26	P5QN4	37.3	21.5	38.3	21.3	28.7	20.7	24.3	29.3	41.3	21.3	27.7	26.7	21.7
27	P5QN6	29.0	16.5	30.0	23.3	27.3	22.0	26.3	27.3	36.7	28.3	28.3	22.0	27.7
28	P5QN7	24.7	21.7	21.5	18.7	26.3	24.0	12.0	16.3	31.7	21.3	23.0	16.3	26.7
29	P5QN11	22.7	21.3	26.0	21.3	22.0	19.0	13.7	11.0	26.3	26.7	26.0	15.3	22.7
30	P5QN12	27.3	23.3	21.0	16.3	15.3	22.0	10.7	11.3	28.7	31.3	24.0	14.3	27.3
31	P5QN13	29.0	15.7	31.0	22.0	24.3	20.0	26.0	25.3	33.5	28.0	20.0	21.7	28.7
32	P6QN2	31.0	17.3	28.3	23.7	27.7	21.3	29.7	28.0	35.0	30.0	28.7	23.3	27.3
33	PHN4	22.5	21.3	18.7	17.0	20.7	20.7	10.3	9.3	27.3	14.7	19.3	8.7	15.0

*Values are reported as the means of triplicates. Gentamicin (GEN, 10µg), Tetracycline (TE, 30 µg), Kanamycin (K, 30 µg), Rifampicin (RA, 30 µg), Erythromycin (E, 15 µg), Clindamycin (CM, 2 µg), Amoxicillin (AMX, 10 µg), Ampicillin (AMP, 10 µg), Ciprofloxacin (CIP, 5 µg), Streptomycin (S, 10 µg), Chloramphenicol (C, 30 µg), Cefoxitin (FOX, 30 µg) and Amikacin.

4. CONCLUSIONS

In conclusion, the findings showed the antibiotic susceptibility of potential probiotic *Bacillus* strains. *Bacillus* species isolated from chicken and pig feces showed promising *in vitro* probiotic characteristics such as survival at acid tolerance (88.1 %) and good ability to produce extracellular enzymes (90.4 %). Thirty-three promising probiotic strains were susceptible to tested antibiotics including gentamycin, ciprofloxacin, kanamycin, streptomycin, chloramphenicol, tetracycline, amikacin, clindamycin and were resistant to erythromycin, ampicillin, cefoxitin, amoxicillin, and rifampicin. The results obtained from this study would serve as the basis for further screening and demonstrating potential applications of *Bacillus* spp. as feed supplements in animal production.

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Declaration of competing interest. The authors declare that they have no conflict of interest.

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