HEALTH RISK ANALYSIS IN EPIDEMIOLOGY

UDC 613.26
DOI: 10.21668/health.risk/2025.3.09.eng

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Research article

OCCURRENCE AND CHARACTERIZATION OF BACILLUS LICHENIFORMIS AND BACILLUS SUBTILIS IN YAM POWDER PRODUCTS FROM VIETNAM AS A HEALTH RISK FACTOR

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Yam (Dioscorea alata L.) is a carbohydrate-rich tuber crop widely consumed in tropical countries. This includes Vietnam, where both fresh yam and yam powder are part of traditional diets. Despite its popularity, the microbiological safety of yam-derived products has received limited scientific attention.

This study investigated the microbial contamination and characterized Bacillus licheniformis and Bacillus subtilis isolated from commercially available yam powder products in Vietnam. The results showed that while B. cereus, E. coli, S. aureus, or Salmonella spp. were not detected, varying levels of total aerobic bacteria, yeasts, and molds were present in most samples. B. licheniformis was the predominant species, isolated from five out of seven powder samples, while B. subtilis appeared in two. It should be noted that all B. licheniformis strains harbored the licA, licB, and licC genes responsible for lichenysin synthesis and displayed multidrug resistance to antibiotics such as erythromycin, amoxicillin-clavulanate, and cephalosporins. In contrast, B. subtilis strains lacked diarrheagenic toxin genes (nhe, hbl, cytK) and showed higher susceptibility to most antibiotics. These findings suggest that microbial contamination may occur during processing or storage and highlight the urgent need for updated microbial guidelines, targeted hygienic practices, and routine toxin gene screening to ensure safety and quality of yam-based food products in Vietnam.

Keywords: *Bacillus licheniformis, Bacillus subtilis, yam, antibiotic resistance, M45 CLSI, bceT, cytK, hbl, nhe, licABC.*

Yam (*Dioscorea alata L.*), also known locally in Vietnam as “khoai vạc”, “củ mỡ”, or “củ cái”, belongs to the genus *Dioscorea* in the family Dioscoreaceae. It is a monocotyledonous crop cultivated primarily in regions such as Africa, the United States, the South Pacific, and Asia, and serves as a staple food in many tropical countries [1]. There are two main varieties based on flesh color: white-fleshed and purple-fleshed yams. Yam tubers are rich in carbohydrates, particularly starch, making them an important energy source in the human diet [2]. According to the International Institute of Tropical Agriculture (IITA), West Africa ac-

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counts for 94 % of global yam production, with Nigeria alone contributing 71 % of the total output in 2021[1]. In Vietnam, yams are widely adapted and found across all seven agro-ecological zones. Among the yam cultivars grown domestically, two high-yielding varieties include the white yam (41.4 tons/ha), and purple yam (32.5 tons/ha)[2]. Production of yam powder generally includes such steps as harvesting, cleaning, cutting, grinding, pressing, drying, sieving, and packaging [3]. However, during harvesting and small-scale or household-level processing, the risk of microbial contamination remains due to inadequate hygiene and storage conditions. Microorganisms such as aerobic bacteria, yeasts, molds, and coliforms may proliferate during drying and storage. In particular, some *Bacillus* species can form heat-resistant spores that survive in dried products, posing potential food safety risks.

Bacillus licheniformis (B. licheniformis) is a gram-positive, rod-shaped, aerobic bacterium that was first isolated from soil in 1890. It is ubiquitous in nature and can be found in soil, water, air, and on the bodies of various animals [4]. This species is known for its ability to produce a range of useful compounds including α-amylase (starch degradation), β-galactosidase (lactose hydrolysis), alkaline proteases (protein degradation), anti-biofilm agents, and keratinase (keratin breakdown) [5]. Despite its industrial applications, *B. licheniformis* can also cause foodborne illness due to the production of lichenysin, a lipopeptide cytotoxin structurally and functionally similar to cereulide produced by *B. cereus*. The licABC gene cluster (previously known as *lchAA, lchAB, lchAC*), which encodes lichenysin synthetase enzymes, has been detected in numerous *B. licheniformis* strains isolated from food and environmental sources [6]. Yeak et al. (2022) reported that the concentration of lichenysin required to inhibit 50 % viability of human intestinal Caco-2 cells and pig ileum organ cells was 16.6 μg/ml and 16.8 μg/ml, respectively [6]. A fatal case involving infant formula contaminated with *B. licheniformis* was reported by Salkinoja-Salonen et al. in 1999[3].

Bacillus subtilis (B. subtilis), a spore-forming probiotic bacterium, is commonly found in soil and in the gastrointestinal tracts of certain mammals. This microorganism contributes to maintaining a favorable microbial balance within the digestive system. *B. subtilis* produces extracellular matrix components that provide protective effects and support growth and activity of other beneficial probiotic bacteria [7]. Although *B. subtilis* is generally regarded as a beneficial bacterium, certain strains may carry toxin genes homologous to those found in *B. cereus*, such as *nhe, hbl*, and *cytK*, highlighting the need for thorough virulence screening in safety assessments [8–10].

The aim of this study was: 1) investigate the prevalence of microbial contamination in fresh yam and commercially available yam powder in Vietnam; 2) isolate and identify *B. licheniformis* and *B. subtilis* strains; 3) evaluate their antibiotic resistance profiles, and 4) determine the presence of toxin-encoding genes.

The findings aim to provide early-warning data and contribute to development of microbiological safety standards tailored to production and consumption of yam-based products in Vietnam.

Materials and methods. Four fresh purple yam tuber samples, comprising both white-fleshed and purple-fleshed varieties, were collected directly from cultivated fields in Bac Ninh province, Vietnam. In addition, seven dried purple yam powder samples were pur-

[1] Yam. *International Institute of Tropical Agriculture (IITA)*. Available at: https://www.iita.org/cropsnew/dioscoria/ (August 12, 2024).

[2] Kết quả đánh giá đa dạng nguồn gen cây khoai mỡ (dioscorea alata L.) đang bảo quản tại ngân hàng gen cây trồng quốc gia năm 2009. *Trung Tâm Tài Nguyên Thực Vật [Plant Resources Center (PRC)]*. Available at: https://prc.org.vn/?p=587 (August 12, 2024).

[3] Salkinoja-Salonen M.S., Vuorio R., Andersson M.A., Kämpfer P., Andersson M.C., Honkanen-Buzalski T., Scoging A.C. Toxigenic Strains of Bacillus licheniformis Related to Food Poisoning. *Appl. Environ. Microbiol.*, 1999, vol. 65, no. 10, pp. 4637–4645. DOI: 10.1128/AEM.65.10.4637-4645.1999

chased from local retail stores in the same region. All samples were transported under chilled conditions and analyzed at the National Institute for Food Control (NIFC).

Microbial contamination in purple yam and yam-derived products. Total aerobic bacteria, yeasts and molds, coliforms, *Bacillus cereus (B. cereus), Escherichia coli (E. coli), Staphylococcus aureus (S. aureus)*, and *Salmonella spp.* were qualitatively and quantitatively analyzed according to the current Vietnamese national standards:

- Enumeration of total aerobic microorganisms in food: TCVN 11039-1:2015;
- Enumeration of yeasts and molds in food: TCVN 8275-2:2010;
- Enumeration of coliforms: TCVN 6848:2007;
- Enumeration of *Escherichia coli*: TCVN 7924-2:2008;
- Detection of *Salmonella spp.*: TCVN 10780-1,2:2017;
- Detection of presumptive *Bacillus cereus*: TCVN 4992:2005.

Isolation and identification of B. licheniformis and B. subtilis in yam-derived products. 10 g of each food sample was homogenized in 90 mL of peptone water (Merck). A dilution series up to 10^{-5} was prepared for each sample, and 100 µL was inoculated onto Mannitol Egg Yolk Polymyxin Agar (MYP; Merck) at each dilution. All plates were incubated overnight at 37 °C. Colonies exhibiting morphology, namely, yellow, mucoid or volcano-like in appearance were streaked onto Tryptone Soya Agar (TSA; Merck), incubated for 24 hours at 37 °C, and then identified using MALDI-TOF Matrix-Assisted Laser Desorption Ionization – Time of Flight on the VITEK® MS system (BioMérieux SA, Marcy l'Etoile, France). E. coli ATCC 8739 was used as a control strain during the run.

Total DNA of *B. licheniformis* and *B. subtilis* was isolated according to the protocol for gram-positive bacteria of the GeneJET Genomic DNA Purification kit (Thermofisher; C5042). The total DNA concentration of *B. cereus* strains was quantified using a nanodrop spectrophotometer at 260 nm. To confirm the presence of *B. licheniformis* among the isolates obtained from purple yam powder samples, four species-specific gene markers – *ligD, rfaB, BL00303,* and *sera* – were employed in PCR assays [11]. In parallel, the presence of *B. subtilis* was verified using the species-specific marker *aprE* [12]. Primer pairs used for the confirmation of *B. licheniformis* and *B. subtilis* are listed in Table 1.

Evaluation of antibiotic resistance characteristics of B. licheniformis and B. subtilis in yam-derived products. The antibiotic resistance of *B. licheniformis* and *B. subtilis* strains was tested using the Kirby-Bauer disk diffusion method [11]. *B. licheniformis* and *B. subtilis* was grown in 10 mL of sterile BHI medium until a cell density equivalent to a 0.5 McFarland standard was reached, approximately $1.5 \cdot 10^8$ CFU/mL. A sterile swab was

Table 1

Specific gene markers to confirm *B. licheniformis* and *B. subtilis*

Name	Sequence (5'-3')	Gene	Size (bp)	Reference
BL00303-F	CGT ATC GGT CGT TCA CTC GG	*BL00303*	332	[11]
BL00303-R	GTT GAT TTT CCG TAG CCT GGG			
serA2F	GAC AAG AGA AAT TTC TAC GAG CAA GTA CAA T	*serA2*	247	
serA2R	GCA GCC TTC CAA TTA CTC CTT CTG CCA CAG T			
rfaBF	TAC GCT AAG GAG GGG C	*rfaB*	376	
rfaBR	GTT TTT ATT GCT TCA TCG GCT			
ligDF	CTA TCA GCA CTT ATG GCA G	*ligD*	216	
ligDR	ACT CCT AGC GGT GTT CC			
BsF	ACC ATT GCG GTA GGT GCG	*aprE*	581	[12]
BsR	GCG TTT GTC CAA GTC GGG			

used to spread the bacterial suspension on Muller- Hinton agar. A total of 9 types of antibiotics were tested according to M45 CLSI, including ampicillin (AMP, 10 μg/disk), chloramphenicol (C, 30 μg/disk), ciprofloxacin (CIP, 5 μg/disk), erythromycin (ERY, 15 μg/disk), imipenem (IPM, 10 μg/disk), meropenem (MRP, 10 μg/disk), tetracycline (TE, 30 μg/disk), vancomycin (VAN, 30 μg/disk), and gentamicin (CN, 10 μg/disk). In addition to the antibiotics listed in the CLSI guidelines, additional antimicrobial agents commonly used in clinical and food safety monitoring were also tested to broaden the resistance profiling. These included: Amoxicillin-clavulanic acid (AMC, 30 μg/10 μg/disk), Aztreonam (ATM, 30 μg/disk), Cefotaxime (CTX, 30 μg/disk), Cefoxitin (FOX, 30 μg/disk), Ceftazidime + Cloxacillin (CAC), Ceftriaxone (CRO, 30 μg/disk), Cefuroxime (CXM, 30 μg/disk), Methicillin (MET, 5 μg/disk), Nalidixic acid (NA, 30 μg/disk), Ofloxacin (OFX, 5 μg/disk), Oxacillin (OX, 5 μg/disk), Penicillin (PRL, 10 IU/disk), Piperacillin (PIP, 30 μg/disk), Polymyxin B (PB, 100 IU/disk), Spectinomycin (SPC, 100 μg/disk), Streptomycin (S, 10 μg/disk), Sulfamethoxazole (MX, 100 μg/disk), and Trimethoprim (TM, 5 μg/disk). The disks were placed evenly on the agar surface using sterile forceps and incubated at 37 °C for 18 hours[4]. The antibiotic susceptibility results were determined by measuring the diameter of the inhibition zone, which is the transparent area surrounding the antibiotic disk.

Detection of toxin genes of B. licheniformis and B. subtilis in yam-derived products. DNA extraction method. Isolated *B. licheniformis* and *B. subtilis* strains were cultured in BHI (Brain Heart Infusion broth) broth and incubated for 18–24 hours at 37 °C. Total DNA of *B. licheniformis* and *B. subtilis* was extracted according to the protocol for gram-positive bacteria of the GeneJET Genomic DNA Purification kit (Thermofisher; C5042). The total DNA concentration of *B. licheniformis* and *B. subtilis* strains was quantified using a nanodrop spectrophotometer at 260 nm. The DNA solution was stored at -20 °C until use.

PCR and multiplex PCR reactions. Primer pairs used for detecting toxin genes of *B. licheniformis* and *B. subtilis* in yam-derived products are listed in Table 2. Detection of toxin genes in *B. licheniformis* was performed using species-specific primers targeting *licA, licB,* and *licC*, which encode components of the lichenysin synthetase complex [13]. To evaluate potential pathogenicity of *B. subtilis*, isolates were screened for the presence of diarrheal toxin genes typically found in *B. cereus*, including the *nhe, hbl,* and *cytK* gene clusters [14]. Conventional PCR was used for amplification of *licA, licB, licC,* and *cytK* genes, while multiplex PCR was applied for detecting the remaining enterotoxin genes (*nheA, nheB, nheC, hblA, hblC, hblD*).

Results and discussion. ***Microbial contamination rates in purple yam and yam-derived products.*** The analysis results revealed that microbial contamination was notably higher in processed purple yam powder products compared to fresh yam tubers harvested from the field. Coliforms were detected only on peel of fresh tubers (up to $2.1 \cdot 10^5$ CFU/g) but not in the flesh, whereas only one powdered sample showed minimal coliform presence ($2.9 \cdot 10^2$ CFU/g), likely due to post-processing contamination (Tables 3 and 4).

Common foodborne pathogens (*B. cereus, E. coli, S. aureus, Salmonella spp.*) were absent in all samples. However, yeast and mold counts were significantly elevated in powdered samples (ranging from $3.8 \cdot 10^1$ to $5.0 \cdot 10^2$ CFU/g) compared to field samples. Likewise, aerobic bacteria were abundant in powdered products (up to $6.8 \cdot 10^6$ CFU/g), whereas the flesh of raw yams showed much lower levels (as low as $4.0 \cdot 10^1$ CFU/g) (Tables 3, 4). Notably, *B. licheniformis* was detected in 5 out of 7 powdered samples, while *B. subtilis* was found in 2, but neither species was present in raw tubers thus suggesting contamination

[4] Biemer J.J. Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. *Ann. Clin. Lab. Sci. (1971)*, 1973, vol. 3, no. 2, pp. 135–140.

Table 2

Primer pairs used for detecting toxin genes of *B. licheniformis* and *B. subtilis*

Name	Sequence (5'-3')	Gene	Size (bp)	Reference
nheAF	TAC GCT AAG GAG GGG C	*nheA*	499	[14]
nheAR	GTT TTT ATT GCT TCA TCG GCT			
nheBF	CTA TCA GCA CTT ATG GCA G	*nheB*	769	
nheBR	ACT CCT AGC GGT GTT CC			
nheCF	CGG TAG TGA TTG CTG GG	*nheC*	581	
nheCR	CAG CAT TCG TAC TTG CCA A			
hblAF	GTG CAG ATG TTG ATG CCG AT	*hblA*	1154	
hblAR	ATG CCA CTG CCT GGA CAT A			
HbICF	GAT ACT AAT GTG GCA ACT GC	*hblC*	740	
HbICR	TTG AGA CTG CTC GTT AGT TG			
HbIDF	AAT CAA GAG CTG TCA CGA AT	*hblD*	829	
HbIDR	CAC CAA TTG ACC ATG CTA AT			
*cytK*F	CGA CGT CAC AAG TTG TAA CA	*cytK*	565	
*cytK*R	CGT GTG TAA ATA CCC CAG TT			
*LicA*F	GTGCCTGATGTAACGAATG	*licA*	735	[13]
*LicA*R	CACTTCCTGCCATATACC			
*LicB*2F	TGATCAGCCGGCCGTTGTCT	*licB*	904	
*LicB*2R	GGCGAATTGTCCGATCATGTCC			
*LicC*F	GCCTATCTGCCGATTGAC	*licC*	1195	
*LicC*R	TATATGCATCCGGCACCA			

Table 3

Microbial contamination rates of purple yams harvested from the field (CFU/g)

№	Criteria	Peel of white-fleshed purple yam	Peel of purple-fleshed purple yam	Flesh of white-fleshed purple yam	Flesh of purple-fleshed purple yam
1	*Coliform*	$2.1 \cdot 10^5$	$2.9 \cdot 10^3$	N/D	N/D
2	*B.cereus*	N/D	N/D	N/D	N/D
3	*E.coli*	N/D	N/D	N/D	N/D
4	*S.aureus*	N/D	N/D	N/D	N/D
5	*Samonella* spp.	N/D	N/D	N/D	N/D
6	Yeast and mold	$5 \cdot 10^4$	$2 \cdot 10^4$	N/D	$1 \cdot 10^1$
7	Total aerobic microorganisms	$6.1 \cdot 10^6$	$7.7 \cdot 10^6$	$4 \cdot 10^1$	$7 \cdot 10^1$
8	*B. licheniformis*	N/D	N/D	N/D	N/D
9	*B. subtilis*	N/D	N/D	N/D	N/D

Note: N/D – not detected.

Table 4

Microbial contamination rates of purple yam powder purchased from local stores (CFU/g)

№	Criteria	1	2	3	4	5	6	7
1	*Coliform*	$2.9 \cdot 10^2$	N/D	N/D	N/D	N/D	N/D	N/D
2	*B.cereus*	N/D	N/D	N/D	N/D	N/D	N/D	N/D
3	*E.coli*	N/D	N/D	N/D	N/D	N/D	N/D	N/D
4	*S.aureus*	N/D	N/D	N/D	N/D	N/D	N/D	N/D
5	*Samonella* spp.	N/D	N/D	N/D	N/D	N/D	N/D	N/D
6	Yeast and mold	$1.6 \cdot 10^2$	$1.6 \cdot 10^2$	$3.8 \cdot 10^1$	$4.0 \cdot 10^2$	$4.2 \cdot 10^2$	$3.0 \cdot 10^1$	$5.0 \cdot 10^2$
7	Total aerobic microorganisms	$3.8 \cdot 10^6$	$9.0 \cdot 10^5$	$3.4 \cdot 10^6$	$7.4 \cdot 10^4$	$1.1 \cdot 10^5$	$6.8 \cdot 10^6$	$2.7 \cdot 10^5$
8	*B. licheniformis*	$2.1 \cdot 10^3$	$4 \cdot 10^2$	$9.1 \cdot 10^1$	$2 \cdot 10^3$	$4 \cdot 10^3$	N/D	N/D
9	*B. subtilis*	N/D	N/D	N/D	N/D	N/D	$3.0 \cdot 10^1$	$4.0 \cdot 10^1$

Note: N/D – not detected.

likely occurred during processing or storage. These findings also highlight a regulatory gap in microbiological assessment: current national microbiological criteria (QCVN 8-3:2012/BYT) do not provide specific limits for root vegetables like purple yam, making it difficult to evaluate compliance or safety[5]. This lack of clear standards poses challenges for food hygiene control and consumer protection, especially for traditional products without established benchmarks. From the powdered yam samples, a total of 10 *B. licheniformis* and 7 *B. subtilis* representative strains were selected for further analyses.

Isolation and identification of *B. licheniformis* and *B. subtilis* in yam-derived products. ***Identification by MALDI-TOF mass spectrometry.*** A total of 17 bacterial colonies isolated from purple yam powder samples were initially identified using MALDI-TOF mass spectrometry. All isolates achieved MS scores above 2.0, enabling reliable identification at the species level. Among them, 10 isolates were identified as *B. licheniformis* and 7 as *B. subtilis*. These preliminary identifications were subsequently confirmed through species-specific PCR assays targeting marker genes unique to *B. licheniformis* and *B. subtilis* ensuring accuracy at the DNA level.

Confirmation by specific DNA markers. All 10 isolates (lanes 1–10) exhibited clear and distinct amplicons corresponding to the expected sizes of each primer pair: *ligD* (216 bp), *rfaB* (376 bp), *BL00303* (332 bp), and *serA* (247 bp) with the exception of isolate number 9, which failed to yield a PCR product for the *rfaB* gene.

Absent amplification in isolate 9 for *rfaB*, while maintaining positive signals for the other three genes, suggests the possibility of a sequence variation at the primer binding site or a deletion of the *rfaB* gene in this particular strain. Nevertheless, the detection of three out of four species-specific markers strongly supports the identification of isolate 9 as *B. licheniformis*. These results collectively confirm the identity of the isolates at the DNA level and are consistent with the MALDI-TOF identification results.

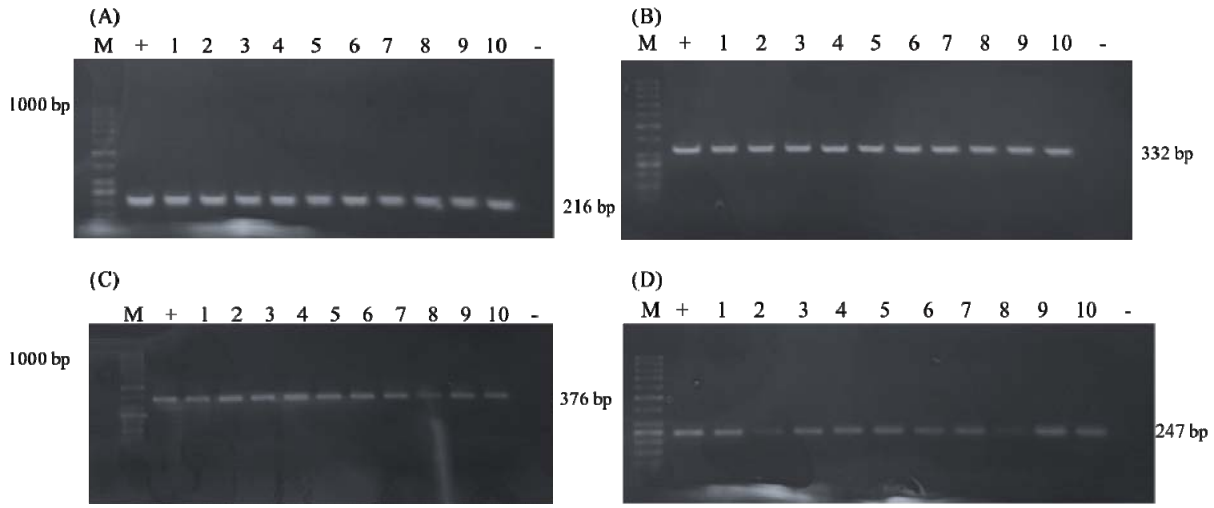

Figure 1. PCR product electrophoresis of the 4 *B. licheniformis*-specific DNA markers: (A) *ligD*, (B) *BL00303*, (C) *rfaB* and (D) *serA* (The PCR products *ligD*, *BL00303*, *rfaB*, and *serA* sequence sizes are 216, 332, 376, and 247 bp, respectively.
M: DNA ladder 50 bp; (+): Positive control; (-): Negative control; 1–10: isolated *B. licheniformis* strains)

[5] Giới hạn ô nhiễm vi sinh vật trong thực phẩm theo QCVN 8-3:2012/BYT. *Thư Viện Pháp Luật*. Available at: https://thuvienphapluat.vn/chinh-sach-phap-luat-moi/vn/ho-tro-phap-luat/tu-van-phap-luat/58299/gioi-han-o-nhiem-vi-sinh-vat-trong-thuc-pham-theo-qcvn-8-3-2012-byt (August 12, 2025).

Figure 2. PCR product electrophoresis of the *B. subtilis*-specific DNA markers (M: DNA ladder 50 bp; (-): Negative control; 1–10: isolated *B. licheniformis* strains)

As shown in Figure 2, all tested samples (lines 1–10) exhibited clear amplification bands at the expected size, consistent with the DNA ladder (M), while the negative control (–) showed no amplification, confirming the absence of contamination in the PCR assay.

These results indicate that all tested isolates possess the species-specific sequence for *B. subtilis* further corroborating the protein-level identification previously obtained using MALDI-TOF mass spectrometry. These strains were subsequently subjected to antibiotic susceptibility testing to assess their resistance profiles.

Antibiotic resistance profiles of *B. licheniformis* and *B. subtilis* strains isolated from purple yam powder products. Antibiotic resistance profiles of *B. licheniformis* are provided in Figure 3.

The antibiotic resistance patterns of 11 *B. licheniformis* strains isolated from purple yam powder were evaluated using the Kirby – Bauer disk diffusion method against 28 antibiotics from 14 antimicrobial classes. All strains demonstrated complete resistance (100 %) to eight antibiotics, including aztreonam, amoxicillin-clavulanic acid, cefotaxime, erythromycin, nalidixic acid, methicillin, ceftazidime, and polymyxin B revealing a concerning broad-spectrum resistance profile. Notably, resistance extended to several antibiotics primarily targeting gram-positive bacteria: high resistance rates were observed for β-lactams (methicillin 100 %, penicillin 70 %, cefuroxime 60 %) and erythromycin (100 %), suggesting the potential presence of mecA genes encoding altered PBPs or erm genes conferring macrolide resistance [15]. Additionally, streptomycin resistance (80 %) may indicate aminoglycoside-modifying enzyme production. The concurrent resistance to both gram-positive-targeting drugs (e.g., β-lactams) and gram-negative-specific agents (e.g., aztreonam) suggests accumulation of multiple resistance mechanisms, possibly through horizontal gene transfer in agricultural environments. The

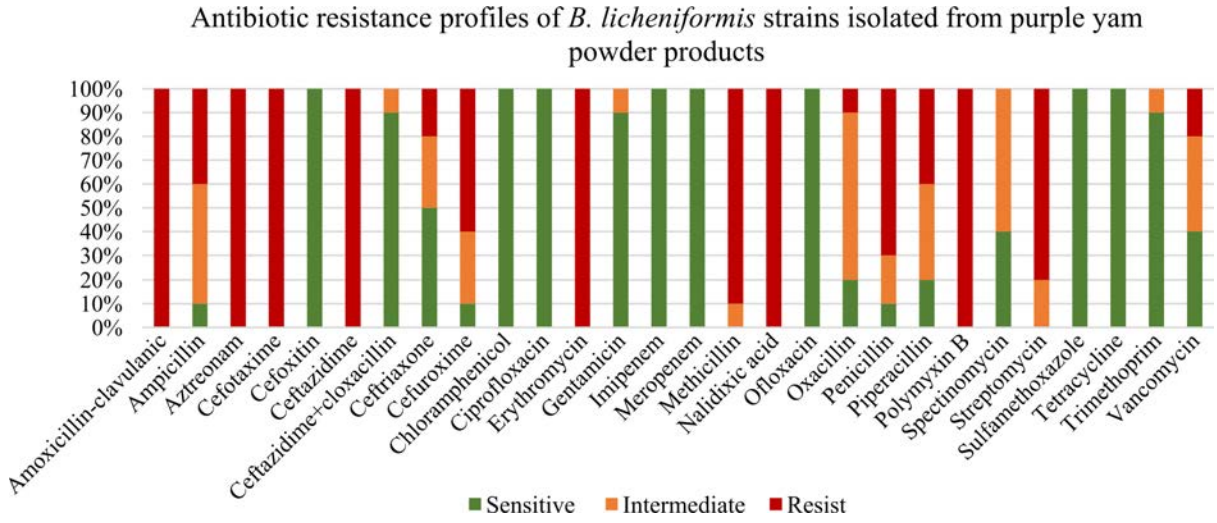

Figure 3. Antibiotic resistance profile of *B. licheniformis* based on inhibition zone diameter (mm); red: resistant, orange: intermediate, green: susceptible.

universal susceptibility to imipenem, ciprofloxacin, and chloramphenicol is consistent with CLSI benchmarks for Bacillus (CLSI, 2023) and corresponds with observations by Ozkocaman et al. (2006) and Banerjee et al. (2007), though the preserved carbapenem sensitivity is noteworthy given the rising global resistance to this drug class [16, 17]. Of particular significance were the unexpected susceptibility patterns observed for spectinomycin (40 % susceptible), sulfamethoxazole (100 %), and trimethoprim (90 %). This atypical sensitivity may be explained by: (1) absence of aadA resistance genes combined with unique membrane permeability characteristics enabling spectinomycin access to its ribosomal target (J. Davies & G.D. Wright, 1997)[6] and (2) obligate dependence on endogenous folate synthesis pathways due to deficient exogenous folate uptake mechanisms (P. Huovinen et al., 1995)[7]. The strain-specific variation in spectinomycin susceptibility (40 % sensitive vs 60 % resistant) may reflect differential expression of ribosomal RNA methyltransferases, while the universal trimethoprim sensitivity contrasts with typical dfr gene prevalence in soil bacilli, warranting further genomic investigation. These findings underscore the importance of regional antimicrobial resistance surveillance, particularly for foodborne *Bacillus* species that may serve as reservoirs for resistance gene dissemination.

Antibiotic resistance profiles of *B. subtilis* are provided in Figure 4.

The antibiotic susceptibility testing of 7 *B. subtilis* strains demonstrated complete sensitivity (100 %) to chloramphenicol, ciprofloxacin, erythromycin, carbapenems (imipenem, meropenem), gentamicin, amoxicillin-clavulanate, cefoxitin, nalidixic acid, ofloxacin, and oxacillin indicating that these drugs remain effective treatment options. However, concerning resistance patterns were observed, including universal resistance (100 %) to ampicillin, aztreonam, third-generation cephalosporins (ceftazidime, ceftriaxone, cefotaxime),

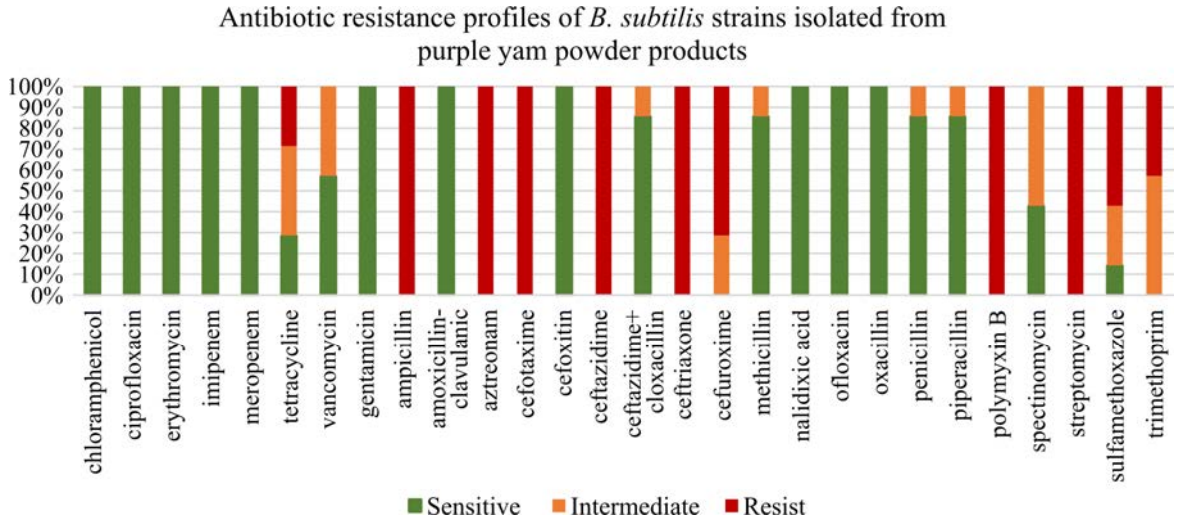

Figure 4. Antibiotic resistance profile of B. subtilis based on inhibition zone diameter (mm); red: resistant, orange: intermediate, green: susceptible.

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polymyxin B, and streptomycin, consistent with both intrinsic and acquired resistance mechanisms. Striking restoration of susceptibility to ceftazidime when combined with cloxacillin (86 % sensitive versus 0 % for ceftazidime alone) demonstrates β-lactamase-mediated resistance can be the predominant mechanism [18]. Of particular clinical significance is the intermediate vancomycin susceptibility in 43 % of strains, potentially indicating emerging resistance through peptidoglycan precursor modification (e.g., D-Ala-D-Lac substitution) [19]. The strains exhibited variable resistance to tetracycline (29 % resistant, 43 % intermediate) and spectinomycin (57 % intermediate) suggesting heterogeneous distribution of *tet* (M/O) and *spcN* resistance determinants. These findings underscore the importance of ongoing surveillance of foodborne *B. subtilis* strains.

Presence of toxin genes in *B. licheniformis* and *B. subtilis* strains isolated from purple yam powder products. Our results showed that all 10 *B. licheniformis* strains isolated from purple yam powder products carried all three toxin genes *licA*, *licB*, and *licC* (Figure 5). This 100 % detection rate suggests high prevalence of the lichenysin biosynthetic gene cluster among food-derived *B. licheniformis* strains in Vietnam.

These findings are consistent with those of Madslien et al. (2013), who reported that all 53 *B. licheniformis* strains isolated from food and environmental sources carried the *lchAA* gene (homologous to *licA*), which encodes lichenysin synthetase A [20]. Additionally, another study confirmed the presence of *licA*, *licB*, and *licC* genes in *B. licheniformis* strains isolated from bovine mastitis milk samples in Iran indicating that the distribution of these genes is not limited to food but also extends to animal sources [13]. The prevalence of the *licABC* gene cluster observed in our study is comparable to or even higher than previously reported levels highlighting a potential food safety concern associated with purple yam powder products in Vietnam.

All *B. subtilis* strains isolated from purple yam powder samples in this study tested negative for three major enterotoxin gene groups commonly associated with foodborne illness, namely, *nhe*, *hbl*, and *cytK* genes (Figure 6).

These results suggest that the *B. subtilis* strains do not harbor key virulence factors and are unlikely to cause diarrheal illness in humans. This finding is consistent with the study

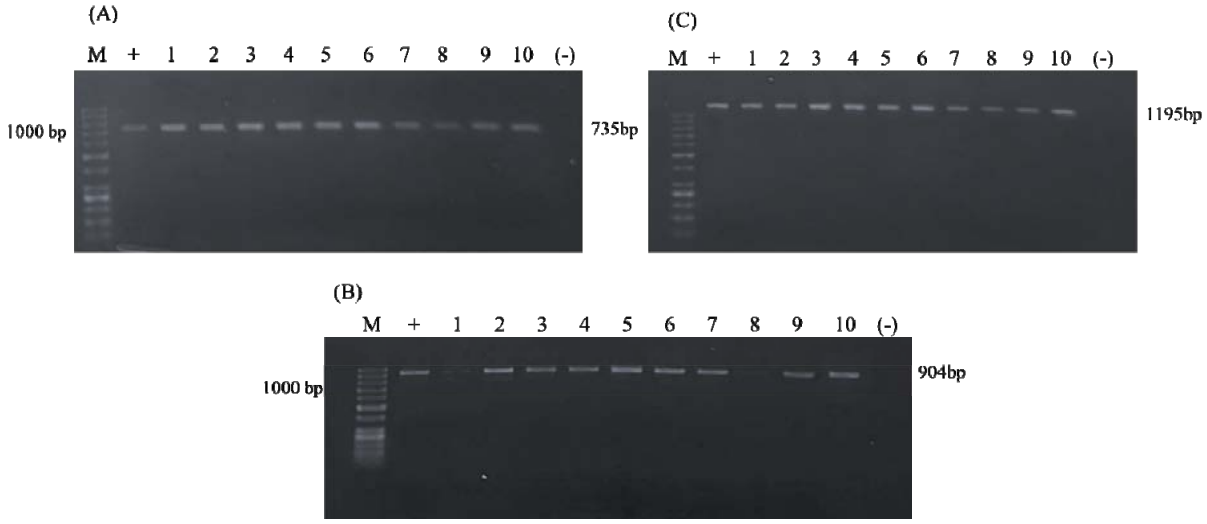
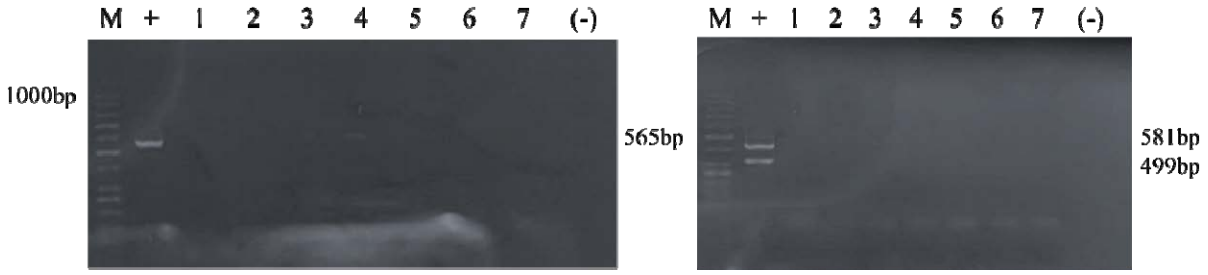


Figure 5. PCR product electrophoresis of the 3 *B. licheniformis* toxin genes: (A) *licA*, (B) *licB*, and (C) *licC* (the PCR products *licA*, *licB* and *licC* sequence sizes are 735, 904, and 1195 bp, respectively; M: DNA ladder 50 bp; (+): positive control; (-): Negative control; 1–10: isolated *B. licheniformis* strains)



A B

Figure 6. PCR product electrophoresis of (A) *cytK*, (B) *hbl* and *nhe* toxin genes (M: DNA ladder 50 bp; (-): Negative control; 1–7: isolated *B. subtilis* strains)

by Ceuppens et al. (2013), which reported that most *B. subtilis* strains isolated from food lacked these toxin genes and rarely produced toxins similar to those found in *B. cereus* [21]. Likewise, Logan (2011) noted that *B. subtilis* poses a very low risk of foodborne illness due to the general absence of major enterotoxin genes. Although the presence of *B. subtilis* in food products may indicate suboptimal hygiene during production, the current results suggest that this species does not constitute a significant virulence threat in purple yam powder products.

Conclusion. This study revealed a higher microbial load in commercially available yam powder compared to freshly harvested yam, with notable detection of *B. licheniformis* and *B. subtilis* only in the powdered samples. All *B. licheniformis* strains carried the licABC toxin gene cluster and showed multi-drug resistance to critical antibiotics, which can be considered a risk factor for consumers' health.

Meanwhile, *B. subtilis* strains lacked diarrheagenic toxin genes and posed lower virulence risk. These findings raise concerns over the microbial safety of yam-derived products, particularly under current regulatory gaps in Vietnam, where microbiological criteria specific to yam are not yet defined. The study results underscore the urgent need to establish national microbial standards for yam-based products and to strengthen hygienic control measures during processing, especially for small-scale and household-level production, to ensure food safety for Vietnamese consumers.

Funding. The authors express their deepest gratitude for financial support provided by the National Institute for Food Control, Vietnam.

Competing interests. The authors declare no competing interests.

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Received: 16.06.2025
Approved: 30.08.2025
Accepted for publication: 26.09.2025