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Short communication

Distribution of psychrophilic and mesophilic histamine-producing bacteria in retailed fish in Japan

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ABSTRACT

Cases of histamine fish poisoning (HFP) have been reported worldwide. Although most cases of HFP are caused by mesophilic histamine-producing bacteria (HPB) when temperature abuse occurs, HFP could also be caused by psychrophilic HPB when fish are stored at low temperatures. In this study, mesophilic and psychrophilic HPB were isolated from a wide range of fish samples and identified by 16S rRNA and *gyrB* sequencing. Most of the mesophilic isolates were *Photobacterium damselae* subsp. *damselae* (42.7%) and *Morganella morganii* (24.0%), and most of the psychrophilic HPB were *Photobacterium* (94.9%). Whereas 76.0% of the mesophilic HPB produced more than 500 mg/kg of histamine (Hm), psychrophilic HPB generally produced lower Hm level, but 43.2% of these isolates still produced more than 500 mg/kg Hm. This study showed the importance of investigating not only mesophilic but psychrophilic HPB as well.

1. Introduction

Histamine fish poisoning (HFP) is food poisoning with allergy-like symptoms, e.g., rash, urticaria, flushing, nausea, and headache (Taylor, 1985). Histamine (Hm), the causative agent of HFP, is produced via decarboxylation of the free histidine present in fish meat and is catalyzed by histidine decarboxylase (HDC) in Hm-producing bacteria (HPB) (Lerke, Werner, Taylor, & Guthertz, 1978). HFP is more frequently caused by red-fleshed fish that contain high amounts of free histidine, such as bonito, tuna, sardine, saury, and mackerel (reviewed by Hungerford, 2010), than by white-fleshed fish.

HFP cases caused by red-fleshed fish and its processed products, such as smoked and canned fish, have been reported worldwide (Lehane & Olley, 2000). Between 2000 and 2006, 187 cases of HFP, associated with tuna, mahi–mahi, escolar, and big-eye, were reported in the United States, and 14 cases, mainly caused by tuna, were reported in Australia between 2001 and 2006. In Japan, 89 cases of HFP were reported between 1998 and 2008. Most cases were caused by tuna, followed by swordfish and mackerel (reviewed by Toda, Yamamoto, Uneyama, & Morikawa, 2009).

It is important to identify HPB to determine the source of HFPs and prevent future HFPs. A number of bacterial species, both Gram-positive and Gram-negative, have been reported as HPB. Gram-positive HPB, such as *Lactobacillus buchneri* (Martín, Fernández, Linares, & Alvarez, 2005), *Tetragenococcus muriaticus* (Kimura, Konagaya, & Fujii, 2001), and *Leuconostoc œnos* (Coton, Rollan, & Lonvaud-Funel, 1998), have pyruvoyl-dependent HDCs (Recsei & Snell, 1985) and are mainly isolated from fermented products, such as wine and fish sauce. In contrast, Gram-negative HPB have pyridoxal phosphate-dependent HDCs (Kamath, Vaaler, & Snell, 1991). Enteric bacteria, such as *Enterobacter aerogenes* (Enjalbert, Richard, Attisso, & Crémieux, 1979), *Raoultella planticola* (Kanki, Yoda, Tsukamoto, & Shibata, 2002), *Morganella morganii* (Kawabata, Ishizaka, Miura, & Sasaki, 1956), and marine bacteria, such as *Photobacterium phosphoreum* (Fujii, Hiraishi, Kobayashi, Yoguchi, & Okuzumi, 1997) and *Photobacterium damselae* subsp. *damselae* (Kimura, Hokimoto, Takahashi, & Fujii, 2000; Takahashi et al. 2008) have been reported as HPB. These are mainly isolated from raw fish and unfermented fish products.

Most of the bacteria that have caused reported cases of HFP are mesophilic (Behling & Taylor, 1982). These cases are likely caused by failure to control the temperature of fish and fish products (Lehane & Olley, 2000). Therefore, controlling the storage temperature of fish is important for the prevention of HFP. However,

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temperature abuse might not be the only factor that contributes to HFP. In fact, in a study that examined Hm accumulation in seafood during chilled storage, 31% of the investigated seafood samples contained more than 500 mg/kg Hm (Dalgaard, Emborg, Kjølby, Sørensen, & Ballin, 2008). Although these fish samples may have been at higher temperatures during distribution, the possibility of Hm accumulation by psychrophilic HPB at lower temperatures needs to be investigated. *P. phosphoreum* and *Morganella psychrotolerans*, are known as psychrophilic HPB (Dalgaard, Madsen, Samieian, & Emborg, 2006; Emborg, Dalgaard, & Ahrens, 2006); however, only a few cases of HFP cases have been reported to be caused by these HPB (Emborg & Dalgaard, 2006; Kanki, Yoda, Ishibashi, & Tsukamoto, 2004).

A number of studies have isolated and identified HPB from seafood (Allen, Green, Bolton, Jaykus, & Cope, 2005; Kim, Mah, & Hwang, 2009; López-Sabater, Rodríguez-Jerez, Roig-Sagués, & Mora-Ventura, 1994). However, most of these studies focused only on mesophilic HPB, and the few studies on psychrophilic HPB isolated from a very limited number of fish species. In the present study, both mesophilic and psychrophilic HPB were isolated from a wide range of same fish samples. Moreover, whereas most of these studies identified HPB isolates by culture method, this study used molecular method, which is more accurate at the species-level identification.

2. Materials and methods

2.1. Fish samples

The fish samples used in this study included tuna (*Thunnus* spp.), horse mackerel (*Trachurus japonicus*), mackerel (*Scomber* spp.), bonito (*Katsuwonus pelamis*), saury (*Cololabis saira*), sardine (*Sardinops melanostictus*, *Etrumeus teres*), grunt (*Parapristipoma trilineatum*), yellowtail (*Seriola quinqueradiata*), swordfish (*Xiphiidae gladius*), flying fish (*Cypselurus pinnatibarbatus japonicus*), and herring (*Clupea pallasii*) (Table 1). These fish species were chosen from those that were previously reported to cause HFP (Toda et al., 2009) or red-fleshed fish with high levels of free histidine (Lehane & Olley, 2000). The fish samples were kept on ice during transportation and processed within 1 h of the purchase.

2.2. Isolation of HPB from fish samples

HPB were isolated following a protocol described in a previous study (Takahashi, Kimura, Yoshikawa, & Fujii, 2003) with some minor modifications. Muscle tissue underneath the skin (10 g) was aseptically removed from the samples and placed in 90 mL of histidine broth containing (per liter) 10 g of Bacto Peptone (Becton, Dickinson, and Company, [BD] Franklin Lakes, New Jersey), 3 g of Bacto Yeast Extract (BD), 5 g of glucose (Wako Pure Chemical Ind. Ltd., Osaka, Japan), and 5 g of L-histidine (Wako) in 50% artificial seawater (ASW) at pH 5 (Smith, Sutton, Fuerst, & Reichelt, 1991). After homogenates of mixture were incubated for 24 h at 30 °C for mesophilic HPB or 48 h at 15 °C for psychrophilic, 5 μL of the culture was used to assay for the presence of Hm using paper chromatography described previously (Takahashi et al., 2003). Briefly, 5 μL of the homogenates was applied to Advantec filter paper (no.51B; 40 cm by 40 cm; Toyo Roshi, Tokyo, Japan). A solvent consisting of 100% 1-butanol and 10% NH_4OH (1:1) was applied for 90 min. After dried at room temperature, applying Pauly's diazo reagent made the histamine spots visualized. Homogenates that tested positive for Hm were streaked on Niven medium containing (per liter) 5 g of Bacto Tryptone (BD), 5 g of Yeast extract (BD), 27 g of L-histidine (Wako), 1 g of $CaCO_3$ (Kanto Chemical Co., Inc., Tokyo, Japan), 15 mL of 0.4% bromocresol purple (Tokyo Kasei Kougyou, Tokyo, Japan), and 20 g of agar (Kokusan Chemical Co. Ltd., Tokyo, Japan) (pH 5) in 50% ASW (Niven, Jeffrey, & Corlett, 1981) and incubated at 30 °C for 24 h or 15 °C for 72 h. Five to ten light purple colonies were picked and inoculated into histidine broth. After incubation at 30 °C for 24 h or 15 °C for 48 h, 5 μL of the culture was used to confirm the presence of Hm by paper chromatography. Strains that tested positive for Hm were defined as Hm-positive strains.

Table 1
Prevalence of histamine-producing bacteria in fish samples from retail markets in and near Tokyo Japan.

Source	No. of samples	No. of histamine-positive homogenate samples (%)		No. of histamine-producing bacterial isolates	
		30 °C	15 °C	30 °C	15 °C
Tuna	24	13 (54.2)	7 (29.2)	18	11
Horse mackerel	21	8 (38.1)	19 (90.5)	12	25
Mackerel	16	8 (50.0)	13 (81.3)	10	20
Bonito	14	5 (35.7)	3 (21.4)	7	5
Saury	14	6 (42.9)	10 (71.4)	7	12
Sardine	13	7 (53.8)	10 (76.9)	11	15
Grunt	11	5 (45.5)	7 (63.6)	7	13
Yellowtail	10	5 (50.0)	1 (10.0)	8	1
Swordfish	10	6 (60.0)	5 (50.0)	10	7
Flying fish	6	4 (66.7)	4 (66.7)	4	5
Herring	4	2 (50.0)	3 (75.0)	2	4
Total	143	69 (48.3)	82 (57.3)	96	118

2.3. Measurement of the Hm-producing ability of HPB isolates

Hm-positive strains isolated at 30 °C were inoculated at an initial density of 10^6 CFU/mL into histidine broth and incubated at 30 °C for 24 h, whereas those isolated at 15 °C were inoculated at the same initial density and incubated at 15 °C for 48 h. After incubation, cultures were sterilized with a 0.2-μm filter (Toyo Roshi). Hm accumulation in the culture filtrate was measured using Check color Histamine (Kikkoman Corp., Chiba, Japan) according to the manufacturer's instructions. Strains that produced more than 100 mg/kg Hm were defined as HPB. When 2 or more strains were isolated from the same fish sample and showed equal Hm producing abilities, only 1 strain was selected for identification because they could be clonal isolates. Strains isolated at 30 °C were defined as mesophilic HPB, whereas those isolated at 15 °C were defined as psychrophilic HPB.

2.4. Identification of HPB

The method used for the identification of HPB was described previously (Takahashi et al., 2003). Mesophilic and psychrophilic HPB were inoculated into histidine broth and incubated at 30 °C for 24 h and 15 °C for 48 h, respectively. After the DNA of these strains was extracted using a DNA extraction kit (Mag Extractor-Genome; Toyobo Co. Ltd., Tokyo, Japan), the 16S ribosomal RNA (rRNA) was amplified in a Veriti thermal cycler (Life Technologies Corp., Carlsbad, California). PCR products (5 μL) were separated by 1% agarose gel electrophoresis along with suitable molecular markers (100-bp ladder; Amersham Biosciences Corp., Piscataway, New Jersey). After electrophoresis, the gels were stained with ethidium bromide and visualized under a UV (245 nm) trans-illuminator.

The isolates were identified by amplifying 400–450 bp of the 16S rRNA using the universal primers 27F and 1492R (Weisburg, Barns, Pelletier, & Lane, 1991), and then directly sequencing the purified products using primer 27F. The BLAST 2.0 algorithm was used to compare the amplified sequences with those in the DNA data bank of Japan (DDBJ; Shizuoka, Japan) (http://www.ddbj.nig.