J.Natn.Sci.Foundation Sri Lanka 2023 51 (2): 215 - 223
DOI: http://dx.doi.org/10.4038/jnsfsr.v51i2.10819

RESEARCH ARTICLE

Food Microbiology

Prevalence of histamine forming bacteria in selected nodes of the supply chain of Sri Lankan Yellowfin tuna

PH Ginigaddarage[1*], GJ Ganegama Arachchi[1], KWS Ariyawansa[1] and CM Nanayakkara[2,3]
[1] *Institute of Post Harvest Technology, National Aquatic Resources Research and Development Agency, Nara Road, Colombo 15, Sri Lanka.*
[2] *Department of Plant Sciences, Faculty of Science, University of Colombo, Colombo 03, Sri Lanka.*
[3] *Sri Lanka Institute of Biotechnology, Pitipana, Homagama, Sri Lanka.*

Submitted: 02 December 2021; Revised: 21 June 2022; Accepted: 23 September 2022

Abstract: This study aimed to identify histamine-forming bacteria (HFB) and the sources of introduction of such bacteria to recommend control measures to mitigate histamine formation in yellowfin tuna (YFT). Field samples were collected from multi-day boats that landed at Dikkowita, Negombo, Trincomalee and Dondra fishery harbours. Ice from the fish holds (n=63) and chill transport vehicles (n=63), and swabs from the fish holds (n=63), the boat decks (n=63) and the skin of YFT (n=63) were collected. Fish loin samples (n=15), ice samples (n=36) and swabs from the skin of YFT (n=18), floor (n=18) and chill transport vehicles (n=18) were collected from fish processing plants. Presumptive HFB isolated from Nivens medium and Violet Red Bile Glucose (VRBG) agar were screened for histamine forming ability in Tripticase soy broth (TSB) supplemented with 1.0% L-histidine. HFB isolates were characterized by sequencing approximately 1400 bp of the 16S rDNA. Seven isolates that produced histamine in the range of 3000–4000 ppm in TSB isolated from ice samples, and a swab sample collected from the boat deck, were confirmed as *Klebsiella aerogens* (n=6) and *Morganella morganii* (n=1) respectively. *Hafnia alvei* (n=1), *Serratia* sp. (n=2), *Citrobacter freundii* (n=1), *Rahnella* sp. (n=1) and *Aeromonas salmonicida* (n=8) were also among the isolated histamine forming bacteria. *Pseudomonas* sp. (n=24) and *Shewanella baltica* (n=7), which are known as spoilage bacteria were also isolated and showed weak histamine formation. Hence, it is evident that histamine-forming bacteria could be introduced into the fish from ice and contacting surfaces. This necessitates the practice of rigorous cleaning procedures and adaptation of proper postharvest handling procedures to minimize contamination of the fish.

Keywords: Fish processing factories, histamine forming bacteria, multiday boats, yellowfin tuna.

INTRODUCTION

Histamine is formed by the bacterial decarboxylation of free histidine in fish muscle when the fish are subjected to temperature abuse during or after harvest. Histamine toxicity or scombroid poisoning is generally a mild illness with a range of symptoms, including rash, nausea, vomiting, diarrhea, flushing and tingling, and itching of the skin (Taylor & Sumner, 1987). Scombroid fish (e.g. tuna, mackerel, bonito) contain high levels of free histidine in their muscles and are related to scombroid poisoning (Taylor *et al*., 1989). Several fish species, which are not categorized as scombroid fish, such as mahi-mahi, bluefish, herring, and sardine are also found to be associated with incidents of scombroid poisoning (Price & Melvin, 1994). In most cases, the histamine levels in illness-causing fish have been above 200 ppm, often above 500 ppm (USFDA, 2021). Scombrotoxin food poisoning will only occur in healthy individuals when a dose of at least 50 mg histamine is consumed, and this generally implies that the fish would have histamine levels exceeding 200 mg/kg (FAO, 2013).

Once the fish is killed, its immune system is inactivated enabling the gill and gut colonizing bacteria to invade the internal tissues. The growth of histamine forming bacteria is more rapid at high-abuse temperatures (e.g. 21.1 °C or higher) than at moderate-abuse temperatures (e.g. 7.2 °C). Most of the histamine forming bacterial growth is particularly rapid at temperatures near 32.2 °C (USFDA, 2021). Despite maintaining the

* Corresponding author (hasangi_g@yahoo.com; https://orcid.org/0000-0003-4815-719X)

recommended chilling conditions, the production of toxic concentrations of histamine by psychrotolerant bacteria is the main reason for fish spoilage and poisoning at 0–5 ^{0}C (Dalgaard & Emborg, 2008). The enzyme histidine decarboxylase can remain stable in frozen fish and can be reactivated after thawing. Once it is formed, the production of histamine can continue at or near refrigeration temperatures (Visciano *et al*., 2014).

A variety of histamine forming bacteria have been isolated and identified in different studies carried out around the world. Gram-negative enteric and marine bacteria are mainly associated with histamine formation in fish (Kim *et al*., 2003). Taylor and Speckard (1983) have reported that *Morganella morganii*, *Klebsiella pneumonia* and *Hafnia alvei* were isolated from fish, which caused scromboid poisoning. Several studies have reported that some enteric bacteria, including *Proteus vulgaris*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Serratia fonticola*, *Serratia liquefaciens* and *Citrobacter freundii* were able to produce histamine (Yoshinaga & Frank 1982; Taylor & Speckard 1983; Middlebrooks *et al*., 1988; Kim *et al*., 2003; Tsai *et al*., 2004). *Clostridium* sp., *Vibrio alginolyticus*, *Acinetobacter lowffi*, *Plesiomonas, shigelloides*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Aeromonas* sp. and *Photobacterium* sp. have also been reported as histamine producers (Middlebrooks *et al*., 1988; Yatsunami & Echigo 1991; Okuzumi *et al*., 1994). Tsai *et al*., in 2004 reported that *Proteus*, *Enterobacter*, *Klebsiella*, *Rahnella* and *Acinetobacter* isolated from sailfish fillets are histamine producers.

Sri Lanka is a small tropical island in the Indian Ocean claiming an Exclusive Economic Zone (EEZ) area of 517,000 km^2. According to the Ministry of Fisheries and Aquatic Resources of Sri Lanka (MFAR, 2021), the total fisheries production of the marine sector in 2019 was 415,490 Mt. with tuna exports amounting to 14,252 Mt. Approximately 40% of the Yellowfin tuna (YFT) has been rejected when selecting for exports because of their poor quality (*NARA, 2017, Unpublished data*). Elevated histamine content and improper handling practices are some of the reasons for tuna fish rejections. Since histamine is formed due to the presence of contaminant bacteria, identification of histamine producers and investigation of their sources would help to control histamine formation in fish to a certain extent. Large size YFT (>30 kg) have a high demand in export markets including the European countries, USA, and Japan. Hence controlling the growth of histamine forming bacteria in fish can increase the fish quality resulting in an expansion of the export market for fish. However, detailed information is scarce on large size YFT (>30 kg) contamination by histamine forming bacteria in the supply chain. Thus, the aim of this study was to investigate histamine forming bacteria in export size YFT in selected nodes of the supply chain to isolate, characterize, and identify the sources of contamination.

MATERIALS AND METHODS

Field sampling

Fish, ice and swab samples were collected from multi-day boats and chill transport vehicles during unloading of fish at Dikkowita (DK) (7.0049°N, 79.8664°E), Negombo (NE) (7.2040°N,79.8277°E), Trincomalee (TM) (8.5693°N,81.1958°E), and Dondra (DO) (5.921°N,80.5837°E) fishery harbours, and from selected fish processing plants located in the Western Province of Sri Lanka. The number of samples and sampling locations are given in Table 1.

Table 1: Sampling points and number of samples collected from different locations

Sample description	Number of samples						
	Fishery harbours				Processing plants		
	Dikkowita	Negombo	Trincomalee	Dondra	P1	P2	P3
Ice from fish holds of multiday boats	24	15	15	09	-	-	-
Swabs from fish holds	24	15	15	09	-	-	-
Swabs from boat decks	24	15	15	09	-	-	-
Swabs from skin of YFT	24	15	15	09	06	06	06
Ice from chill transport vehicles	24	15	15	09	06	06	06
Fish loin samples	-	-	-	-	06	03	06
Swabs of floor of processing plant	-	-	-	-	06	06	06
Ice from processing plant	-	-	-	-	06	06	06
Swabs from chill trucks	-	-	-	-	06	06	6

Surface sampling using swabs

Surface sampling was done as per ISO 18593 (2018). A stick swab was removed from its sterile wrapping and dipped in a tube containing the neutralizer (Dey-Engley neutralizing broth, Himedia M1062) to moisten the tip of the swab. The tip of the swab was pressed against the wall of the tube to remove any excess neutralizer. The moistened tip of the swab was placed on the sampling surface to be examined and an area of 100 cm^2 was streaked using a sterile template. The stick swab was returned to the tube containing sterile phosphate buffer as the diluent and the tube was closed properly to ensure that the swab was moist until analysis. Samples were brought to the Quality Control Laboratory (Microbiology Unit) of the National Aquatic Resources Research and Development Agency (NARA) under chilled conditions for analysis.

Collection of ice and fish samples

About 500 g of ice samples and 500 g of fish loin samples of YFT with a weight in the range of 30–64 kg were collected aseptically in sterile polythene bags and brought to the laboratory under chilled conditions for analysis.

Isolation and identification of histamine forming bacteria

Isolation of histamine-forming bacteria was done using two selective media: Nivens medium fortified with L-histidine (Niven *et al*., 1981) and Violet Red Bile Glucose (VRBG) agar (OXOID CM0485). Sample dilutions (10^{-1}, 10^{-2} and 10^{-3}) were carried out, and 1 mL of the diluted solution was spread on the respective medium. The Nivens medium plates were incubated at 35 °C and 25 °C for 4 days, and the VRBG agar plates were incubated at 25 °C for 48 h. Colonies that were blue or purple color on the Niven's plates and purple/pink on the VRBG agar plates were picked and streaked on Trypticase Soy Agar (TSA). Purple/pink colonies on the VRBG agar plates at 25 °C were considered as Enterobacteriaceae and counted using a colony counter. Repeated streaking was performed to isolate bacteria into pure cultures for further analysis.

Histamine content in fish samples

The histamine content of the fish samples was analysed according to the AOAC 977.13 method (AOAC, 2012).

Histamine formation by bacterial isolates

Histamine production by the bacterial isolates was determined in duplicate by the modified AOAC 977.13 method (Kim *et al*., 2004). One loopful of bacterial culture from a TSA slant was inoculated into 10 mL of Tryptone Soy Broth (TSB), supplemented with 1% L-histidine and 3% NaCl, and incubated at 35 °C for 24 h. After incubation, 1 mL of the bacterial culture broth was added to 9 mL of 75% methanol and heated at 60 °C for 15 minutes in a water bath to extract histamine. The concentration of histamine in the extract was determined by column elution, and o-phthalaldehyde (OPT) derivatization followed by measuring the fluorescence intensity using a spectrofluorometer (RF 1501 - Shimadzu).

Characterization of histamine forming bacterial isolates

Cultures that were positive for histamine formation were biochemically tested using API 20E. These cultures were further confirmed by amplifying and sequencing approximately 1400 bp of the 16S ribosomal DNA (rDNA) for bacteria. Primers used for the amplification were UNI-L-5′-AGAGTTTGATCATGGCTCAG-3′ and UNI-R–5′- GTGTGACGGGCGGTGTGTAC-3′ (Huang *et al*., 2010). PCR amplification was performed in a 50 µL reaction mixture containing 1X buffer, 1.5 mM $MgCl_2$, 0.3 µM of each primer, 200 µM deoxynucleotide triphosphates, 1.25 U of Taq DNA polymerase (Promega), and template DNA (10 ng). Amplifications were conducted for 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s with an initial denaturation at 94 °C for 4 min and a final extension at 72 °C for 7 min (Huang *et al*., 2010). Amplicons were detected by electrophoresis on a 2.0% agarose gel using Diamond Nucleic acid dye (Promega). PCR products were sent to Macrogen Inc., South Korea for purification and sequencing. Extraction of bacterial DNA was done by using HiPurA bacterial genomic DNA extraction and purification kit (Himedia MB505) according to their instructions. *M. morganii* (ATCC25830) and *Klebsiella aerogens* (ATCC13048) were used as positive controls.

Sequences obtained for each isolate were compared with sequences of other reference strains from the US National Center for Biotechnology Information (NCBI) data base using the BLAST tool. MEGA X software with the maximum likelihood method taking *Acidicapsa borealis* as the out group and bootstrap value of 1000 replicates was used to construct the phylogenetic tree of 16S rDNA sequences of major histamine producing bacterial species.

RESULTS AND DISCUSSION

Detection of HFB in fish processing plants

Samples collected from fish processing plants contained *Aeromonas salmonicida*, *Aeromonas* sp., *Pseudomonas* sp., *C. freundii, Shwenella baltica* and *Rahnella* sp. (Table 2). These bacteria were isolated from swabs taken from the surface of fish skin and chill trucks, ice samples, and fish loin samples. The isolated bacteria formed histamine in 1% histidine broth in the range of 4–200 ppm. The weight of fish from which loin samples were obtained was in the range of 30–64 kg and histamine content ranged from 4–49 ppm. Ice samples produced by the fish processing factories and the swabs collected from the floor of the fish processing plants did not give histamine positive isolates.

Table 2: Isolated bacterial species from the fish processing plants and the number of isolates, the place from which the samples were obtained and the amount of histamine formed by each species

No.	Isolated bacterial species	No of isolates	Places from which the samples were obtained	Amount of histamine formed (ppm)
1	*C. freundii*	01	Fish loin	180
2	*A. salmonicida*	07	Surface of fish skin, Ice from chill truck, truck inner surface	04–50
3	*S. baltica*	02	Truck inner surface	08–15
4	*Rahnella* sp.	01	Surface of fish skin	08
5	*Pseudomonas* sp.	01	Surface of fish skin	07
6	*Aeromonas* sp.	01	Surface of fish skin	05

Ice samples collected from the fish processing plants contained <1 CFU/mL of Enterobacteriaceae indicating that the ice produced by the factories was of good quality. Enterobacteriaceae counts of fish loin samples were less than 1.0×10^2 CFU/g.

Table 3: Average Enterobacteriaceae counts in the samples obtained from fish processing plants

Sample location/type	Average Enterobacteriaceae count
Swabs from fish skin	2.0×105 CFU/cm^2
Swabs from inner surface of chill transport vehicles	6.8×103 CFU/cm^2
Swabs from the floor of processing plant	7.0×103 CFU/cm^2
Ice samples from chill transport vehicles	1.8×104 CFU/mL
Ice from processing plant	< 1 CFU/mL
Fish loin samples	<1.0×102 CFU/g

Klebsiella ozaenae, Vibrio alginolyticus and *Aeromonas* sp. have been reported as contaminants in fish processing facilities (Gingerich *et al*., 2001). Hu *et al*., (2015) have reported *C. freundii* as a histamine forming species in Blue Scad (*Decapterus maruadsi*) and Chub Mackerel (*Scomber japonicas*), which corroborates the findings of the present study.

Detection of HFB in fishery harbours

Histamine forming bacteria isolated from samples collected from multiday boats (MDB) landed at the fishery harbours are given in Table 4. Histamine forming *K. aerogens, Pseudomonas* sp., and *Serratia* sp. were detected from the ice samples collected from chill transport vehicles at the Dikkowita fishery harbour. Swab samples collected from boat decks contained *Pseudomonas* sp. and *M. morganii*. Two swab samples taken from fish surface were positive for *Pseudomonas* sp. and *A. salmonicida*. Samples collected from the Negombo fishery harbour contained mostly *Pseudomonas* sp. and formed less than 30 ppm of histamine in the trypticase soy broth supplemented with 1% L-histidine.

H. alvei and *Pseudomonas* sp. were isolated from ice samples collected from the chilled transport vehicles at the Trincomalee fishery harbour. In addition, *Pseudomonas* sp. were also isolated from swab samples collected from fish holds, fish skin and boat decks.

A *Serratia* sp. isolated from an ice sample, was capable of producing histamine at a concentration above 1000 ppm under laboratory conditions; this sample was collected from a multiday boat landed at the Dondra fishery harbour. Further, from the same location, isolates of *S. baltica* were identified in a swab sample collected from the fish hold of a multiday boat, ice samples collected from fish holds, and swab sample collected from fish skin. Pure cultures of *K. aerogenes* (ATCC 13048) and *M. morganii* (ATCC 25830) produced 3300 ppm and 3400 ppm of histamine, respectively, in tryptic soy broth supplemented with 1% L-histidine.

Table 4: Isolated bacterial species from the fishery harbours and the number of isolates, the place from which the samples were obtained and the amount of histamine formed by each species

Isolated bacterial species	No. of isolates	Places from which the samples were obtained	Amount of histamine formed (ppm)
K. aerogenes	06	Ice collected from chill transport vehicles	3000–4000
Pseudomonas sp.	23	Ice collected from chill transport vehicles, swabs from boat decks, swabs from fish skin, ice collected from fish holds, swabs from fish holds	05–35
Serratia sp.	02	Ice collected from chill transport vehicles; ice collected from fish holds	100–1600
M. morganii	01	Swab from boat deck	3500
A. salmonicida	01	Swabs from fish skin	30
H. alvei	01	Ice collected from chill transport vehicles	75
Shewanella sp.	01	Swabs from fish skin	26
S. baltica	05	Swabs from fish holds, ice collected from fish holds, Ice collected from chill transport vehicles	05–17

Hu *et al*., (2014) reported several histamine forming bacterial species such as *Arthrobacter bergeri*, *Pseudomonas* sp., *Psychrobacter*, *S. baltica* and *A. salmonicida* in Blue scad where *A. salmonicida* produced the highest amount, i.e., 115 ppm. In the present study, eight isolates of *A. salmonicida* produced histamine at <50 ppm and *S. baltica* (07 isolates) and *Pseudomonas* sp. (24 isolates) showed weak histamine formation under laboratory conditions. Lou *et al*. (2021) have reported that *S. baltica* has a high potential to decompose nutrients in fish. Even though *S. baltica* does not produce high amounts of histamine, because of its spoilage capacity, these bacteria should be controlled to prevent contamination of fish to reduce spoilage potential. *Pseudomonas* sp. isolated in the present study indicated weak ability to form histamine as the amount of histamine formed was in the range of 5–35 ppm. This is in agreement with the studies of Nor-khaizura *et al*. (2009), Koohdar *et al*. (2011), Soliman *et al*. (2017), and Simora and Peralta (2018). However, Fernandez-No *et al*., (2011) reported that a *Pseudomonas fragi* strain isolated from turbot was a stronger histamine producer than the known histamine producing strain *Enterobacter aerogenes* ATCC 13048. In addition, *Pseudomonas* sp. is also known as an indicator of food quality as it is a spoilage organism (Koutsoumanis & Nychas, 2000; Jeyasekaran *et al*., 2006; Yagoub, 2009). Therefore, growth of such spoilage bacteria on direct fish contacting surfaces should be controlled to minimize contamination. *M. morganii* isolated from a swab collected from a boat deck formed 3500 ppm of histamine under laboratory conditions. Six isolates of *K. aerogens* found in ice samples collected from chill transport vehicles produced histamine in the range of 3000–4000 ppm. These findings on *M. morganii* and *K. aerogens* are supported by the studies of Kim *et al*. (2001), Koohdar *et al*. (2011) and Butler *et al*. (2015).

Kim *et al*., (2001) and Tsai *et al*., (2004) also reported *Serratia* sp., *Rahnella* sp., and *H. alvei* as histamine forming bacteria.

It was noted that the swab samples drawn from the skin of fish had an Enterobacteriaceae count ranging from 2.0×10^5 to 8.0×10^5 CFU/cm^2 (Table 5). Swabs from boat decks and fish holds varied from 1.8×10^5 to 9.6×10^5 and 1.8×10^5 to 7.6×10^5 CFU/cm^2, respectively. Ice samples collected from chill transport vehicles contained an Enterobacteriaceae count ranging from 6.6×10^2 to 1.2×10^5 CFU/mL while ice samples collected from fish holds of multiday boats had counts in the range of 3.0×10^6 to 8.6×10^7 CFU/mL. These results indicate that there is a considerable amount of Enterobacteriaceae present in all the samples analysed.

Table 5: Average Enterobacteriaceae counts (at 25 °C) of samples collected from the fishery harbours

Sampling location/type	Fishery harbour			
	Dikkowita	Negombo	Trincomalee	Dondra
Swabs from fish skin (CFU/cm^2)	5.3×10^5	2.6×10^5	8.0×10^5	2.0×10^5
Swabs from boat decks (CFU/cm^2)	2.1×10^5	9.6×10^5	1.8×10^5	4.7×10^5
Swabs from fish holds (CFU/cm^2)	1.9×10^5	7.6×10^5	4.7×10^5	1.8×10^5
Ice samples from chill transport vehicles (CFU/mL)	3.0×10^4	6.6×10^2	4.8×10^3	1.2×10^5
Ice from fish holds (CFU/mL)	3.0×10^6	8.6×10^7	4.6×10^7	4.2×10^6

Normally, the total number of microorganisms on the skin surface of fish varies in the range of 10^2–10^7 CFU /cm^2, while both the gills and the intestines contain 10^3–10^7 CFU/g (Huss, 1995). The Enterobacteriaceae count is considered as an index of fish quality and can be used for routine monitoring. The presence of Enterobacteriaceae warrants further testing for specific pathogens. The main sources of contaminants are apparently the fish contact surfaces, which suggests the necessity of a good cleaning procedure for the contact surfaces.

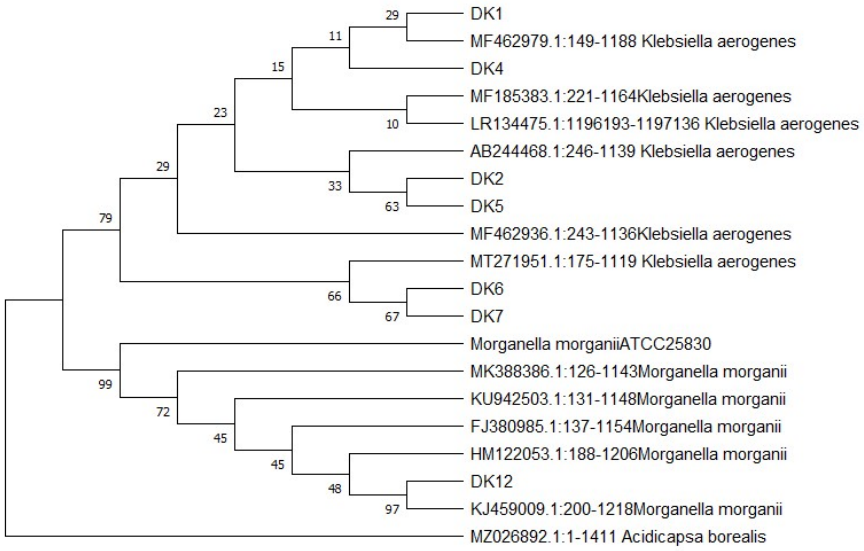

Figure 1: Phylogenetic analysis of the 16S rDNA sequences of major histamine-forming bacteria. Numbers above and below branches indicate bootstrap values from the maximum likelihood method.

In the present study, *K. aerogens,* known as a prolific histamine producer, was isolated on several occasions from ice samples collected from chill transport vehicles. Besides *K. aerogens,* other strong and weak histamine producers were also isolated from ice samples collected from chill transport vehicles and ice from fish holds of

multiday boats. Boat decks and the surface of fish holds were also found to contain histamine forming bacteria. *Pseudomonas* sp. and *S. baltica*, which are weak histamine producers and known to be spoilage bacteria, were also isolated from fish contact surfaces and ice samples. Subburaj *et al*., (1984) have isolated histamine forming bacteria in fish market environments and from ice, water, and baskets used when handling fish in India. In a study conducted in Greece, Economou *et al*., (2016) have also reported that considerable amounts of histamine producing bacteria were present in ice used to preserve fish.

Identification of isolated bacteria by sequencing of 16S rDNA

The phylogenetic tree constructed using the 16S rDNA sequences of isolated major histamine forming bacterial species is given in Figure 1. The six isolates, DK1, DK2, DK4, DK5, DK6 and DK7, were clustered together with *K. aerogens* MF185383.1:221-1164, LR134475.1:1196193-1197136, MF462936.1:243-1136, AB244468.1:246-1139, MF462979.1:149-1188 and MT271951.1:175-1119 strains.

Sequences of the isolated organisms had more than 99% similarity values when compared with the NCBI database (Table 6). The isolate DK12 clustered with KJ459009.1:200-1218 *M. morganii* strain and had more than 98% similarity with the NCBI database.

Table 6: Identification of isolated major histamine producers by using 16s rDNA based on the NCBI database with the % similarity and E value

Isolate	Organism	% Similarity	E value	Accession number
DK1	*K. aerogens*	99.33	0.0	OL468744
DK2	*K. aerogens*	99.79	0.0	OL468745
DK4	*K. aerogens*	99.57	0.0	OL468746
DK5	*K. aerogens*	100.00	0.0	OL468747
DK6	*K. aerogens*	99.59	0.0	OL468748
DK7	*K. aerogens*	99.89	0.0	OL468749
DK12	*M. morganii*	98.92	0.0	OL468707

Out of the six *K. aerogenes* isolates, DK1, DK4 clustered closely with MF462979, while DK 2 and DK 5 clustered separately showing some clear genetic differences. In addition, DK 6 and DK 7 formed a distinctly separate cluster indicating further drifting from the earlier four isolates. The bootstrap percentages for the clustering of DK1 was 29% and DK4 was 11% while for DK6 and DK7 it was 67%. Divergence of DK 6 and 7 from the main cluster has a bootstrap percentage of 66. Hence, although different clustering patterns were observed, it can be concluded that all the isolates are *K. aerogenes*. Interestingly, although clustering with *M. morganii*, the current isolate of DK 12 showed a distinct genetic diversity from the recorded isolates as evident by a bootstrap percentage of 97.

CONCLUSION

It can be concluded that histamine forming bacteria were present on direct fish contacting surfaces such as ice, boat deck, etc. Similarly, the direct fish contacting surfaces were the sources of *Pseudomonas* sp. and *S. baltica*, which are known as weak histamine producers (<35 ppm of histamine was produced in this study) and spoilage bacteria. Hence a risk of cross-contamination of fish is evident. It is necessary to control the occurrence and growth of these bacteria by adopting proper handling and cleaning practices. The quality of ice used in the fishery industry should be improved using good quality water for manufacturing ice and providing clean and hygienic storage facilities. Crushing and transportation of ice should also be done in a hygienic manner to reduce bacterial contaminations.

Acknowledgement

The authors gratefully acknowledge the staff of Institute of Post-Harvest Technology of the National Aquatic Resources Research and Development Agency (NARA), Sri Lanka and staff of fishery harbours and fish processing plants for the support given during this study.

REFERENCES

AOAC (2012). *Histamine in Sea food, AOAC Official Method 977.13*. AOAC International, Gaithersburg, MD, USA.

Butler K.B., Bowers J.C. & Benner Jr R.A. (2015). Prevalence and characterization of high histamine–producing bacteria in Gulf of Mexico fish species. *Journal of Food Protection* **78**(7): 1335–1342. DOI: https://doi.org/10.4315/0362-028X.JFP-15-012

Dalgaard P. & Emborg J. (2008). Histamine and biogenic amines: formation and importance in seafood. In: *Improving Seafood Products for the Consumer* (ed. Torger BØrresen), pp. 292–324. Woodhead publishing Limited, Cambridge, England.

Economou V., Gousia P., Kemenetzi D., Sakkas H. & Papadopoulou C. (2016). Microbial quality and histamine producing Microflora analysis of the ice used for fish preservation. *Journal of Food Safety* **37**: 1–8. DOI: https://doi.org/10.1111/jfs.12285.

Fernández-No I.C., Böhme K., Calo-Mata P. & Barros-Velázquez J. (2011). Characterisation of histamine-producing bacteria from farmed blackspot seabream (*Pagellus bogaraveo*) and turbot (*Psetta maxima*). *International Journal of Food Microbiology* **151**(2011): 182–189. DOI: https://doi.org/10.1016/j.ijfoodmicro.2011.08.024.

FAO (2013). *Public Health Risks of Histamine and other Biogenic Amines from Fish and Fishery Products*. Meeting report. FA O Headquarters, Rome, Italy. Available at *https://www.fao.org/3/i3390e/i3390e.pdf* . Accessed 16 June 2021.

Gingerich T.M., Lorca T., Flick Jr G.J., Mc Nairand H.M. & Pierson M.D. (2001). Isolation of histamine-producing bacteria from fish-processing facilities and fishing vessels. *Journal of Aquatic Food Product Technology* **10**(3): 61–66. DOI: https://doi.org/DOI:10.1300/J030v10n03_06.

Hu J.W, Cao M.J., Guo S.C., Zhang L.J., Su W.J. & Liu G.M. (2015). Identification and inhibition of histamine-forming bacteria in Blue Scad (*Decapterus maruadsi*) and Chub Mackerel (*Scomber japonicus*). *Journal of Food Protection* **78**(2): 383–389. DOI: https://doi.org/10.4315/0362-028X.JFP-14-296

Hu Y., Huang Z. & Chen X. (2014). Histamine producing bacteria in Blue Scad (*Decapterus maruadsi*) and their abilities to produce histamine and other biogenic amines. *World Journal of Microbiology and Biotechnology* **30**(8): 2213–2221. DOI: https://doi.org/10.1007/s11274-014-1642-z.

Huang Y.R., Liu K.J., Hsieh H.S., Hsieh C.H., Hwang D.F. & Tsai Y.H. (2010). Histamine level and histamine-forming bacteria in dried fish products sold in Penghu Island of Taiwan. *Food Control* **21**: 1234–1239. DOI: https://doi.org/10.1016/j.foodcont.2010.02.008.

Huss H.H. (1995). *Quality And Quality Changes in Fresh Fish*, FAO Fisheries Technical Paper (FAO), p. 348. FAO Headquarters, Rome, Italy.

International Organization for Standardization (ISO) (2018). Microbiology of the food chain — Horizontal methods for surface sampling ISO Standard No.18593:2018

Jeyasekaran G., Ganesan P., Anandaraj R., Shakila R.J. & Sukumar D. (2006). Quantitative and qualitative studies on the bacteriological quality of Indian white shrimp (*Penaeus indicus*) stored in dry ice. *Food Microbiology* **23**(6): 526–533. DOI: https://doi.org/10.1016/j.fm.2005.09.009

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

Kim S.H., Eun J.B., Chen T.Y., Wei C.I., Clemens R.A. & An H. (2004). Evaluation of histamine and other biogenic amines and bacterial isolation in canned anchovies recalled by the USFDA. *Journal of Food Science* **69**: 157–162.

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 fresh and temperature-abused Albacore. *Journal of Food Protection* **64**(7): 1035–1044. DOI: https://doi.org/10.4315/0362-028x-64.7.1035.

Koohdar V.A., Razavilar V., Motalebi A.A., Mosakhani F. & Valinassab T. (2011). Isolation and identification of histamine-forming bacteria in frozen Skipjack tuna (*Katsuwonus pelamis*). *Iranian Journal of Fisheries Sciences* **10**(4): 678–688.

Koutsoumanis K. & Nychas G.J. (2000). Application of systemic experimental procedure to develop a microbial model for rapid fish shelf-life predictions. *International Journal of Food Microbiology* **60**(2–3): 171–184. DOI: https://doi.org/10.1016/s0168-1605(00)00309-3.

Lou X., Zhaia D. & Yanga H. (2021). Changes of metabolite profiles of fish models inoculated with *Shewanella baltica* during spoilage. *Food Control* **123**(2021)107697: 1–12. DOI: https://doi.org/0.1016/j.foodcont.2020.107697

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**(4): 1024–1029.
DOI: https://doi.org/10.1111/j.1365-2621.1988.tb13522.x

MFAR (2021). Fisheries Statistics 2020. Available at: *https://www.fisheries.gov.lk/web/images/statistics/annual_report/FISHERIES_STATISTICS_2020-min.pdf*. Accessed on 16 June 2021.

Niven C.F., Jeffreg M.B. & Corlett D.A. (1981). Differential plating medium for quantitative detection of histamine-producing bacteria. *Applied Environment and Microbiology* **41**(1): 321–322.

Nor-khaizura M.A.R., Zaiton H., Jamilah B., Rusul R.A.G. & Smail-Fitry M.R. (2009). Histamine and histamine-forming bacteria in *Keropoklekor* (Malaysian Fish Sausage) during processing. *Food Science and Technology Research* **15**(4): 395–402.

Okuzumi M., Hiraishi A., Kobayashi T. & Fujii T. (1994). *Photobacterium histaminum* sp. *nov*., a histamine-producing marine bacterium. *International Journal of Systematic Bacteriology* **44**(4): 631–636.
DOI: https://doi.org/10.1099/00207713-44-4-631.

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, USA.

Simora R.M.C & Peralta E.M. (2018). Occurrence of Histamine and Histamine-forming bacteria in Philippine traditional dried-salted fish products. *Asian Fisheries Science* **31**(2018): 73–88.
DOI: https://doi.org/10.33997/j.afs.2018.31.2.001

Soliman W.S., Shaapan R.F., Mohamed L.A., Younes A.M., Elgendy M.Y. & El din D.A.S. (2017). Laboratory screening of biogenic amines producing bacteria potentially threatens human health in some Egyptian fish and fish products. *Journal of Fisheries and Aquatic Science* **12**(3): 134–140.
DOI: https://doi.org/10.3923/jfas.2017.134.140

Subburaj M., Karunasagar I. & Karunasagar I. (1984) Incidence of histidine decarboxylating bacteria in fish and market environs. *Food Microbiology* **1**: 263–267.
DOI: https://doi.org/10.1016/0740-0020(84)90060-1

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

Taylor S.L., Stratton J.E. & Nordlee J.A. (1989). Histamine poisoning (scombroid fish poisoning): An allergy-like intoxication. *Clinical Toxicology* **27**(4&5): 225–240.
DOI: https://doi.org/10.3109/15563658908994420

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

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**(2): 407–412.
DOI: https://doi.org/10.4315/0362-028x-67.2.407

USFDA (2021). *Fish and Fishery Products Hazards and Controls Guidance*, chapter 7, fourth edition. Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Food Safety, USA. Available at *https://www.fda.gov/media/80637/download*. Accessed 16 June 2021.

Visciano P., Schirone M., Tofalo R. & Suzzi G. (2014). Histamine poisoning and control measures in fish and fishery products. *Frontiers in Microbiology* **5**: 1–3.
DOI: https://doi.org/10.3389/fmicb.2014.00500

Yagoub S.O. (2009). Isolation of *Enterobacteriaceae* and *Pseudomonas spp*. from raw fish sold in fish market in Khartoum state. *Journal of Bacteriology Research* **1**(7): 85–88.

Yatsunami K. & Echigo T. (1991). Isolation of salt tolerant histamine forming bacteria from commercial rice-bran pickle sardine. *Nippon Suisan Gakkaishi* **57**(9): 1723–1728.
DOI: https://doi.org/10.2331/SUISAN.57.1723

Yoshinaga D.H. & Frank H.A. (1982). Histamine-producing bacteria in decomposing skipjack tuna (*Katsuwonus pelamis*). *Applied and Environmental Microbiology* **44**(2): 447–452.
DOI: https://doi.org/10.1128/aem.44.2.447-452.1982