Journal of Food Protection, Vol. 72, No. 9, 2009, Pages 1987–1991

Research Note

Detection of Gram-Negative Histamine-Producing Bacteria in Fish: A Comparative Study

KRISTIN BJORNSDOTTIR,[1] GREGORY E. BOLTON,[1] PATRICIA D. McCLELLAN-GREEN,[2] LEE-ANN JAYKUS,[3] AND DAVID P. GREEN[1]*

[1]*Department of Food, Bioprocessing and Nutrition Sciences and* [2]*Department of Environmental and Molecular Toxicology, North Carolina State University, c/o Center for Marine Sciences and Technology, 303 College Circle, Morehead City, North Carolina 28557; and* [3]*Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, North Carolina 27695, USA*

MS 09-072: Received 13 February 2009/Accepted 10 April 2009

ABSTRACT

Poisoning due to ingestion of foods with elevated levels of biogenic amines (histamine, putrescine, cadaverine, and tyramine) is well documented. Histamine fish poisoning largely is due to growth of naturally occurring bacteria associated with scombroid fish species. A rapid and reliable method is needed to screen for the presence of histamine-forming bacteria in fish. This study included a comparison of three methods for the detection of histamine-producing bacteria. A total of 152 histamine-producing and non–histamine-producing bacteria from multiple sources were screened using a modified Niven's agar method, a potentiometric method, and a PCR-based assay targeting a 709-bp fragment of the histidine decarboxylase gene. Histamine production by bacterial isolates was confirmed by high-performance liquid chromatography (HPLC). Bacterial strains were categorized as producing high amounts of histamine, low amounts of histamine, or no histamine. Of the 152 strains tested, 128 (84%) were positive with the Niven's agar method, 73 (48%) were positive with the potentiometric technique, and 74 (49%) were positive with the PCR assay. Overall, a 38% false-positive rate was observed with the modified Niven's agar method, although this method detected both low-histamine and high-histamine strains. There was a high degree of concordance (>99%) between results of the potentiometric and PCR methods, but neither of these methods detected low-histamine bacteria. These observations support the need for a simple and straightforward yet sensitive method for detecting histamine-producing bacteria in seafood and environmental samples.

Poisoning due to the ingestion of foods with elevated levels of biogenic amines (histamine, putrescine, cadaverine, and tyramine) is well documented *(2)*. Of these amines, histamine is a common cause of foodborne disease, particularly in association with the consumption of fish. For example, between 1990 and 2003, histamine fish poisoning accounted for 7.5% of all foodborne disease outbreaks and 38% of all seafood-related illnesses reported to the U.S. Centers for Disease Control and Prevention *(10)*.

Excess histamine in foods occurs as a result of the activity of amino acid–specific enzymes derived from spoilage bacteria and has been associated with fermented products such as salami, cheese, sauerkraut, and wine. In fish, histamine is sometimes called scombrotoxin because of its common association with scombroid fishes (i.e., tuna, mackerel, and bonito) but has also been associated with nonscombroid fish (mahi-mahi and bluefish), which have high levels of amino acid precursors in their flesh. Histamine fish poisoning occurs largely due to the growth of naturally occurring gram-negative bacteria, e.g., *Morganella morganii*, *Raoultella planticola,* and *Enterobacter aerogenes*. Most of these organisms are mesophiles. Therefore, tight temperature control is important for the prevention of histamine formation in fish, as reflected in the hazard analysis critical control point guidelines of the U.S. Food and Drug Administration *(2)*. Unfortunately, rapid chilling alone may not prevent the formation of high concentrations of biogenic amines by a few psychrotrophic histamine-producing bacteria *(14)*. Once formed, histamine is resistant to commonly used food preservation methods, including freezing, cooking, retorting, and smoking *(12)*.

A rapid and reliable method is needed to screen for the presence of histamine-forming bacteria. Historically, fish products have been screened for the presence of histamine-producing bacteria using Niven's agar, a differential growth medium containing the pH indicator bromocresol purple, tryptone, L-histidine hydrochloride, and a few other components *(5, 24, 26)*. Histamine accumulation occurs during the growth of bacteria and results in a rise in pH, which induces a color change, allowing for visualization of positive bacterial colonies. This method is relatively easy to use and inexpensive. However, some researchers have reported loss of histamine production in bacterial strains after cultivation in culture-based media *(20)*. False-positive reactions also are frequent, largely because of the formation of other (nonhistamine) alkaline compounds during microbial metabolism *(3, 5, 21)*. Investigators have sought to

* Author for correspondence. Tel: 252-222-6304; Fax: 252-222-6335; E-mail: dpg@ncsu.edu.

reduce such false-positive reactions by making various modifications to the differential medium. By manipulating the pH and the incubation time and temperature, Mavromatis and Quantick *(23)* produced a modified Niven's medium with increased selectivity.

The development of streamlined methods for detecting histamine and/or gram-negative histamine-producing bacteria in fish has been an area of active research. A potentiometric method has been reported in which histamine is detected based on an increase in conductance after growth of histamine-producing microorganisms in histidine decarboxylase broth *(19)*. More recently, investigators have focused efforts on the development of molecular techniques (reviewed by Landete et al. *(20)*), which usually target the gene *hdc* encoding the histidine decarboxylase enzyme *(8, 9, 16, 17, 25)*. Takahashi et al. *(25)* developed a rapid molecular method for detection of gram-negative histamine-producing bacteria using PCR followed by single-strand conformation polymorphism analysis.

The purpose of this study was to compare three different methods used for the detection of histamine-producing bacteria. Gram-negative bacteria with and without histamine production capabilities were screened on modified Niven's agar, by conductance change after incubation in histidine decarboxylase broth, and with a PCR-based identification assay. These results were compared directly with histamine production levels obtained using high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Culture library. Histamine-producing and non–histamine-producing bacterial strains were obtained from multiples sources: the American Type Culture Collection (ATCC; Manassas, VA), Dr. Graham Fletcher (New Zealand Institute for Plant and Food Research Ltd., Auckland), Dr. Rachel Nobles (University of North Carolina–Chapel Hill Institute of Marine Sciences, Morehead City), Dr. John Kaneko (PacMar Inc., Honolulu, HI), and Dr. George Flick (Virginia Polytechnic Institute and State University, Blacksburg). Some strains were isolated from fish (gills, belly cavity, and muscle tissue of yellowfin tuna [*Thunnus albacares*], mahi-mahi [*Coryphaena hippurus*], bluefish [*Pomatomus saltatrix*], and wahoo [*Acanthocybium solandri*]) and from environmental samples collected in North Carolina and Hawaii.

Media components were obtained from Becton Dickinson (Sparks, MD) unless otherwise specified. For isolation of naturally occurring histamine-producing bacteria, surface swabs (32 cm^2) or tissue samples (5 g) were enriched in 10 ml of histidine broth (pH 6.5) containing 1% Bacto Proteose Peptone, 0.3% yeast extract, 1.5% NaCl (all obtained from EMD, Gibbstown, NJ), and 0.5% histidine (L-histidine hydrochloride monohydrate, Acros, Morris Plains, NJ) and incubated at 37°C for 24 h. A 1-ml subsample of the enrichment culture was incubated with 9 ml of Trypticase soy broth (TSB) containing 2% histidine, 2% NaCl, and 0.0005% pyridoxal HCl (TSB+; Alexis, Plymouth Meeting, PA) (pH 5.8) for 24 h at 37°C. Histamine formation in the subculture was tentatively identified using the Veratox histamine test kit (Neogen Corp., Lansing, MI). Those enrichment cultures testing positive with the Veratox kit were serially diluted in saline (0.85% NaCl), and 0.1 ml was spread on Trypticase soy agar plates containing 2% NaCl. Ten representative isolates were identified based on a combination of cell morphology, gram stain results, and results from the Enteric and Nonfermenter BBL crystal identification test kit (Becton Dickinson). Gram-negative histamine-producing and non–histamine-producing bacteria were used for further screening.

Histamine detection by HPLC. HPLC chemicals were obtained from J. T. Barker (Hayward, CA) unless otherwise specified. Histamine concentration was determined by the HPLC method of Cinquina et al. *(6)*. Bacterial isolates were first inoculated in duplicate into TSB+ (pH 6.5) and incubated at 37°C for 48 h, and a 200-μl aliquot of the culture broth was extracted in 800 μl of 1 M perchloric acid (VWR, West Chester, PA). The diluted sample was vortexed for 1 min, sonicated for 15 min, and centrifuged for 15 min at 4,160 × *g* at 4°C. The supernatant was filtered through a 0.45-μm-pore-size polytetrafluoroethylene filter (VWR) and diluted 1:10 in HPLC grade water. This diluted sample was then injected onto a Luna C18(2) column (Phenomenex, Torrance, CA) using an Agilent 1050 HPLC and separated under isocratic conditions with 85% eluent A (85% phosphate buffer, pH 6.9, and 15% methanol) and 15% eluent B (acetonitrile). Flow rate was set at 0.5 ml/min, and detection was achieved using a diode array detector at 214 nm. The detection limit for this method was 125 ppm based on a histamine standard curve constructed from serially diluted histamine suspensions of 2.5 to 200 ppm.

Culture-based method. Bacterial strains ($n = 152$) from the culture library were streaked onto Niven's agar *(24)* as modified by the method of Mavromatis and Quantick *(23)*, i.e., containing 0.5% tryptone, 0.5% yeast extract, 2.7% histidine, 0.5% NaCl, 0.1% $CaCO_3$ (Mallinckrodt, Hazelwood, MO), 3% agar, and 0.006% bromocresol purple (Fisher Scientific, Pittsburgh, PA) and incubated at 30°C for 48 h. Isolates were considered positive for histamine production when the color of the medium changed from green to purple.

Potentiometric method. Strains were evaluated for their ability to increase conductance after growth in histidine decarboxylase broth (HDB), in accordance with the method developed by Klausen and Huss *(19)* with slight modifications. Single isolates were incubated in 9 ml of TSB for 24 h at 30°C. Samples were diluted 1:10 in TSB, and 1 ml of the culture was added to 9 ml of HDB containing 0.2% peptone, 0.1% Lab-Lemco (Oxoid, Basingstoke, UK), 0.81% histidine, 0.5% NaCl, and 0.0005% pyridoxal·HCl in potentiometric vials. Vials were placed into the BacTrac instrument (SY-Lab, Neupurkersdorf, Austria), and conductance was measured automatically at 10-min intervals for 24 h at 30°C. Results were expressed graphically as the percent increase in medium conductance (%M) versus time. Positive isolates were defined as those that increased the conductance of the HDB above 5% of baseline within 24 h.

Molecular method. Culture strains were inoculated into 5 ml of TSB containing 2% NaCl for 24 to 48 h, and DNA was isolated using the UltraClean Microbial DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA) in accordance with manufacturer's instructions. Absorbance was recorded at 260 and 280 nm (Spectra Max 190, Molecular Devices, Sunnyvale, CA) in a 96-well quartz plate, and DNA concentration and purity were calculated using standard formulae.

A 709-bp fragment of the *hdc* gene was amplified using primers designed by Takahashi et al. *(25)*: *hdc-f* (5′-TCH ATY ARY AAC TGY GGT GAC TGG RG-3′) and *hdc-r* (5′-CCC ACA KCA TBA RWG GDG TRT GRC C-3′). Amplification was performed in 50-μl reactions that included 25 μl of PCR master

mix (50 U/ml *Taq* DNA polymerase, 400 μM concentrations of each of the four deoxynucleoside triphosphates, reaction buffers, and 3 mM $MgCl_2$, pH 8.5; all provided by Promega Corp., Madison, WI), 75 pmol of each primer, and 20 ng of DNA template. Amplifications were carried out for 40 cycles (94°C for 1 min, 52°C for 1 min, and 72°C for 1 min) in a GTC-2 thermal cycler (Precision Scientific, Chicago, IL). PCR products were separated on a 1% agarose gel at 86 V in 1× TBE (89 mM Tris-borate, 2 mM EDTA, pH 8.3) for 1 h. Gels were stained with ethidium bromide (0.3 μg/ml) and visualized with a UV transilluminator (UVP, Upland, CA). Product size was confirmed by comparison with 100-bp molecular weight markers (Invitrogen, Carlsbad, CA).

RESULTS AND DISCUSSION

A total of 152 isolates were screened in this study. On the basis of HPLC analysis (which is considered the ''gold standard''), the isolates were subclassified into three groups: high histamine (>1,000 ppm) producers (73 of 152, 48%), low histamine (126 to 500 ppm) producers (6 of 152, 4%), and nonproducers (<125 ppm) (73 of 152, 48%). Using the modified Niven's method, 128 (84%) of the 152 cultures screened were positive and 24 (16%) were negative for histamine production. These 128 histamine-positive isolates consisted of 73 high producers (57%), 6 low producers (5%), and 49 nonproducers (38%); all 24 isolates that were negative for histamine with the Niven's method were nonproducers (Table 1). Thus, when compared with the HPLC gold standard method, the modified Niven's screening method produced a 38% (49 of 128) false-positive rate. This finding is consistent with the work of Lopez-Sabater et al. *(21)* and Fletcher et al. *(13),* who similarly reported that detection of histamine-producing bacteria using Niven's agar resulted in 63 and 15% false-positive rates, respectively. False-positive results are likely due to the production of one or more basic compound(s) capable of increasing the pH of the medium, resulting in the characteristic color change also created by histamine-producing strains *(1)*.

With the potentiometric method, 73 (48%) of the 152 strains were positive; this group included all of the high histamine producers. The remaining 79 strains (52%) were negative for histamine production by the potentiometric method and included all the strains in the library that were classified as low producers and nonproducers as determined by HPLC. This finding is consistent with the work of Klausen and Huss *(19),* who also obtained a positive response using the potentiometric method when screening high-histamine-producing strains of *Morganella* but a negative response for nonproducing strains of *Pseudomonas* and *Alteromonas*.

Using the PCR assay targeting the *hdc* gene, 74 (49%) of the 152 strains screened were positive and 78 (51%) were negative for histamine production. The isolates that were histamine positive by PCR included all of the strains designated as high histamine producers by HPLC and a single isolate (*Citrobacter freundii* HW7.4) that was classified as a nonproducer by both the potentiometric method and HPLC. The strains that were negative for histamine production by PCR included the remaining 72 nonproducers and the 6 low producers classified by HPLC. Takahashi et al. *(25)* similarly found that all high histamine producers in their study produced positive PCR results when targeting the *hdc* gene with the same primer set. These investigators also identified low-histamine-producing strains of *Citrobacter braakii* and *Hafnia alvei* that produced negative and positive PCR results, respectively. Using different primers based on the *hdc* gene for the *R. planticola* HDC protein, Kanki et al. *(15)* successfully amplified a 724-bp fragment from all the histamine-producing *R. planticola* and *Raoutella ornithinolytica* strains in their library, as did De las Rivas et al. *(9)* for a 534-bp amplification product. However, neither of these research groups examined low-histamine-producing bacteria such as *C. freundii* and *H. alvei* for amplification of the *hdc* gene.

Both the potentiometric and PCR methods produced results that were similar to those obtained with the HPLC gold standard when screening strains that produce high amounts of histamine; concordance between either of these methods and HPLC exceeded 99%. Similarly high concordance was found when comparing the potentiometric and PCR-based methods to one another. High histamine producers are more likely than the low histamine producers to produce toxic levels of histamine under conditions of temperature abuse *(18)*. Therefore, the presence of high-histamine-producing bacteria suggests a higher likelihood for product adulteration, which is compelling justification for the use of either the potentiometric or the PCR-based methods for routine screening for histamine-producing bacteria in natural environments.

However, neither the potentiometric nor the PCR method was acceptable for the identification of low-histamine-producing bacteria. With the potentiometric method, the small amounts of histamine (<500 ppm) produced by these strains may have been insufficient to result in detectable changes in conductance. Another explanation is that other bacterial amino acid decarboxylases, such as the arginine decarboxylase, were able to metabolize histidine in addition to their natural substrates; this phenomenon has been observed for strains of *Salmonella* Typhimurium and *Escherichia coli (4, 7)*. Either or both of these explanations may account for the potentiometric results. However, we also were unable to detect the *hdc* gene in these same low histamine producers. The significance of this finding is currently unknown. The *hdc* gene associated with similar low-histamine-producing stains of species such as *C. freundii* and *H. alvei* has yet to be identified and may be plasmid associated *(11, 20)*. Histamine producers may lose their ability to form histamine during prolonged storage or cultivation of isolated strains in synthetic media *(22),* and this loss of histamine production may be related to loss of plasmids containing the *hdc* gene. Other possible explanations for these findings are the absence of an *hdc* gene, presence of an alternative *hdc* gene sequence, and/or loss of a plasmid-borne gene.

The modified Niven's method is the easiest and least costly of the three approaches evaluated in this study. Advantages of the modified Niven's method are its adaptability to quantitative assay, making it useful for the

TABLE 1. *Histamine production by various bacterial strains as determined using the modified Niven's, potentiometric, and PCR-based methods*

Bacterial species or strain	No. of strains positive/no. of strains tested			Histamine (ppm)[a]		Source[b]
	Niven's	Potentiometric	PCR	Mean	Range	
Morganella morganii	30/30	30/30	30/30	4,466	2,880–6,353	ATCC, NC, HI, NZ, ICPB
Providencia rustigianii	3/3	3/3	3/3	4,269	3,778–4,383	NC, HI
Proteus mirabilis	2/2	2/2	2/2	4,114	4,024–4,204	NC
Raoutella planticola	1/1	1/1	1/1	6,143		ATCC
R. ornithinolytica	2/2	2/2	2/2	5,624	5,459–5,789	NC
Enterobacter aerogenes	6/6	6/6	6/6	6,660	2,833–7,779	ATCC, NC, HI
E. gergoviae	2/2	2/2	2/2	5,634	3,508–7,760	HI
Photobacterium damselae	27/27	27/27	27/27	3,372	1,622–7,711	NC, HI
Klebsiella oxitoca	1/1	0/1	0/1	<125		ATCC
Hafnia alvei	3/3	0/3	0/3			
ATCC 13337, NZ1	2/2	0/2	0/2	181	171–204	ATCC, NZ
HW46.3	1/1	0/1	0/1	<125		HI
Vibrio alginolyticus	2/2	0/2	0/2	<125		ATCC, NC
Citrobacter freundii	21/21	0/21	1/21			
BO216, A4077, A4086, FT761	4/4	0/4	0/4	265	126–448	NC
ATCC 8090, BR121, BR129, BO240, BO251, BO201, BO222, HPP304, A4083, FT730, BR902, HW12.1, HW16.3, HW17.1, HW24.1, HW1.4, HW7.4[c]	17/17	0/17	1[c]/17	<125		ATCC, NC HI
C. amalonaticus	1/1	0/1	0/1	<125		HI
Serratia marcescens	1/1	0/1	0/1	<125		HI
V. mimicus	1/1	0/1	0/1	<125		NC
Pseudomonas putida	1/1	0/1	0/1	<125		NC
Shewanella putrefaciens	6/6	0/6	0/6	<125		NC
E. cloacae	11/11	0/11	0/11	<125		NC
Escherichia hermannii	2/2	0/2	0/2	<125		HI
V. parahemolyticus	1/1	0/1	0/1	<125		NC
V. fluvialis	2/2	0/2	0/2	<125		HI
Pseudomonas aeruginosa	2/6	0/6	0/6	<125		
ATCC 27853, HW19.3	2/2	0/2	0/2	<125		HI, ATCC
HW22.3, HW28.6, NZ8, NZ10	0/4	0/4	0/4	<125		HI, NZ
Escherichia coli	0/13	0/13	0/13	<125		ATCC, NC
Listeria innocua	0/3	0/3	0/3	<125		NC
Shigella flexneri	0/1	0/1	0/1	<125		ATCC

[a] Histamine production of isolates as determined by HPLC (the gold standard). Values are mean histamine produced by a species based on two independent replicates for each strain and range of histamine produced by each species.

[b] ATCC, American Type Culture Collection; ICPB, International Collection of Phytopathogenic Bacteria, Davis, CA; NC, North Carolina; HI, Hawaii; NZ, New Zealand.

[c] PCR-positive isolate.

enumeration of histamine-producing bacteria from natural (fish and environmental) samples, and its reliability for identification of low-histamine-producing strains, although these strains may not produce histamine of toxicological significance (>500 ppm). Nonetheless, the high incidence of false-positive results means that detection of histamine producers using modified Niven's medium should be considered presumptive only, requiring further confirmation. This necessity complicates the assay and calls into question the interpretation of enumerative results. The potentiometric and PCR methods require more expensive equipment and a higher degree of training for the user but are faster (<24 h). Although these methods do not reliably detect strains producing low amounts of histamine, they accurately detect high-histamine-producing strains that are more likely to produce histamine levels of toxicological significance. These observations support the need for a straightforward method for identifying histamine-producing bacteria; this method should combine cultural methods with potentiometric or molecular methods. A logical approach would be colony lift hybridization, which uses the combined power of microbiological culture with nucleic acid hybridization. This method is uniquely suited to situations in which the performance of selective and/or differential media

is less than perfect. Colony lift hybridization provides more accurate quantitative results because the target organism can be confirmed without the need for subculturing. Efforts to develop such a method are currently underway.

ACKNOWLEDGMENTS

This study was funded in part by grants from the National Fisheries Institute Fisheries Scholarship Foundation, North Carolina Sea Grant, and the North Carolina Agricultural Research Service. We thank Dr. Graham Fletcher (New Zealand Institute for Plant and Food Research Ltd.), Dr. Rachel Nobles (University of North Carolina–Chapel Hill, Institute of Marine Sciences), Dr. John Kaneko (PacMar Inc.), and Dr. George Flick (Virginia Polytechnic Institute and State University) for culture and sample donations and Drs. Jay Levine, Fred Breidt, and Damian Shea (North Carolina State University) for the loan of laboratory equipment used in this study.

REFERENCES

1. Actis, L. A., J. C. Smoot, C. E. Barancin, and R. H. Findlay. 1999. Comparison of differential plating media and two chromatography techniques for the detection of histamine production in bacteria. *J. Microbiol. Methods* 39:79–90.
2. Anonymous. 2001. Scombrotoxin (histamine) formation, p. 73–102. *In* Fish and fisheries products hazards and controls guidance, 3rd ed. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, DC.
3. Baranowski, J. D., P. A. Brust, and H. A. Frank. 1985. Growth of *Klebsiella pneumonia* UH-2 and properties of its histidine-decarboxylase system in resting cells. *J. Food Biochem.* 9:349–360.
4. Blethen, S. L., E. A. Boeker, and E. E. Snell. 1968. Arginine decarboxylase from *Escherichia coli*. I. Purification and specificity for substrates and coenzyme. *J. Biol. Chem.* 243:1671–1677.
5. Chen, C. M., C.-I. Wei, J. A. Koburger, and M. R. Marshall. 1989. Comparison of four agar media for detection of histamine-producing bacteria in tuna. *J. Food Prot.* 52:808–813.
6. Cinquina, A. L., F. Longo, A. Cali, L. De Santis, R. Baccelliere, and R. Cozzani. 2004. Validation and comparison of analytical methods for the determination of histamine in tuna fish samples. *J. Chromatogr. A* 1032:79–85.
7. Cundell, D. R., J. L. Devalia, M. Wilks, S. Tabaqchali, and R. J. Davies. 1991. Histidine decarboxylase from bacteria that colonize the human respiratory tract. *J. Med. Microbiol.* 35:363–366.
8. De las Rivas, B., A. Marcobal, A. V. Carrascosa, and R. Muñoz. 2006. PCR detection of foodborne bacteria producing the biogenic amines histamine, tyramine, putrescine, and cadaverine. *J. Food Prot.* 69:2509–2514.
9. De las Rivas, B., A. Marcobal, and R. Muñoz. 2005. Improved multiplex-PCR method for the simultaneous detection of food bacteria producing biogenic amines. *FEMS Microbiol. Lett.* 244: 367–372.
10. Dewaal, C. S., G. Hicks, K. Barlow, L. Alderton, and L. Vegosen. 2006. Foods associated with foodborne illness outbreaks from 1990 through 2003. *Food Prot. Trends* 26:466–473.
11. Di Lorenzo, M., M. Stork, M. E. Tolmasky, L. A. Actis, D. Farrell, T. J. Welch, L. M. Crosa, A. A. Wertheimer, Q. Chen, P. Salinas, L. Waldbeser, and J. H. Crosa. 2003. Complete sequence of virulence plasmid pJM1 from the marine fish pathogen *Vibrio anguillarum* strain 775. *J. Bacteriol.* 185:5822–5830.
12. Etkind, P., M. E. Wilson, K. Gallagher, and J. Cournoyer. 1987. Bluefish-associated scombroid poisoning—an example of the expanding spectrum of food poisoning from seafood. *JAMA* 258:3409–3410.
13. Fletcher, G. C., G. Summers, and P. W. C. van Veghel. 1998. Levels of histamine and histamine-producing bacteria in smoked fish from New Zealand markets. *J. Food Prot.* 61:1064–1070.
14. Halasz, A., A. Barath, L. Simonsarkadi, and W. Holzapfel. 1994. Biogenic-amines and their production by microorganisms in food. *Trends Food Sci. Technol.* 5:42–49.
15. Kanki, M., T. Yoda, T. Tsukamoto, and T. Shibata. 2002. *Klebsiella pneumoniae* produces no histamine: *Raoultella planticola* and *Raoultella ornithinolytica* strains are histamine producers. *Appl. Environ. Microbiol.* 68:3462–3466.
16. Kim, S.-H., H. An, K. G. Field, C.-I. Wei, J. B. Velazquez, B. Ben-Gigirey, M. T. Morrissey, R. J. Price, and T. P. Pitta. 2003. Detection of *Morganella morganii*, a prolific histamine former, by the polymerase chain reaction assay with 16S rDNA–targeted primers. *J. Food Prot.* 66:1385–1392.
17. Kim, S.-H., H. An, C.-I. Wei, W. Visessanguan, S. Benjakul, M. T. Morrissey, Y.-C. Su, and T. P. Pitta. 2003. Molecular detection of a histamine former, *Morganella morganii*, in albacore, mackerel, sardine, and a processing plant. *J. Food Sci.* 68:453–457.
18. Kim, S.-H., J. Barros-Velazquez, B. Ben-Gigrey, J.-B. Eun, S. H. Jun, C.-I. Wie, and H. An. 2003. Identification of the main bacteria contributing to histamine formation in seafood to ensure product safety. *Food Sci. Biotechnol.* 12:451–460.
19. Klausen, N. K., and H. H. Huss. 1987. A rapid method for detection of histamine-producing bacteria. *Int. J. Food Microbiol.* 5:137–146.
20. Landete, J. M., B. De Las Rivas, A. Marcobal, and R. Muñoz. 2008. Updated molecular knowledge about histamine biosynthesis by bacteria. *Crit. Rev. Food Sci. Nutr.* 48:697–714.
21. Lopez-Sabater, E. I., J. J. Rodriguez-Jerez, M. Hernandez-Herrero, and M. T. Mora-Ventura. 1996. Incidence of histamine-forming bacteria and histamine content in scombroid fish species from retail markets in the Barcelona area. *Int. J. Food Microbiol.* 28:411–418.
22. Lucas, P. M., W. A. M. Wolken, O. Claisse, J. S. Lolkema, and A. Lonvaud-Funel. 2005. Histamine-producing pathway encoded on an unstable plasmid in *Lactobacillus hilgardii* 0006. *Appl. Environ. Microbiol.* 71:1417–1424.
23. Mavromatis, P., and P. C. Quantick. 2002. Modification of Niven's medium for the enumeration of histamine-forming bacteria and discussion of the parameters associated with its use. *J. Food Prot.* 65: 546–551.
24. Niven, C. F., M. B. Jeffrey, and D. A. Corlett. 1981. Differential plating medium for quantitative detection of histamine-producing bacteria. *Appl. Environ. Microbiol.* 41:321–322.
25. Takahashi, H., B. Kimura, M. Yoshikawa, and T. Fujii. 2003. Cloning and sequencing of the histidine decarboxylase genes of gram-negative, histamine-producing bacteria and their application in detection and identification of these organisms in fish. *Appl. Environ. Microbiol.* 69:2568–2579.
26. Yoshinaga, D. H., and H. A. Frank. 1982. Histamine-producing bacteria in decomposing skipjack tuna (*Katsuwonus pelamis*). *Appl. Environ. Microbiol.* 44:447–452.