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Determination of histamine and histamine-forming bacteria in tuna dumpling implicated in a food-borne poisoning

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Abstract

An incident of food-borne poisoning causing illness in seven victims, due to ingestion of tuna dumpling, occurred in March 2006, in Chiayi Prefecture, southern Taiwan. The leftovers of the victims' tuna dumpling and the five other tuna dumpling samples from five other retail stores were collected and tested to determine the occurrence of histamine and histamine-forming bacteria. The levels of pH, salt content, aerobic plate count (APC), total volatile basic nitrogen (TVBN), total coliform (TC) and *Escherichia coli* in all samples ranged from 6.08 to 6.43, 0.46% to 0.81%, 5.90 to 8.95 log CFU/g, 6.38 to 21.29 mg/100 g, 750 to 8000 most probable number (MPN)/g, and <3 to 1000 MPN/g, respectively. The suspected tuna dumpling contained 160.8 mg/100 g of histamine greater than the hazard action level of 50 mg/100 g set by the US Food and Drug Administration (FDA) for tuna fish. Given the allergy-like symptoms of the victims and the high histamine content in the suspected tuna dumpling, this food-borne poisoning was strongly suspected to be due to histamine intoxication. In addition, although thirteen histamine-producing bacteria strains capable of producing 8.1–19.7 ppm of histamine in trypticase soy broth (TSB) supplemented with 1.0% L-histidine (TSBH), were identified as *Enterobacter* sp. (three strains), *Pantoea agglomerans* (two strains), *Klebsiella variicola* (four strains) and *Serratia marcescens* (four strains), by 16S rDNA sequencing with PCR amplification, they were not determined to be the main contributors to histamine accumulation in suspected tuna dumpling.

Keywords: Histamine; Tuna dumpling; Histamine-forming bacteria; Food poisoning

1. Introduction

Histamine is the causative agent of scombroid poisoning, a food-borne chemical hazard. Scombroid poisoning is usually a mild illness with a variety of symptoms including rash, urticaria, nausea, vomiting, diarrhea, flushing, and tingling and itching of the skin (Taylor, 1986). Severity of the symptoms can vary considerably with the amount of histamine ingested and the individual's sensitivity to histamine (Russell & Maretic, 1986). Scombroid fish such as tuna, mackerel, bonito, and saury that contain high levels of free histidine in their muscle are often implicated in scombroid poisoning incidents (Taylor, 1986). However, several species of nonscombroid fish such as mahi-mahi, bluefish, herring, and sardine have often been implicated in incidents of scombroid poisoning (Price & Melvin, 1994). In Taiwan, scombroid poisoning occurs occasionally (Chen & Malison, 1987; Murray, Hobbs, & Gilbert, 1982; Tsai, Kung, et al., 2005; Tsai, Kung, Chen, Chang, & Wei, in press), and the fish implicated in these outbreaks are

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tuna, mackerel, milkfish and black marlin. Recently, due to their popularity in Taiwanese people, sailfish, swordfish and marlin fillets have become the most frequently implicated fish species in scombroid outbreaks in Taiwan (Chang, Kung, Chen, Lin, & Tsai, in press; Hwang, Chang, Shiau, & Cheng, 1995; Hwang, Chang, Shiau, & Chai, 1997; Hwang et al., 1999; Tsai, Hsieh, et al., 2007).

Biogenic amines are formed mainly through the decarboxylation of specific free amino acids by exogenous decarboxylases released by the microbial species associated with the seafood (Rawles, Flick, & Martin, 1996). Many different bacterial species are known to possess histidine decarboxylase and have the ability to produce histamine (An & Ben-Gigirey, 1998). Although only *Morganella morganii*, *Klebsiella pneumoniae* and *Hafnia alvei* have been isolated from the fish incriminated in scombroid poisoning (Taylor & Speckard, 1983), a variety of other bacterial species capable of producing histamine have been identified in fish (Eitenmiller, Wallis, Orr, & Phillips, 1981; Middlebrooks, Toom, Douglas, Harrison, & McDowell, 1988; Taylor & Speckard, 1983; Yoshinaga & Frank, 1982). Among them are the enteric bacteria that include *Proteus vulgaris*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Serratia fonticola*, *Serratia liquefaciens*, *Raoultella* (formerly *Klebsiella*) *planticola*, *Raoultella ornithinolytica* and *Citrobacter freundii* (Ababouch, Afila, Rhafiri, & Busta, 1991; Kim et al., 2003; Lopez-Sabater, Rodriguez-Jerez, Roig-Sagues, & Mora-Ventura, 1994; Tsai, Lin, et al., 2005). In addition to the enteric bacteria, *Clostridium* spp., *Vibrio alginolyticus*, *Acinetobacter lowffi*, *Plesiomonas shigelloides*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Aeromonas* spp., and *Photobacterium* spp. have also been reported as histamine producers (Lopez-Sabater, Rodriguez-Jerez, Hernandez-Herrero, & Mora-Ventura, 1994; Middlebrooks et al., 1988; Okuzumi, Hiraishi, Kobayashi, & Fujii, 1994; Ryser, Marth, & Taylor, 1984; Yatsunami & Echigo, 1991). Recently, we demonstrated the presence of histamine-forming *Proteus*, *Enterobacter*, *Klebsiella*, *Rahnella* and *Acinetobacter* in sailfish fillets in Taiwan, but failed to isolate any of the three above mentioned major histamine-formers, the *H. alvei*, *M. morganii* and *K. pneumoniae* (Tsai, Kung, Lee, Lin, & Hwang, 2004).

An incident of food-borne poisoning due to ingestion of tuna dumpling, occurred in Chiayi Prefecture, southern Taiwan, in March 2006. The incident caused seven victims to become ill. They all suffered from allergy-like symptoms, including rash, nausea, diarrhea, flushing, and tingling and itching of skin, but all recovered within 24 h. To elucidate the causative agent, the suspected tuna dumpling was collected from the victims' leftovers. Additionally, the five other tuna dumpling samples were purchased from five other retail stores in southern Taiwan and processed for analyses for biogenic amine levels. In addition, the chemical and microbiological quality, and histamine-forming bacteria in these tuna dumpling samples were also investigated.

2. Materials and methods

2.1. Samples

A 150-g portion of the leftovers from the victims' tuna dumpling was collected. The five other tuna dumpling samples were obtained from five other retail stores in southern Taiwan. All the collected and frozen samples were wrapped in aseptic bags, placed in ice, and immediately transported to the laboratory for use within 8 h.

2.2. pH value and salt content determination

The tuna dumpling samples (10 g) were homogenized in sterile blenders with 10 ml of distilled water to make a thick slurry. The pH of this slurry was then measured using a Corning 145 pH meter (Corning Glass Works, Medfield, MA, USA). The salt content in each sample was determined according to the AOAC procedures (1995) by homogenizing 2 g of tuna dumpling sample, with 18 ml of distilled water. The homogenate was titrated with 0.1 M $AgNO_3$ using 10% w/v K_2CrO_4 solution as an indicator.

2.3. Microbiological analysis and isolation of histamine-forming bacteria

A 25-g portion of the tuna dumpling sample was homogenized at high speed for 2 min in a sterile blender with 225 ml of sterile potassium phosphate buffer (0.05 M, pH 7.0). The sterile blender was prepared by autoclaving for 15 min at 121 °C. The homogenates were serially diluted with a sterile phosphate buffer, and 1.0 ml aliquots of the dilutes were inoculated into aerobic plate count (APC) agar (Difco, Detroit, MI, USA) containing 0.5% NaCl. Bacterial colonies were counted after the plates were incubated at 35 °C for 48 h. The bacterial numbers in the tuna dumpling samples were expressed as $\log_{10}$ colony forming units (CFU)/g.

To isolate histamine-forming bacteria, a 0.1 ml aliquot of the sample dilute was spread on histamine-forming bacterium isolation agar (HBI agar) fortified with L-histidine (Niven, Jeffreg, & Corlett, 1981). Following incubation of the differential agar plates for 4 d at 35 °C, colonies with blue or purple color on the plates were picked and further streaked on trypticase soy agar (TSA) (Difco) to obtain pure cultures. Their ability to produce biogenic amines was determined by inoculating the isolates in trypticase soy broth (TSB) (Difco) supplemented with 1% L-histidine (TSBH) and incubated without shaking at 35 °C for 24 h. Two millilitres of the culture broth were taken for quantitation of biogenic amines.

Analyses of total coliform and *Escherichia coli* in these tuna dumpling samples were conducted using the three-tube most probable number (MPN) methods (FDA, 1992). Lauryl sulphate tryptose broth (LST broth) and brilliant green lactose bile (2%) broth (BGLB broth) were used for presumptive and confirmed tests for total coliform,

respectively. *E. coli* was determined by using the LST broth and EC broth. Cultures that showed positive production of gas were then confirmed by eosine methylene blue agar (EMBA) and IMViC test.

2.4. Identification of histamine-forming isolates

The presumptive histamine-forming isolates were identified on the basis of morphology, Gram stain, endospore stain, catalase and oxidase reaction. The identity of histamine-forming isolates was further confirmed by amplifying and sequencing approximately 1400 bp of the 16S ribosomal DNA (rDNA) for bacteria (Kuhnert, Capaul, Nicolet, & Frey, 1996; Kuhnert, Heyberger-Meyer, Nicolet, & Frey, 2000). Amplification of histamine-forming bacteria was performed using the universal primers UNI-L (5′-AGAGTTTGATCATGGCTCAG-3′) and UNI-R (5′-GTGTGACGGGCGGTGTGTAC-3′) (Kuhnert et al., 1996, 2000). Bacterial cells were cultured overnight in 2 ml of TSB at 35 °C and centrifuged at 5000*g* for 10 min. The cell pellet was washed and resuspended in 0.5 ml of TE-buffer (10 mM Tris–HCl, 1 mM EDTA; pH 8.0), and lysed by 20% sodium dodecyl sulfate (SDS). After the solution was boiled for 20 min and the cellular debris was discarded following centrifugation at 13,000*g* for 3 min, the total DNA in the supernatant was precipitated with 70% ethanol and used as template DNA for PCR.

PCR amplification was performed in 20 μl reaction mixture containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 20 pmol of each primer, a 0.2 mM concentration for each of the four deoxynucleotide triphosphates, 0.5 U of Taq DNA polymerase (Applied Biosystems, Foster City, CA, USA), and template DNA (10 ng). Amplifications were carried out for 35 cycles (94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s) in a GeneAmp PCR 2400 Thermal Cycler (Applied Biosystems) with an initial denaturation at 94 °C for 4 min and a final extension at 72 °C for 7 min (Kuhnert et al., 1996, 2000). Amplicons were detected by electrophoresis on a 1.5% agarose gel staining with ethidium bromide. Amplicons were purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) eluted in Tris–HCl (10 mM, pH 8.5) prior to sequencing. The amplified DNA was directly sequenced with the ABI Taq-Dye Deoxy Terminator Cycle sequencing kit and ABI Model 377 automated DNA sequencer (Applied Biosystems). The sequences were analyzed with the BLAST (NCBI) for identification of histamine-forming bacteria.

2.5. Determination of total volatile base nitrogen (TVBN)

The TVBN content of the tuna dumpling sample was measured by the method of Conway's dish (Cobb, Aoaniz, & Thompson, 1973). The TVBN extract of the tuna dumpling sample in 6% trichloroacetic acid (TCA, Sigma, St. Louis, MO, USA) was absorbed by boric acid and titrated with 0.02 N HCl. The TVBN content was expressed in mg/100 g fish.

2.6. Biogenic amine analysis

Each tuna dumpling sample was ground in a Waring Blender for 3 min. The ground samples (5 g) were transferred to 50 ml centrifuge tubes and homogenized with 20 ml of 6% trichloroacetic acid (TCA) for 3 min. The homogenates were centrifuged (10,000*g*, 10 min, 4 °C) and filtered through Whatman No. 2 filter paper (Whatman, Maidstone, England). The filtrates were placed in volumetric flasks, and TCA was added to bring to a final volume of 50 ml. Samples of standard biogenic amine solutions and 2 ml aliquots of the tuna dumpling extracts were derivatized with benzoyl chloride according to the previously described method (Hwang et al., 1997). Two millilitres of each bacterial culture broth were also benzoylated using the same procedures for tuna dumpling extracts. The benzoyl derivatives were dissolved in 1 ml of methanol, and 20 μl aliquots were used for HPLC injection.

The contents of biogenic amines in the tuna dumpling samples were determined with a Hitachi liquid chromatograph (Hitachi, Tokyo, Japan) consisting of a Model L-7100 pump, a Rheodyne Model 7125 syringe loading sample injector, a Model L-4000 UV–Vis detector (set at 254 nm), and a Model D-2500 Chromato-integrator. A LiChrospher 100 RP-18 reversed-phase column (5 μm, 125 × 4.6 mm, E. Merck, Damstadt, Germany) was used for chromatographic separation. The gradient elution program began with 50:50 (v/v) methanol:water at a flow rate of 0.8 ml/min for the first 0.5 min, followed by a linear increase to 85:15 methanol:water (0.8 ml/min) during the next 6.5 min. The methanol:water mix was held constant at 85:15 (0.8 ml/min) for 5 min, and then decreased to 50:50 (0.8 ml/min) during the next 2 min.

3. Results and discussion

The values of the pH, salt content, APC, TVBN, total coliform (TC), and *E. coli* in the suspected tuna dumpling implicated in food-borne poisoning, and the five other tuna dumpling samples from five other retail stores are presented in Table 1. The levels of pH, salt content, aerobic plate count (APC), total volatile basic nitrogen (TVBN), TC and *E. coli* in all six samples ranged from 6.08 to 6.43, 0.46% to 0.81%, 5.90 to 8.95 log CFU/g, 6.38 to 21.29 mg/100 g, 750 to 8000 most probable number (MPN)/g, and <3 to 1000 MPN/g, respectively. Based on the Taiwanese regulatory standard of 6.47 log CFU/g of APC for frozen foods to be served after cooking, 50% (3/6) of the tuna dumpling samples were unacceptable. Although Taiwanese regulatory standard of TC for frozen foods to be served after cooking is not set, all tuna dumpling samples contained more than the 750 MPN/g of total coliform (Table 1). Four of the six tuna dumpling samples (66.7%) contained more than 100 MPN/g of *E. coli*, which are more than the 50 MPN/g Taiwan allowable limit for frozen foods to be served after cooking (Table 1). The contents of TVBN in two the tuna dumpling samples were

Table 1
Values of the pH, salt content, aerobic plate count (APC), total volatile basic nitrogen (TVBN), total coliform (TC), and *E. coli* in the tuna dumpling implicated in food poisoning, and the five other tuna dumpling samples from five other retail stores

Source and type of tuna dumpling	pH	Salt content (%)	APC (log CFU/g)	TVBN (mg/100 g)	TC (MPN/g)	*E. coli* (MPN/g)
Victims' leftover	6.08	0.81	6.35	15.37	2100	1000
Retail store samples, No. 1	6.10	0.48	6.02	10.21	750	100
No. 2	6.41	0.48	6.90	10.59	1100	1000
No. 3	6.43	0.46	7.70	6.38	4600	1000
No. 4	6.38	0.61	5.90	10.10	8000	<3
No. 5	6.13	0.46	8.95	21.29	7200	<3

above the Taiwanese regulatory level of 15 mg/100 g (Table 1). Based on the finding that high levels of APC, TC and *E. coli* were detected in the suspected tuna dumpling and the five other tuna dumpling samples, we postulate that the commercial tuna dumpling had been seriously contaminated during its processing.

The levels of biogenic amines in the suspected tuna dumpling sample responsible for histamine poisoning illness, and the five other tuna dumpling samples from five other retail stores are summarized in Table 2. While the suspected tuna dumpling sample had a histamine content of 160.8 mg/g, two of five other tuna dumpling samples had 8.6 and 10.4 mg/100 g of histamine, that are greater than the 5.0 mg/100 g allowable limit suggested by the US Food and Drug Administration (FDA) for scombroid fish and/or products (USFDA, 2001, chap. 7). These six tuna dumpling samples contained less than 8.6 mg/100 g of the other biogenic amines. The US Food and Drug Administration (FDA) has established a "hazard action level" of 50 mg histamine/100 g (500 ppm) for tuna fish based on data collected from numerous outbreaks (Taylor, 1989). Bartholomev, Berry, Rodhouse, and Gilhouse (1987) demonstrated that histamine at greater than 100 mg/100 g in fish would be toxic and unsafe for consumption. Thus, the high level of histamine in tuna dumpling samples along with the allergy-like symptoms developed in the victims supported the conclusion that histamine was the causative agent of this food-borne poisoning incident.

High levels of histamine content have been found in various types of fish implicated in scombroid poisoning. The marlin implicated in a poisoning incident had a histamine content ranging between 93.5 and 276 mg/100 g (Morrow, Margolies, Rowland, & Robert, 1991); the hot-smoked mackerel implicated in a scombrotoxic incident had a histamine content of 270 mg/100 g (Clifford, Walker, & Wright, 1989); and the canned tuna implicated in a poisoning, had a histamine content of 116 mg/100 g, while that of wholesome canned tuna had only 2.74 mg/100 g (Kim & Bjeldanes, 1979). In Taiwan, incidences of scombroid poisoning only occurred occasionally, and the fish implicated in those occasional outbreaks are tuna, mackerel, milkfish and black marlin (Chen & Malison, 1987; Murray et al., 1982; Tsai, Kung, et al., 2005; Tsai, Kung, et al., in press). Sailfish, swordfish and marlin fillets have recently become the most frequently implicated fish species in scombroid outbreaks in Taiwan (Chang et al., in press; Hwang et al., 1995, 1997, 1999; Tsai, Hsieh, et al., 2007). Quality loss and histamine accumulation often occur after frozen fish of the above mentioned species are thawed and kept for long periods of time at room temperature before further processing. Since histamine is heat resistant, it can remain intact in canned or cooked fish products (Lopez-Sabater, Rodriguez-Jerez, Roig-Sagues, et al., 1994). In this study, the use of poor quality tuna fish for production or the application of a defective processing technique during production resulted in the presence of toxic levels of histamine in the suspected tuna dumpling.

However, it is not clear whether this high content of histamine, in the tested tuna dumpling, is the primary causative agent of scombrotoxicosis. Some studies with volunteers on the role of histamine in suspected scombro-

Table 2
The levels of biogenic amines in the tuna dumpling implicated in food poisoning and the five other tuna dumpling samples from five other retail stores

Source and type of tuna dumpling	Levels of biogenic amine (mg/100 g)								
	Put[a]	Cad	Try	Phe	Spd	Spm	His	Tyr	Agm
Victims' leftover	0.8 ± 0.2[b]	5.2 ± 0.3	ND[c]	ND	ND	8.6 ± 1.8	160.8 ± 5.9	0.3 ± 0.2	1.2 ± 0.6
Retail store samples, No. 1	ND	ND	ND	ND	ND	6.8 ± 1.4	8.6 ± 1.6	5.0 ± 0.9	5.4 ± 2.8
No. 2	ND	5.5 ± 1.3	ND	ND	ND	ND	ND	ND	ND
No. 3	ND	1.7 ± 0.8	ND	ND	ND	ND	ND	ND	ND
No. 4	ND	0.5 ± 0.4	ND	ND	ND	ND	ND	ND	ND
No. 5	ND	3.3 ± 1.4	ND	ND	ND	8.1 ± 1.0	10.4 ± 2.1	ND	ND

[a] Put: putrescine; Cad: cadaverine; Try: tryptamine; Phe: 2-phenylethylamine; Spd: spermidine; Spm: spermine; His: histamine; Tyr: tyramine; and Agm: agmatine.
[b] Mean ± SD for triplicate assays.
[c] ND: not detected (amine level less than 0.1 mg/100 g).

toxicosis, revealed that histamine alone is unlikely to be the causative agent of such poisoning (Clifford et al., 1989). There is compelling evidence to implicate that other factors, such as other biogenic amines, can potentiate histamine toxicity (Clifford et al., 1991; Ijomah et al., 1991), as spoiled fish containing histamine tends to be more toxic than the equivalent amount of pure histamine that is ingested orally (Lehane & Olley, 2000). Putrescine and cadaverine were shown to enhance histamine toxicity when present in spoiled fish by inhibiting the intestinal histamine metabolizing enzyme, including diamine oxidase (Antoine et al., 2002; Bjeldanes, Schutz, & Morris, 1978; Taylor & Sumner, 1987). Putrescine, cadaverine, spermine, tyramine and agmatine were also found in the suspected tuna dumpling samples in this study, and their possible contribution in potentiating histamine toxicity deserves further studies. Other researchers also suggested, the influence of other marine toxins such as saxitoxin-like substances and urocanic acid on histamine food poisoning (Clifford et al., 1992; Lehane & Olley, 2000). Therefore, more studies are needed to elucidate the exact mechanism of histamine poisoning, especially the interaction of high histamine content in fish flesh with other biogenic amines and marine toxins in inducing this type of food poisoning (Lopez-Sabater, Rodriguez-Jerez, Hernandez-Herrero, Roig-Sagues, & Mora-Ventura, 1996).

Table 3 listed the identity of 13 histamine-forming bacteria as determined by 16S rDNA sequences, following comparison to reference strains, using NCBI database analysis. The PCR amplicon from strains TB, TI, and TJ had a 100% homology with *Enterobacter* sp., while those from strains TC and TH aligned with *Pantoea agglomerans* at 100%. The PCR amplicon from strains T1-9, T7-6, T5-2 and T5-4 had a homology with *Klebsiella variicola* at 100%, whereas those from strains T3-6, T6-1, T6-5 and T6-6 had a homology with *Serratia marcescens* at 99%. These 13 histamine-forming isolates produced substantial amounts of histamine (8.1–19.7 ppm) in TSBH medium. Some of them also produced different amounts of putrescine, cadaverine and tyramine through the action of their respective decarboxylase enzymes on various amino acids that also existed in culture medium (Table 3).

The *Enterobacter* sp. and *P. agglomerans* cultures isolated from suspected tuna dumpling in this study produced 11.5–16.8 ppm of histamine in TSBH medium. The *E. cloacae* isolated from salted anchovies by Rodriguez-Jerez, Lopez-Sabater, Roig-Sagues, and Mora-Ventura (1994), Rodriguez-Jerez, Mora-Ventura, Lopez-Sabater, and Hernandez-Herrero (1994) Hernandez-Herrero, Roig-Sagues, Rodriguez-Jerez, and Mora-Ventura (1999) produced histamine at 27.1, 18.1 and 21.4 ppm, respectively. The recently isolated *E. cloacae* from salted mackerel products in Taiwan was also identified as weak histamine-forming bacteria (Tsai, Lin, et al., 2005). The *P. agglomerans* that Kim et al. (2001) isolated from temperature-abused albacore was a weak histamine-former. Recently, *P. agglomerans*, that was isolated from salted mackerel products in Taiwan, was found to be weak histamine-former (Tsai, Lin, et al., 2005).

Klebsiella spp. were identified as prolific histamine-formers, capable of producing in a culture broth more than 1000 ppm of histamine (Lopez-Sabater et al., 1996; Taylor, Lieber, & Leatherwood, 1978). For example, *K. oxytoca* and *K. pneumoniae* isolated from tuna during canning produced more than 2000 ppm of histamine in TSBH broth (Lopez-Sabater, Rodriguez-Jerez, Roig-Sagues, et al., 1994). However, the isolated *K. oxytoca* from albacore tuna and salted anchovy was a weak histamine-former, producing only 97.9 ppm and 26.7 ppm, respectively (Hernandez-Herrero et al., 1999; Kim et al., 2001). Similarly, the *K. variicola*, which was isolated in this study, was also a weak histamine-former, capable of producing 8.4–11.4 ppm of histamine in TSBH (Table 3). The *S. marcescens* strains T3-6, T6-1, T6-5 and T6-6 that were isolated

Table 3
Identification of histamine-forming bacteria isolated from the tuna dumpling implicated in food poisoning and the five other tuna dumpling samples collected from five other retail stores by 16S rDNA, based on the output results from NCBI database analysis, and their production of histamine and other biogenic amines (ppm) in culture broth

Source	Strain	Organism identified	Percentage identity (%)	Gene bank accession number	His[a]	Put	Cad	Tyr
Victims' leftover	TB	*Enterobacter* sp.	100	EF025346.1	14.4	31.9	ND[b]	ND
	TI	*Enterobacter* sp.	100	EF025346.1	15.1	36.0	136.9	ND
	TJ	*Enterobacter* sp.	100	EF025346.1	11.8	37.7	182.3	ND
	TC	*Pantoea agglomerans*	100	DQ307453.1	16.8	70.1	216.4	ND
	TH	*P. agglomerans*	100	DQ307453.1	11.5	ND	200.3	ND
Retail stores	T1-9	*Klebsiella variicola*	100	AJ783916.1	8.4	ND	162.1	ND
	T7-6	*K. variicola*	100	AJ783916.1	10.6	ND	136.6	8.3
	T5-2	*K. variicola*	100	AJ783916.1	11.4	ND	153.5	3.9
	T5-4	*K. variicola*	100	AJ783916.1	10.9	ND	110.5	ND
	T3-6	*Serratia marcescens*	99	AB061685.1	19.7	ND	136.1	ND
	T6-1	*S. marcescens*	99	AB061685.1	16.5	ND	110.5	ND
	T6-5	*S. marcescens*	99	AB061685.1	8.1	ND	129.7	ND
	T6-6	*S. marcescens*	99	AB061685.1	13.8	ND	96.9	ND

[a] His: histamine, Put: putrescine; Cad: cadaverine, and Tyr: tyramine.
[b] ND: not detected (amine level less than 1 ppm).

the five other tuna dumpling samples in this study were weak histamine-formers, and they produced only 8.1–19.7 ppm of histamine in TSBH. *S. marcescens*, *S. plymuthica* and *S. fonticola* have been isolated from tuna as weak histamine-formers, producing 5.5–134.8 ppm of histamine in culture broth (Lopez-Sabater et al., 1996). *S. grimesii* isolated from dried milkfish products implicated in a food-borne poisoning in Taiwan was also shown to be weak histamine-formers (Tsai, Kung, et al., in press).

H. alvei, *M. morganii* and *K. pneumoniae* have been implicated as causative organisms in the formation of toxicologically significant levels of histamine in outbreaks of scombroid fish poisoning (Taylor & Speckard, 1983). Recently, *R. ornithinolytica* was the major histamine-producing bacterium responsible for the high content of histamine in the implicated milkfish products in Taiwan (Tsai, Kung, et al., in press). Nevertheless, none of above four major histamine-formers was isolated from tuna dumpling samples in this study. The histamine-forming isolates produced small amounts of histamine in culture broth, indicating that they are not the main contributors to histamine accumulation in the suspected tuna dumpling. It was possible that the major histamine-forming bacteria that contributed to the higher levels of histamine in the suspected tuna dumpling were killed or inhibited during thawing or re-freezing process and storage condition, or could not grow on the HBI agar or TSBH medium that were used.

4. Conclusion

This study showed that suspected tuna dumpling sample had TVBN and *E. coli* levels greater than Taiwanese regulatory limit of 15 mg/100 g and 50 MPN/g, respectively. The high content of histamine at 160.8 mg/100 g in the suspected tuna dumpling sample could be the etiological factor for this food-borne poisoning. *Enterobacter* sp. (three strains), *P. agglomerans* (two strains), *K. variicola* (four strains) and *S. marcescens* (four strains) were the 13 weak histamine-formers isolated from the suspected tuna dumpling and the five other tuna dumpling samples.

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References

Ababouch, L., Afila, M. E., Rhafiri, S., & Busta, F. F. (1991). Identification of histamine-producing bacteria isolated from sardine (*Sardina pilchardus*) stored in ice and at ambient temperature (25 °C). *Food Microbiology, 8*, 127–136.

An, H., & Ben-Gigirey, B. (1998). Scombrotoxin poisoning. In I. Millar, D. Gray, & N. Strachan (Eds.), *Microbiology of seafoods*. London: Chapman & Hall Ltd.

Antoine, F. R., Wei, C. I., Otwell, W. S., Sims, C. A., Littell, R. C., Hogle, A. D., et al. (2002). Analysis of biogenic amines and their precursor free amino acids in mahi-mahi (*Coryphaena hippurus*). *Journal of Food Biochemistry, 26*, 131–152.

AOAC (1995). *Official methods of analysis of AOAC International* (16th ed.). Arlington, VA: AOAC International.

Bartholomev, B. A., Berry, P. R., Rodhouse, J. C., & Gilhouse, R. J. (1987). Scombrotoxic fish poisoning in Britain: Features of over 250 suspected incidents from 1976 to 1986. *Epidemiology and Infection, 99*, 775–782.

Bjeldanes, L. F., Schutz, D. E., & Morris, M. M. (1978). On the aetiology of scombroid poisoning: Cadaverine potentiation of histamine toxicity in the guinea pig. *Food and Cosmetics Toxicology, 16*, 157–159.

Chang, S. C., Kung, H. F., Chen, H. C., Lin, C. S., & Tsai, Y. H. (in press). Determination of histamine and bacterial isolation in swordfish fillets (*Xiphias gladius*) implicated in a food borne poisoning. *Food Control*.

Chen, K. T., & Malison, M. D. (1987). Outbreak of scombroid fish poisoning, Taiwan. *American Journal of Public Health, 77*, 1335–1336.

Clifford, M. N., Walker, R., Ijomah, P., Wright, J., Murray, C. K., & Hardy, R. (1991). Is there a role for amines other than histamines in the aetiology of scombrotoxicosis? *Food Additives and Contaminants, 8*, 641–651.

Clifford, M. N., Walker, R., Ijomah, P., Wright, J., Murray, C. K., Hardy, R., et al. (1992). Do saxitoxin-like substances have a role in scombrotoxicosis? *Food Additives and Contaminants, 9*, 657–667.

Clifford, M. N., Walker, R., & Wright, J. (1989). Studies with volunteers on the role of histamine in suspected scombrotoxicosis. *Journal of the Science of Food and Agriculture, 47*, 363–375.

Cobb, B. F., Aoaniz, I., & Thompson, C. A. (1973). Biochemical and microbial studies on shrimp: Volatile nitrogen and amino nitrogen analysis. *Journal of Food Science, 38*, 431–435.

Eitenmiller, R. R., Wallis, J. W. J., Orr, H., & Phillips, R. D. (1981). Production of histidine decarboxylase and histamine by *Proteus morganii*. *Journal of Food Protection, 44*, 815–820.

FDA (1992). *Bacteriological analytical manual*. Arlington, VA: AOAC International.

Hernandez-Herrero, M. M., Roig-Sagues, A. X., Rodriguez-Jerez, J. J., & Mora-Ventura, M. T. (1999). Halotolerant and halophilic histamine-forming bacteria isolated during the ripeing of salted anchovies. *Journal of Food Protection, 62*, 509–514.

Hwang, D. F., Chang, S. H., Shiau, C. Y., & Chai, T. (1997). High-performance liquid chromatographic determination of biogenic amines in fish implicated in food poisoning. *Journal of Chromatography B, 693*, 23–30.

Hwang, D. F., Chang, S. H., Shiau, C. Y., & Cheng, C. C. (1995). Biogenic amines in the flesh of sailfish (*Istiophorus platypterus*) responsible for scombroid poisoning. *Journal of Food Science, 60*, 926–928.

Hwang, D. F., Chen, T. Y., Chang, S. H., Chou, S. S., Deng, J. F., & Chai, T. (1999). Biogenic amines and bacterial isolates of marlin implicated in food poisoning. *Food Science and Agricultural Chemistry, 1*, 223–228.

Ijomah, P., Clifford, M. N., Walker, R., Wright, J., Hardy, R., & Murray, C. K. (1991). The importance of endogenous histamine relative to dietary histamine in the aetiology of scombrotoxicosis. *Food Additives and Contaminants, 8*, 531–532.

Kim, S. H., Barros-Velazquez, J., Ben-Gigirey, B., Eun, J. B., Jun, S. H., Wei, C. I., et al. (2003). Identification of the main bacteria contributing to histamine formation in seafood to ensure product safety. *Food Science and Biotechnology, 12*, 451–460.

Kim, J. S., & Bjeldanes, L. F. (1979). Amine content of toxic and wholesome canned tuna-fish. *Journal of Food Science, 44*, 922–923.

Kim, S. H., Field, K. G., Morrissey, M. T., Price, R. J., Wei, C. I., & An, H. (2001). Source and identification of histamine-producing bacteria from temperature-abused albacore. *Journal of Food Protection, 64*, 1035–1044.

Kuhnert, P., Capaul, S., Nicolet, J., & Frey, J. (1996). Phylogenetic positions of *Clostridium chauvoei* and *Clostridium septicum* based on 16S rRNA gene sequences. *International Journal of Systematic Bacteriology, 46*, 1174–1176.

Kuhnert, P., Heyberger-Meyer, B., Nicolet, J., & Frey, J. (2000). Characterization of PaxA and Its operon: A cohemolytic RTX toxin determinant from pathogenic *Pasteurella aerogenes*. *Infection and Immunity, 68*, 6–12.

Lehane, L., & Olley, J. (2000). Review. Histamine fish poisoning revisited. *International Journal of Food Microbiology, 58*, 1–37.

Lopez-Sabater, E. I., Rodriguez-Jerez, J. J., Hernandez-Herrero, M., & Mora-Ventura, M. A. T. (1994). Evaluation of histidine decarboxylase activity of bacteria isolated from sardine (*Sardina pilchardus*) by an enzymatic method. *Letter in Applied Microbiology, 19*, 70–75.

Lopez-Sabater, E. I., Rodriguez-Jerez, J. J., Hernandez-Herrero, M., Roig-Sagues, A. X., & Mora-Ventura, M. A. T. (1996). Sensory quality and histamine formation during controlled decomposition of tuna (*Thunnus thynnus*). *Journal of Food Protection, 59*, 167–174.

Lopez-Sabater, E. I., Rodriguez-Jerez, J. J., Roig-Sagues, A. X., & Mora-Ventura, M. A. T. (1994). Bacteriological quality of tuna fish (*Thunnus thynnus*) destined for canning: Effect of tuna handling on presence of histidine decarboxylase bacteria and histamine level. *Journal of Food Protection, 57*, 318–323.

Middlebrooks, B. L., Toom, P. M., Douglas, W. L., Harrison, R. F., & McDowell, S. (1988). Effects of storage time and temperature on the microflora and amine development in Spanish mackerel (*Scomberomorus maculatus*). *Journal of Food Science, 53*, 1024–1029.

Morrow, J. D., Margolies, G. R., Rowland, J., & Robert, L. J. (1991). Evidence that histamine is the causative toxin of scombroid-fish poisoning. *New England Journal of Medicine, 324*, 716–720.

Murray, C. K., Hobbs, G., & Gilbert, R. J. (1982). Scombrotoxin and scombrotoxin-like poisoning from canned fish. *Journal of Hygiene Cambridge, 88*, 215–220.

Niven, C. F., Jeffreg, M. B., & Corlett, D. A. (1981). Differential plating medium for quantitative detection of histamine-producing bacteria. *Applied and Environmental Microbiology, 41*, 321–322.

Okuzumi, M., Hiraishi, A., Kobayashi, T., & Fujii, T. (1994). *Photobacterium histaminum* sp. *nov*., a histamine-producing marine bacterium. *International Journal of Systemic Bacteriology, 44*, 631–636.

Price, R. J., & Melvin, E. F. (1994). Recommendations for on Board Handling of Albacore Tuna. Sea Grant Extension Program, Food Science and Technology Extension, University of California, Davis.

Rawles, D. D., Flick, G. J., & Martin, R. E. (1996). Biogenic amines in fish and shellfish. *Advances in Food and Nutrition Research, 39*, 329–365.

Rodriguez-Jerez, J. J., Lopez-Sabater, E. I., Roig-Sagues, A. X., & Mora-Ventura, M. T. (1994). Histamine, cadaverine and putrescine forming bacteria from ripened Spanish semipreserved anchovies. *Journal of Food Science, 59*, 998–1001.

Rodriguez-Jerez, J. J., Mora-Ventura, M. T., Lopez-Sabater, E. I., & Hernandez-Herrero, M. M. (1994). Histidine, lysine and ornithine decarboxylase bacteria in spanish salted semi-preserved anchovies. *Journal of Food Protection, 57*, 784–787.

Russell, F. E., & Maretic, Z. (1986). Scombroid poisoning: Mini-review with case histories. *Toxicon, 24*, 967–973.

Ryser, E. T., Marth, E. H., & Taylor, S. L. (1984). Histamine production by psychrotrophic pseudomonads isolated from tuna fish. *Journal of Food Protection, 47*, 378–380.

Taylor, S. L. (1986). Histamine food poisoning: Toxicology and clinical aspects. *Critical Reviews in Toxicology, 17*, 91–117.

Taylor, S. L. (1989). Histamine poisoning (scombroid fish poisoning): An allergy-like intoxication. *Clinical Toxicology, 27*, 225–240.

Taylor, S. L., Lieber, E. R., & Leatherwood, M. (1978). A simplified method for histamine analysis in food. *Journal of Food Science, 43*, 247–250.

Taylor, S. L., & Speckard, M. (1983). Isolation of histamine-producing bacteria from frozen tuna. *Marine Fisheries Review, 45*, 35–39.

Taylor, S. L., & Sumner, S. S. (1987). Determination of histamine, putrescine and cadaverine. In D. E. Kramer & J. Liston (Eds.), *Seafood quality determination* (pp. 235–245). New York: Elsevier.

Tsai, Y. H., Hsieh, H. S., Chen, H. C., Cheng, S. H., Chai, T., & Hwang, D. F. (2007). Histamine level and species identification of billfish meats implicated in two food borne poisonings. *Food Chemistry, 104*, 1366–1371.

Tsai, Y. H., Kung, H. F., Chen, H. C., Chang, S. C., & Wei, C. I. (in press). Determination of histamine and histamine-forming bacteria in dried milkfish (*Chanos chanos*) implicated in a food-borne poisoning. *Food Chemistry*.

Tsai, Y. H., Kung, H. F., Lee, T. M., Chen, H. C., Chou, S. S., Wei, C. I., et al. (2005). Determination of histamine in canned mackerel implicated in a food borne poisoning. *Food Control, 16*, 579–585.

Tsai, Y. H., Kung, H. F., Lee, T. M., Lin, G. T., & Hwang, D. F. (2004). Histamine related hygienic qualities and bacteria found in popular commercial scombroid fish fillets in Taiwan. *Journal of Food Protection, 67*, 407–412.

Tsai, Y. H., Lin, C. Y., Chang, S. C., Chen, H. C., Kung, H. F., Wei, C. I., et al. (2005). Occurrence of histamine and histamine-forming bacteria in salted mackerel in Taiwan. *Food Microbiology, 22*, 461–467.

USFDA (US Food and Drug Administration) (2001). Scombrotoxin (histamine) formation. In: Fish and fishery products hazards and controls *guide*. (3rd ed., pp. 73–93). Washington, DC: Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood.

Yatsunami, K., & Echigo, T. (1991). Isolation of salt tolerant histamine-forming bacteria from commercial rice-bran pickle sardine. *Bulletin of the Japanese Society of Scientific Fisheries, 57*, 1723–1728.

Yoshinaga, D. H., & Frank, H. A. (1982). Histamine-producing bacteria in decomposing skipjack tuna (*Katsuwonus pelamia*). *Applied and Environmental Microbiology, 44*, 447–452.