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Prevalence of Antibiotic-resistant *Salmonella* in Vegetables and Fermented Foods and their Control by Lactic Acid Bacteria

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Abstract

Salmonella is the leading cause of foodborne disease and a public health concern. In this study, food samples were collected from fresh markets within northeastern Thailand. Antibiotic-resistant *Salmonella* were detected in fresh vegetables and fermented meat products. Of 172 collected food samples, 25 (14.53%) were contaminated with *Salmonella* resistant to at least 1 of 9 antibiotics. Eight strains of 7 serovars, including *S.* Derby, *S.* Paratyphi B, *S.* Stanley, *S.* Dabou, *S.* Rissen, *S.* Hvittingfoss, and *S.* Brenderup, were resistant to S, TE, CN, SXT, and NA, accounting for 20%, 16%, 12%, 12%, and 12% of all antibiotics, respectively. To determine the anti-*Salmonella* activity of lactic acid bacteria, *Pediococcus pentosaceus* PS34 isolated from fermented fish was selected from 5 strains of food-derived lactic acid bacteria according to the antibacterial and acid-resistance properties. When added to 3 log CFU/ml cultures of *Salmonella*, the PS34 cell-free supernatant (CFS) reduced the numbers of *Salmonella* after 1, 15, 30, and 60 minutes of incubation; the reduction reached 99.9% within 30 minutes. The minimal arbitrary inhibition concentration of freeze-dried PS34 CFS was a 1:4 dilution. With the exception of neutralization to pH 6.5, treatment with proteolytic enzymes and heating at 100°C and 120°C did not reduce the freeze-dried PS34 CFS anti-*Salmonella* activity, indicating that the extracellular PS34 bioactive substances were heat stable and highly effective against *Salmonella in vitro*. Thus, bioactive substances produced by *P. pentosaceus* PS34 have the potential to reduce the risk of antibiotic-resistant *Salmonella* from contaminated food reaching consumers.

Keywords: Antibiotic-resistant *Salmonella*, *Pediococcus pentosaceus*, bioactive substance, fresh vegetable, fermented food.

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INTRODUCTION

Salmonella is a leading cause of foodborne disease and a public health concern worldwide. In humans and animals, *Salmonella* infection causes high morbidity and mortality rates as well as large economic losses[1]. The source of infection is mainly cross-contamination from animal faces and affects a variety of foods, such as poultry, pork, beef, fish, seafood, eggs, milk, cheese, fresh products, and fruit[2-4]. *Salmonella* is becoming increasingly resistant to various antibiotics via resistance genes against 3rd- and 4th-generation beta-lactams[5], which is becoming a serious problem in Thailand and many countries[6]. The inappropriate uses of antibiotics in animal production is the main reason for antimicrobial resistance in *Salmonella,* increasing the virulence and invasiveness of strains, making the treatment of the associated infections increasingly difficult and leading to prolonged illnesses[7,8]. In Thailand, *Salmonella* is present in food products at high levels due to improper hygiene practices during handling and food processing[9]. However, there is still limited information on the incidence of antibiotic-resistant *Salmonella* in food products[10].

In recent years, people have begun to demand natural foods without chemical preservatives[11] due to health concerns regarding chemical food additives[12]. There is increasing interest in the use of natural antimicrobials for food protection and safety that can control both spoilage and pathogenic microorganisms[12,13]. Thus, the use of natural antimicrobials derived from probiotics has become appealing[14]. Many probiotics are lactic acid bacteria (LAB) mainly comprised of *Bifidobacterium* and *Lactobacillus*[15]. Other species, such as *Pediococcus acidilactici* and *Pediococcus pentosaceus*[16], also have probiotic properties. In addition to organic acids, probiotics produce a variety of antimicrobial compounds, such as hydrogen peroxide, carbon dioxide, diacetyl, acetaldehyde, D-isomer amino acids, biosurfactants, bacteriocin, and reuterin[13].

In a previous study, several LAB strains were isolated from various traditional Thai fermented foods and Mekong freshwater fish. Among these, *P. pentosaceus* PS34 showed the greatest potential probiotic properties, producing many antimicrobial peptides, proteolytic and lipolytic enzymes and several health-benefit-associated substances. These strains also inhibited the growth of the standard *S.* Typhimurium DMST 562 (ATCC13311) strain[17]. Thus, the analysis of the ability of potential LAB strains to inhibit the spread of antibiotic-resistant *Salmonella* serovars without causing toxicity to food and the environment has gained our attention. The aims of this study were to determine the prevalence of antibiotic-resistant *Salmonella* in fermented foods and vegetables and to evaluate the antimicrobial activity of the selected probiotic, *P. pentosaceus* PS34, against antibiotic-resistant *Salmonella* isolated from food.

MATERIALS AND METHODS

Bacterial strains

Salmonella Typhimurium DMST562 is a standard strain. The antibiotic-resistant *Salmonella* isolates used in this study originated from fermented foods and vegetables. Five LAB strains of *Pediococcus pentosaceus* PS34, NP6, BD33, CF32 and SW5, were isolated from fermented fish, fermented pork, Mae Kong river fish[18].

Collection of food samples

A total of 172 food samples, including 100 vegetables and 72 Thai fermented foods (pla-som and nham), were purchased from fresh markets in Khon Kaen, Kalasin, Roiet and Mahasarakham provinces, Northeastern Thailand. After collection, the samples were tightly sealed and kept in plastic bags and chilled at 5-8°C in a delivery cooler box and analyzed in the laboratory within 24 h.

Isolation and identification of *Salmonella*

The isolation and identification of *Salmonella* was performed according to ISO 6579:2002. Briefly, 25 g of each food sample was added to 225 ml of buffered peptone water (BPW; Oxoid, Basingstoke, Hampshire, UK) and mixed using a stomacher for 2 minutes. The samples were pre-enriched at 37°C for 18 ± 2 h. The cultures obtained from the pre-enrichment were subcultured in two selective enrichment broths: Rappaport-Vassiliadis medium with soy and a Müllere-Kauffmann tetrathionate broth (Oxoid), and incubated for 24 ± 3 h at 41.5 ± 0.5°C and 37 ± 1°C, respectively. The enriched cultures were streaked on the XLD and Hextoen (Oxoid) selective plating media and incubated at 37°C for 24 h. *Salmonella* colonies were confirmed by biochemical tests using triple iron agar (TSI), motile indole lysine agar (MIL), urea agar and indole

tests. The presence of the invasion-associated gene *iag*A in presumptive colonies displaying typical *Salmonella* biochemical properties was confirmed by an iQ-Check *Salmonella* II real-time PCR detection kit (Bio-Rad) according to the manufacturer's instructions. All *Salmonella* isolates were stored at -80°C in 20% glycerol until further examination. Presumptive *Salmonella* colonies were further confirmed by serological tests. The serological somatic O and H antigen tests for *Salmonella* were determined by the Kauffmann-White method[19] using commercial antisera (S&A, Thailand).

Screening of antibiotic-resistant *Salmonella* by disc diffusion

The antibiotic susceptibility was determined on Muller-Hinton agar using the standard disc diffusion method, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI; Clinical and Laboratory Standards Institute, 2012). A total of 172 *Salmonella* isolates were screened with nine antibiotic discs (Oxoid, UK), including gentamycin 10 μg (CN), tetracycline 30 μg (TE), ciprofloxacin 5 μg (CIP), sulfamethoxazole-trimethoprim 25 μg (SXT), nalidixic acid 30 μg (NA), sulfonamides 300 μg (S), cephalothin 30 μg (KF), neomycin 30 μg (N), and nitrofurantoin 300 μg (F). The plates were incubated overnight at 37°C and 30°C. The

diameters of the inhibition zones were measured and compared with CLSI standards (CLSI, 2017). Quality control tests were performed using *E. coli* ATCC 25922 and *S. aureus* ATCC 259223 as standard strains. The experiment was carried out in duplicate.

In vitro* antimicrobial activity of *P. pentosaceus* BD33, CF32, NP6, PS34 and SW5 LAB crude cell-free supernatant (CFS) against antibiotic-resistant *Salmonella

Preparation of crude CFS

One loop full of fresh culture of each of the 5 LAB strains was cultured in 10 ml of MRS agar at 35°C for 24 h and subcultured with 1% of LAB culture in 100 ml of MRS at 35°C for 24 h. After incubation, the CFS filtrate was obtained by centrifugation at 12,000 x g for 15 minutes. The supernatant pH was measured, and the supernatant was sterilized by filtration through a 0.2 μm pore size filter. The antimicrobial activity of the untreated CFS (pH 4.0) and CFS (pH 6.5) neutralized with 1N NaOH was tested.

Antimicrobial activity determination by agar well diffusion

The antimicrobial activity of CFS derived from *P. pentosaceus* BD33, CF32, NP6, PS34 and SW5 was tested against 8 *Salmonella* isolates representing 7 antibiotic-resistant serovars, including *S.* Paratyphi B, *S.* Hvittingfoss, *S.* Stanley,

Table 1. Distribution of *Salmonella* serovars in vegetables and fermented pork and fish

Serogroup (Total)	Serovar	Vegetable	Fermented pork	Fermented fish	Total serotypes
B (n=3)	*S.* Derby	1	0	0	1
	S. Paratyphi B	1	0	0	1
	S. Stanley	0	2	1	3
C (n=8)	*S.* Brenderup	0	1	0	1
	S. Brunei	3	0	1	4
	S. Choleraesuis	1	0	0	1
	S. Dabou	1	0	0	1
	S. Newport	1	0	0	1
	S. Rissen	1	1	0	2
	S. Tennessee	0	0	2	2
	S. Virchow	1	0	0	1
E (n=1)	*S.* Weltevreden	1	0	1	2
I (n=1)	*S.* Hvittingfoss	3	0	0	3
X (n=1)	*S.* Bergen	1	0	0	1
Y (n=1)	*S.* Djakarta	1	0	0	1
Total positive samples		16	4	5	25
Percentage		64	16	20	100

S. Brenderup, *S*. Rissen, *S*. Dabou, and *S*. Derby. A 24 h culture grown in LB at 37°C was adjusted to 0.5 McFarland standards, swabbed on LB agar plates with a sterile cotton swab, allowed to dry, and a hole was punched in the agar with a sterile 6 mm cork-borer. Each well was filled with 60 µl of CFS obtained from the *P. pentosaceus* isolates. After incubation at 37°C for 24, 36 and 48 h, the inhibition zones around the well were measured. A 0.8% lactic acid solution and MRS broth were used as positive and negative controls, respectively. The results were considered positive if the diameter of the inhibition zone was greater than 10 mm. The experiment was carried out in triplicate.

Antimicrobial activity determination by broth method

Eight strains of antibiotic-resistant *Salmonella* were grown in LB broth at 37°C for 24 h. Serial dilutions were performed in LB broth to obtain approximate cell concentrations at 3 Log CFU/ml, and 5 ml of CFS was added. After incubation for 1, 15, 30, and 60 minutes, samples
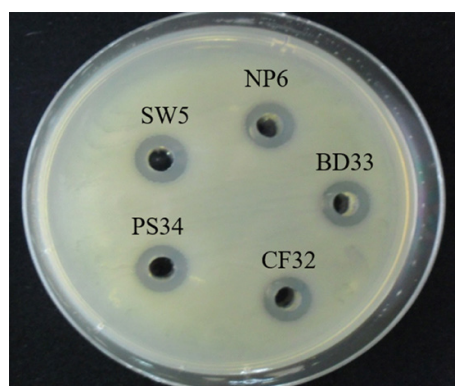

Fig. 1. Inhibitory effect of untreated CFS from overnight cultures of 5 strains of *P. pentosaceus* in MRS broth at pH 4.0 on *S.* Typhimurium DMST562

were taken, and *Salmonella* survival was assessed by plating the samples on LB agar plates and counting the colonies after incubation at 37°C for 24 h. *S.* Typhimurium DMST562 and sterilized distilled water were used as positive and negative controls, respectively. The number of surviving *Salmonella* was calculated as follows:

$$\% \text{ Reduction} = (1-10^{-L}) \times 100, \ L = \text{Log reduction}$$
$$\% \text{ Survival} = 100 - (1-10^{-L}) \times 100$$

In vitro* antimicrobial activity of lyophilized and concentrated PS34 CFS against antibiotic-resistant *Salmonella

One loop full of fresh culture of the 8 LAB strains was cultured in 10 ml of MRS at 35°C for 24 h and subcultured with 1% (v/v) of LAB culture in 100 ml MRS at 35°C for 24 h. The crude P34 CFS was concentrated and preserved by the freeze-drying method. The freeze-dried CFS was resuspended in 5 ml of sterile distilled water and filtered through a 0.2 µm cellulose acetate filter. The optimal inhibitory activity of the CFS against *Salmonella* was assessed. The CFS was serially diluted to 1:1, 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64 ratios in MRS broth, and 10 µl of each was dropped on an LB agar. The inhibition zone was measured. The experiment was carried out in triplicate.

Effects of proteolytic enzymes, pH and temperature on the antimicrobial activity of the CFS

The lyophilized CFS was treated with different conditions as follows: heat by boiling at 100°C for 30 minutes and autoclaving at 121°C for 15 minutes; treated with trypsin, pepsin, proteinase K at a final concentration of 1 mg/ml, and the pH was adjusted to 6.5 using 1 M NaOH. MRS broth was used as the negative control. Then, the antimicrobial activity of the treated CFS was

Table 2. Antibiotic resistance profiles of *Salmonella* isolated from vegetables and fermented meats

Salmonella	Food type	Food origin	Antibiotic-resistance profiles
S. Paratyphi B	Vegetable	Lettuce	CN
S. Dabou	Vegetable	Parsley	NA
S. Hvittingfoss	Vegetable	Water cress	NA, CN
S. Rissen	Vegetable	Lettuce	TE, S, SXT
S. Derby	Vegetable	Coriander	NA, TE, S, SXT
S. Brenderup	Fermented pork	Nham	S
S. Stanley (P)	Fermented pork	Nham	TE, CN
S. Stanley (F)	Fermented fish	Pla-som	TE, S, SXT

tested against *Salmonella* by drop diffusion as described above.

RESULTS

Prevalence and serotyping of antibiotic-resistant *Salmonella* isolated from vegetables and fermented foods

Salmonella were detected in 25 of all 172 samples (14.53%). The isolates were derived from 16 of 100 vegetables (16%) and 9 of 72 fermented meat samples (12.5%). All *Salmonella* isolates expressed the invasion-associated gene *iag*A, as assessed by an iQ-Check *Salmonella* II real-time PCR detection kit. In this study, 25 *Salmonella* isolates were classified into 6 serogroups and 15 serovars. The type and numbers of the serogroups were as follows: B-3, C-8, E-1, I-1, X-1 and Y-1, in which serogroup C (32%) was the most abundant. Antibiotic-resistant *Salmonella* were detected in 64%, 20%, and 16% of vegetable, fermented fish, and fermented pork samples, respectively (Table 1).

Antimicrobial susceptibility of antibiotic-resistant *Salmonella* isolates

In total, the antimicrobial susceptibility of 25 isolates covering 15 serovars was tested against 9 antimicrobial agents. Resistant *Salmonella* isolates were categorized into 8 serovars (*S.* Brenderup *S.* Dabou, *S.* Derby, *S.* Hvittingfoss, *S.* Paratyphi B*, S.* Rissen, *S.* Stanley and *S.* Tennessee).
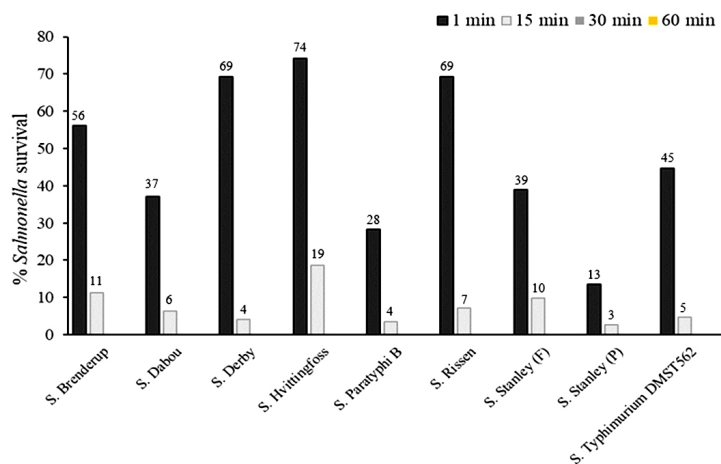

Fig. 2. Survival of antibiotic-resistant *Salmonella* serovars after contact with crude *P. pentosaceus* PS34 CFS for 1, 15, 30 and 60 minutes.

Table 3. Antibiotic-resistance percentages of *Salmonella* isolated from vegetables and fermented meats

Antibiotics (µg)		No. of *Salmonella* isolates (%) (n=25)		
		S	I	R
Cephalothin (30)	KF	25 (100)	0 (0)	0 (0)
Ciprofloxacin (5)	CIP	25 (100)	0 (0)	0 (0)
Gentamycin (10)	CN	22 (88)	0 (0)	3 (12)
Nalidixic acid (30)	NA	20 (80)	2 (0)	3 (12)
Neomycin (30)	N	0 (0)	25 (100)	0 (0)
Nitrofurantoin (300)	F	25 (100)	0 (0)	0 (0)
Sulfonamides (300)	S	17 (68)	3 (12)	5 (20)
Sulfamethoxazole-Trimethoprim (25)	SXT	22 (88)	0 (0)	3 (12)
Tetracycline (30)	TE	20 (80)	1 (4)	4 (16)

S: Susceptible; I: Intermediate; R: Resistant

These resistant *Salmonella* isolates were resistant to at least one antibiotic. The percentages of strains resistant to sulfonamides (300 µg), tetracycline (30 µg), gentamicin (30 µg), nalidixic acid (30 µg), and sulfamethoxazole (25 µg) were 20%, 16%, 12%, 12%, and 12%, respectively. None of the isolates were found to be resistant to ciprofloxacin (50 µg), cephalothin (30 µg), neomycin (30 µg) or nitrofurantoin (300 µg), as shown in Tables 2 & 3.

Antimicrobial activity of LAB strains against antibiotic-resistant *Salmonella*

Due to their potential probiotic properties, including susceptibility to many antibiotics, inhibition of Gram positive and negative bacterial pathogens, and tolerance to low pH in the presence of inulin (a prebiotic)[17,18], the antimicrobial activity of crude *P. pentosaceus* BD33, CF32, NP6, PS34 and SW5 CFS was investigated. The CFS of all *P. pentosaceus* strains was prepared as untreated (pH 4) and neutralized (pH 6.5) and tested against *Salmonella* using the agar well diffusion

Fig. 3. Minimal arbitrary inhibition concentration of freeze-dried PS34 CFS against *S.* Typhimurium DMST562 (A undiluted, B 1:1, C 1:2, D 1:4, E 1:8, F 1:16, G 1:32, and H 1:64).

method. The untreated CFS of all strains showed an inhibition zone of approximately 12.3 mm against *S.* Typhimurium after 24 h of incubation (Fig. 1). However, no inhibition zone was detected for neutralized CFS (pH 6.5). PS34 exhibited the greatest tolerance in the presence of inulin to pH 2 after 24 h[18], therefore PS34 CFS samples were tested against 8 isolates of antibiotic-resistant *Salmonella* and *S.* Typhimurium DMST562. The results showed that after prolonged incubation for 36 and 48 h, the inhibition zone increased slightly (Table 4). The 0.8% lactic acid control showed an inhibition zone of 11.6 mm, and the zone size was not altered after prolonged incubation. Generally, LAB produce the greatest amount of lactic acid at 24 h incubation. Thus, the increase in the inhibition zone after 24 h was derived possibly from other factors, such as bioactive substances.

Antimicrobial activity of crude PS34 CFS when mixed with *Salmonella* cultures for 1, 15, 30, and 60 minutes

PS34 CFS reduced the number of *Salmonella* cells during the first one minute incubation by as much as 87% (survived 13%) for *S.* Stanley (P) (Fig. 2). The numbers of all tested *Salmonella* serovars decreased significantly with the survival at only 3-11% after 15 minutes. Incubation with the CFS reduced the numbers of *Salmonella* by approximately 99.9% within 30 minutes. Finally, the PS34 CFS eliminated all tested *Salmonella* at 60 minutes, in which the cell numbers were reduced by 99.98%. After 24 h, *Salmonella* cell survival was not detected, suggesting that *Salmonella* were completely eliminated.

Determination of the antimicrobial activity of freeze-dried *P. pentosaceus* PS34 CFS by drop diffusion

The 2-fold diluted solution of freeze-dried PS34 CFS was mixed with the cultures of the 8 antibiotic-resistant *Salmonella* isolates, and
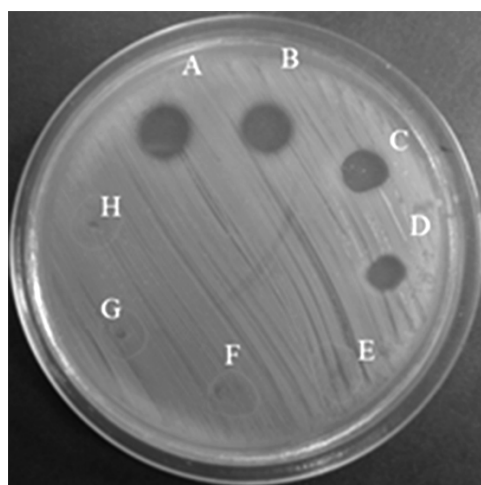
Table 4. The growth inhibition of antibiotic-resistant *Salmonella* and *S.* Typhimurium DMST562 by crude PS34 CFS (pH 4) with 0.8% lactic acid and MRS broth used as positive and negative controls.

Strains	Inhibition zone (mm)				
	24 h	36 h	48 h	0.8% lactic acid	MRS
Antibiotic-resistant *Salmonella*					
S. Typhimurium DMST562	12.5	12.7	13.5	11.6	-

the inhibition zone was measured. The minimal arbitrary inhibition concentration of all samples was found to be a 1:4 dilution, and the growth inhibition effect was lost beyond a 1:8 dilution (Fig. 3).

Effect of heat, proteolysis, and pH on the anti-*Salmonella* activity of the freeze-dried PS34 CFS

When tested with all 8 isolates of *Salmonella*, the anti-*Salmonella* activity of the PS34 CFS remained but decreased slightly after heating (100°C for 30 minutes and 121°C for 15 minutes) and digestion with proteolytic enzymes (trypsin, pepsin, and proteinase K; Table 5). Only a pH of 7 negatively affected the inhibitory activity. It has been reported that lactic acid from LAB fermentation broth is sensitive to heat at approximately 111°C[20]. Thus, the inhibitory activity of PS34-CFS at 121°C was likely derived from antimicrobial agents and/or acid.

Table 5. Diameter of the PS34 CFS-induced *S.* Typhimurium DMST562 inhibition zones after heat treatment, proteolytic enzyme treatment, or pH neutralization

Strains	Diameter of inhibition zone of treated CFS (mm)				
	121°C 15 minutes	100°C 30 minutes	Trypsin, Pepsin, Proteinase K	pH 7	Untreated
S. Typhimurium DMST562	11.25	10	12	12.25	12

DISCUSSION

The resistance of *Salmonella* to many antibiotics has become a serious health problem worldwide[14]. In this study, the prevalence of *Salmonella* in vegetables and traditional Thai fermented pork (nham-mooh) and fermented fish (pla-som fak) sold in the local fresh markets and supermarkets in the northeastern region was 64% in vegetables and 36% in meat/fish. In this study, *S.* Typhimurium and *S.* Enteritidis, major infectious serovars in humans, were not detected. Of the *Salmonella* isolates, 20%, 16%, 12%, 12%, and 12%, were resistant to sulfonamides, tetracycline, gentamicin, nalidixic, and sulfamethoxazole, respectively. Among the antibiotic-resistant isolates, 12.5% and 16% were isolated from fresh vegetables and fermented meats, respectively. In agreement with previous studies, *Salmonella* from the meat products in retail markets in Thailand were resistant to ampicillin, sulfonamides and tetracycline[21].

These results are similar to those in a report from Tunisia (20%)[22] but lower than those detected previously in Morocco (75.43%)[23], Italy (84.2%)[24] and China (74%)[25]. In this study, 14.7% of *Salmonella* isolates (5/34) were resistant to two types of antibiotics. These rates are lower than those found in Botswana (20.3%)[26] and Egypt (100%)[27]. The percentage of isolates resistant to tetracycline was 16.0%. Resistance to these antimicrobials is common in *Salmonella* isolates and has been observed previously in Morocco[23], Tunisia[22], Malaysia[28] and China[29]. The prevalence of sulfonamide resistance is significant (20%), and might be a consequence of the widespread use of these antimicrobials in livestock. This percentage is consistent with that detected in Morocco (20.5%)[30] but higher than that found in Tunisia (1.2%)[22].

The *Salmonella* observed in this present study were determined by the detection of invasion-associated (*iagA*) genes. All positive isolates expressed the *iag*A gene, indicating that the consumption of raw nham-mooh, pla-som fak and fresh vegetables might lead to an increased risk of antibiotic-resistant *Salmonella* infection due to the transfer of resistance genes to other bacteria. However, further epidemiological studies and more data regarding the link between contaminated food consumption, human illness and the presence of virulence genes may be necessary to address food safety concerns.

The presence of several *Salmonella* serovars in foods points to natural cross-contamination in the environment. The prevalence of the predominant *Salmonella* serotypes in this study might be different from that in other geographical regions due to several factors, such as the environment and temperature[30]. The cross-

contamination of the raw material usually occurs at its point of origin, such as the cross-contamination in fish, which usually occurs via exposure of the fish to various pathogens in aquatic environments[31]. The cross-contamination of vegetables usually occurs by fecal contamination of the water used for irrigation and food processing[31]. The low hygienic quality of food processing facilities mainly leads to cross-contamination due to a lack of good handler hygiene practices, such as hand washing during raw material handling, delivery and food processing steps[32,33]. In addition, techniques used for the isolation of pathogenic bacteria might be different[30].

In Thailand, the small-scale production of many traditional fermented Thai meats such as nham-moo and pla-som-fak are processed by indigenous microorganisms, which could be contaminated with pathogens. Traditional Thai fermented food products are commonly produced at home or by small-scale industrial production and sold at small retail markets in upper northeastern Thailand. Hence, contamination with widespread foodborne pathogens, such *Salmonella,* could easily occur, and approaches to prevent this occurrence should be investigated.

In this study, we sought to determine the potential of natural antimicrobials to control pathogenic microorganism growth for food protection and safety as an alternative choice to current food preservatives[12,13]. The CFS from potential probiotic bacteria *P. pentosaceus* PS34 showed strong inhibitory activity against antibiotic-resistant *Salmonella* strains isolated from fresh vegetables and fermented foods. Our results are consistent with those of a previous study reporting that probiotic LAB inhibit the growth of various Gram-positive and Gram-negative pathogenic bacteria. The use of CFS from *L. rhamnosus* and *L. casei* combined with amikacin and gentamicin strongly inhibited the development of a *Pseudomonas aeruginosa* infection[34]. Qureshi et al. (2019)[35] reported that the use of probiotics combined with a conventional antibiotic regimen appeared has great potential for eliminating *Helicobacter pylori* infections. However, the use of probiotics with antibiotic-resistant microorganisms could have both beneficial effects and risks. Therefore, the regulation of the use of probiotics in both humans and animals should be appropriately established[8].

P. pentosaceus PS34 was isolated from fermented fish and is a potential probiotic strain due to its susceptibility to many antibiotics[18]. The CFS from *P. pentosaceus* PS34 may have inhibited the growth of *Salmonella* through the production of various antimicrobial compounds. LAB normally produce weak organic acids in the medium. Weak organic acids, such as lactic acid, can penetrate through the cell membranes of Gram-negative bacteria, causing intracellular acidification after the release of hydrogen ions[36]. This acidification leads to the loss of ATP from the cell. The intracellular hydrogen ions diffuse passively out of the bacterial cells across the membrane, resulting in cell damage and cell death[37]. Bacteriocin is a ribosomally synthesized antimicrobial peptide commonly produced by LAB. Bacteriocin works mainly against Gram-positive bacteria[38]. Many bacteriocins are heat stable, and the widely studied bacteriocin from LAB has gained increased attention as an alternative means to inhibit pathogens growth in foods[39]. In this study, the antimicrobial effect of CFS against *Salmonella* might also involve bacteriocin activity since the inhibition remained after the CFS was heated at high temperatures. Out of the total samples, *Salmonella* were isolated from 36% of fermented meats, which normally have a low pH, indicating that *Salmonella* are naturally able to tolerate acid. The inhibitory effect observed in this study was possibly derived from bioactive substances. However, the loss of CFS activity at neutral pH indicates that the PS34-derived bioactive substance favors pH 4.

The high prevalence of antibiotic-resistant *Salmonella* (at 64%) in fresh vegetables observed in this study is a public safety concern. To minimize the risk of human infection, it is important to continue surveillance and take measures to prevent food and environmental contamination by antimicrobial-resistant bacteria as well as to educate the public that avoiding raw food consumption is important to prevent antimicrobial-resistant bacterial infections. Thus, public health authorities should monitor and provide information and knowledge to customers for safe food consumption. New decontamination

methods are needed to guarantee the safety of various foods[40-43]. Therefore, the regulation of the use of probiotics in both humans and animals should be appropriately established[8].

CONCLUSION

In conclusion, the contamination of vegetables and fermented meats with antibiotic-resistant *Salmonella* is widespread, indicating the inappropriate use of antimicrobial antibiotics in humans and livestock. *Salmonella* cross-contaminate the environment, and thus the transference of antibiotic-resistance genes to other bacteria is possible. This study revealed the antimicrobial properties of CFS from *P. pentosaceus* PS34 originally derived from fermented fish, which was effective against a variety of antibiotic-resistant *Salmonella* serovars isolated from foods. Thus, PS34 has the potential to inhibit the growth of antimicrobial-resistant *Salmonella,* indicating that it may be useful in food or in the environment to improve the quality of food products by inhibiting the growth of contaminating antibiotic-resistant *Salmonella*.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

WS and NR designed the experiments. NR performed the experiments. WS and NR analyzed the data and wrote the manuscript. WS, NR and SS read and approved the manuscript.

FUNDING

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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