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PREVALENCE OF HYDROGEN SULFIDE PRODUCING PSYCHROPHILIC BACTERIA IN CHILLED *MUGIL CEPHALUS "MULLET"* FISH AND THEIR PUBLIC HEALTH SIGNIFICANCE.

(With 4 Tables)

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مدي تواجد البكتيريا المحبة للبرودة المنتجة لكبريتيد الهيدروجين في أسماك البوري المبرد وتاثيرها علي الصحة العامة

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في دراسة لتحديد مدي تواجد البكتيريا المحبة للبرودة المنتجة لكبريتيد الهيدروجين تم فحص خمسون عينة صالحة ظاهريا للاستهلاك الادمي من أسماك البوري المبرد والتي تم جمعها من أسواقِ مدينة بورسعيد بهدف عد وعزل وتصنيف البكتيريا المحبة للبرودة المنتجة لكبريتيد الهيدروجين. اظهرت النتائج ان نسبة العينات الايجابية للبكتيريا المحبة للبرودة المنتجة لكبريتيد الهيدروجين كانت 100 % (50) وكان متوسط العد الكلي للبكتيريا المحبة للبرودة والعد الكلي للبكتيريا المحبة للبرودة المنتجة لكبريتيد الهيدروجين $2,1 \times 10^5 \pm 2,9$ و $1,8 \times 10^4 \pm 3,3 \times 10^3$ خلية/ جرام في العينات موضع الدراسة على التوالي . وكان متوسط نسبة البكتيريا المحبة للبرودة المنتجة لكبريتيد الهيدروجين الي نسبة البكتيريا الكلية المحبة للبرودة 0,86 %. تم تصنيف عترات البكتيريا المحبة للبرودة المنتجة لكبريتيد الهيدروجين المعزولة من أسماك البوري المبرد الي انواع من *سودوموناس* ، *سودوموناس فلوريسنس* وشيوانيلا بيتروفيكانس وكانت بنسبة 77,78% ، 14,44% و 7,78% علي التوالي. تم مناقشة تاثير البكتيريا المحبة للبرودة والمنتجة لكبريتيد الهيدروجين علي الصحة العامة.

SUMMARY

Fifty samples of chilled *Mugil cephalus* "Mullet" were randomly purchased from Port-Said markets. The samples were examined for enumeration and isolation of hydrogen sulfide producing psychrophilic bacteria. The incidence of positive samples for hydrogen sulfide producing psychrophilic bacteria was 100% (50). The mean values of

the total psychrophilic bacterial counts and hydrogen sulfide producing psychrophilic bacterial counts were 2.1 X 10^5 ± 2.9 X 10^4 and 1.8 X 10^3 ± 3.3 X 10^2 CFU/g of chilled *Mugil cephalus* "Mullet" respectively. The incidence of hydrogen sulfide producing psychrophilic bacterial colony in compared to the total psychrophilic bacteria was 0.86%. The hydrogen sulfide producing psychrophilic bacterial isolates in the examined samples were identified as *Pseudomonas spp.*, *Pseudomonas fluorescens* and *Shewanella putrefaciens* with an incidence of 77.78%, 14.44% and 7.78% respectively. The effect of the hydrogen sulfide producing psychrophilic bacteria on the public health was discussed.

***Key words**: Fish, Mugil cephalus, psychrophiles*

INTRODUCTION

Fish is a very perishable, high-protein food that typically contains a high level of free amino acids and volatile nitrogen bases which are essential for human consumption, in addition to the high levels of hydrosoluble and liposoluble vitamins, minerals and polyunsaturated fatty acids (Ashie *et al*., 1996; Haugen and Undeland, 2003; Gonzalez-Fandos, *et al*., 2005).

Although ice storage has been the most widely used methods for the preservation of fresh fish (Chai and Levin, 1975) to keep it fresh during marketing, bacterial activity is generally accepted as the primary cause of fish spoilage (Shewan, 1961) depends on specific organisms, climates, storage conditions, the type of fish and even the place of fish was harvested (Gram and Huss, 1996).

Microbes can metabolize and degrade these compounds resulting in undesirable metabolites and spoilage of the fish (Haugen and Undeland, 2003; Olafsdottir *et al*., 2005). The main spoilage agents of chilled stored fresh fish are the bacteria that produce hydrogen sulfide (Satomi, *et al*., 2006).

Hydrogen sulfide producing psychrophilic bacteria is a group of bacteria including *Shewanella* and *Pseudomonas* species which are the predominant species found as specific spoilage organisms in chilled fresh fish under aerobic conditions (Miller III, *et al*., 1973; Fonnesbech Vogel, *et al*., 2005; Hozbor, *et al*., 2006). Also some species of *Aeromonaceae, Vibrionaceae and Enterobacteriaceae* were able to produce hydrogen sulfide (NMKL, 2006).

These types of psychrophilic bacteria are widely distributed in marine and freshwater environments. They are mostly Gram negative,

motile rods, facultative anaerobic, non spore forming and catalase positive but oxidase and sugar fermentation were variable. The fresh isolates on iron media show black colonies due to the precipitation of the insoluble iron sulfide (Allen and Geldreich, 1975; Baumann and Schubert, 1984; Krieg, 1984; Popoff, 1984; Greenberg and Hunt, 1985; Gennari and Dragotto, 1992 Ziemke *et al*., 1999; Satomi, *et al*., 2006)

In spite of the hydrogen sulfide producing (HSP) bacteria constitute a minor fraction in the initial microflora of the newly caught fish (Jorgensen *et al*., 1988), they can cause organoleptic changes such as offensive, fishy, rotten H_2S off odors in the spoiled aerobically and cold stored fish (Adams, *et al*., 1964; Skjerdal *et al*., 2004).

Different illness in the consumer has been recorded by the hydrogen sulfide producing (HSP) bacteria. Chen *et al*., 1997 recorded that *Shewanella putrefaciens* and *Shewanella algae* have been associated with septicemia, cellulitis, skin, and soft tissue infections. Other *Shewanella* spp., especially *S. baltica*, is commonly associated with food spoilage (Gram and Huss 1996). Patients with liver disease (Otsuka *et al*., 2007) and the immunocompromised individuals appear to be at higher risk for shewanellosisis (Krsnik *et al*., 2002). Some *Pseudomonas* species has been transmitted by food affecting primarily immunocompromised people and those suffering from cystic fibrosis (Morais, *et al*., 1997) leads to several outbreaks (Pererra *et al*., 1977). Food borne *Aeromonas* causing food poisoning and traveler's diarrhea (Cholera-like illness and dysentery like illness). Septicemia and peritonitis can be produced specially in malignancies and immunocompromised host (Palumbo, *et al*., 1992; FDA, 2001). Non-cholera *Vibrio* infections cause self-limiting gastroenteritis lasting 2-3 days and characterized by diarrhea, sometimes bloody stools, abdominal cramps, nausea, vomiting, headache and fever. While necrotizing fascitis and septicemia were associated with chronic liver diseases, adrenal insufficiency, portal hypertension and immunocompromised hosts (Morris, 2003; Yeung and Boor, 2004). The enterotoxigenic strains of the *Enterobactericae* have been isolated from infants and children with acute gastroenteritis while the endotoxins show a bacteraemia in human (Guentzel, 1982; APHA, 1984).

The objective of the present study was to determine safety of chilled *Mugil cephalus "Mullet"* fish for human consumption through the enumeration and identification of the psychrophilic and hydrogen sulfide producing psychrophilic bacteria and their public health significance.

MATERIALS and METHODS

1- Samples collection:

A total of 50 random samples of chilled *Mugil cephalus* "Mullet" were randomly purchased from Port-Said markets. Each individual sample was placed separately into sealed sterile plastic bag on ice, thoroughly identified and delivered to the laboratory to be examined bacteriologically for enumeration, isolation and biochemical identification of the isolated psychrophilic and hydrogen sulfide producing psychrophilic bacteria. All specimens were processed within 4 hours of collection.

2- Preparation, homogenation and enrichment of the samples:

A representative twenty five grams of fish sample were transferred aseptically to a stomacher bag (Seward medical, London, UK) containing 225 ml of sterile 0.1% (W/V) peptone saline (0.1% peptone + 0.85% NaCl) and homogenized for 60 second with stomacher (Lab. Blender 400, Seward Medical, London UK) at room temperature. Serial tenfold dilutions were made in the same dilution up to 10^6 according to the BSI (1996) and ISO/DIS 6887-1 (1997).

3- Isolation and enumeration of psychrophilic bacteria:

0.1 ml of each serial dilution was spread thoroughly and uniformly onto duplicate marked Petri plates of plate count agar (PCA) using surface plate technique. All plates were inverted and incubated at 7°C for 10 days. The psychrophilic bacterial count was calculated and expressed as colony forming units per gram (CFU/g) of fish (Cousin *et al*. (1992).

4- Isolation and enumeration of hydrogen sulfide producing psychrophilic bacteria:

Another 0.1ml of the appropriate dilutions was spread onto duplicate marked petri plates of iron peptone agar (IPA). After solidification the plates were covered with a thin layer of the same growth media. All plates were inverted and incubated at 25°C for 3 days. Plates containing 25-250 black colonies were counted. The hydrogen sulfide producing psychrophilic bacterial count was calculated and expressed as colony forming units per gram (CFU/g) of fish (Gram *et al*., 1987; Gennari and Campanini 1991).

5- Purification and keeping the working isolates:

Approximately 15 typical colonies were picked randomly each from plate count agar and peptone iron agar plates for each sample

(Leroi *et al*., 1998). The picked colonies from plate count agar were purified by reinoculation in nutrient broth and incubated at 25°C for 18-24 hr. A loopful of the inoculated nutrient broth was streaked onto nutrient agar slope (Murray *et al*., 2003). Meanwhile the black colonies from peptone iron agar plates were further purified in brain heart infusion broth supplemented with NaCl (0.5%) and streaked onto peptone iron agar slant (IAS) and incubated at 15°C for 18-24 hr (Gibson and Khoury, 1986; Leroi *et al*., 1998). All purified isolates were maintained at 4°C and subculture periodically each 3 weeks to ensure viability, till the morphological and biochemical identification of the isolates (Harrigan and McCance, 1966).

6- Morphological and biochemical identification of the Isolation:

All isolates were morphologically and biochemically identified at 25°C for Gram stain, cell morphology, oxidase and catalase reactions, motility, oxidation fermentation test (O/F test) and other biochemical tests according to Skerman (1967), Sneath *et al*. (1986) and Gram *et al*. (1987)

7- Statistical methods

Minimum, maximum, mean, standard deviation and standard error of mean as well as frequency distribution were used to describe data. T-test was used to evaluate the relationship between the number of psychrophilic bacteria and hydrogen sulfide producing psychrophilic bacteria in chilled *Mugil cephalus "Mullet".* P value was considered significant if less than 0.05 and 0.01 at 95% and 99% respectively. These tests were analyzed using the Statistical Package for Social Scientists (SPSS) for windows 16.0 (SPSS Inc., Chicago, IL, and USA).

RESULTS

Table 1: Statistical analytical results of the psychrophilic and hydrogen sulfide producing psychrophilic bacterial count (CFU/g) recovered from chilled *Mugil cephalus "Mullet"* (n=50).

	Psychrophilic bacteria	Hydrogen sulfide producing psychrophilic
Min.	1.2×10^4	2.2×10^2
Max.	9.0×10^5	9.7×10^3
Mean	2.1×10^5	1.8×10^3
S.E.	2.9×10^4	3.3×10^2
S.D.	2.1×10^5	2.3×10^3

Min. = Minimum. Max. = Maximum. SE = Standard Error SD = Standard Deviation.

Table 2: Frequency distribution of the examined *Mugil cephalus* based on their psychrophilic and hydrogen sulfide producing psychrophilic bacterial count.

Count range	Psychrophilic bacteria		Hydrogen sulfide Producing psychrophilic	
	No.	%	No.	%
10^2- <10^3	0.00	0.00	31.00	62.00
10^3 - <10^4	0.00	0.00	19.00	38.00
10^4 - <10^5	23.00	46.00	0.00	0.00
10^5 - <10^6	27.00	54.00	0.00	0.00
Total	50.00	100.00	50.00	100.00

Table 3: Relationship between the counts of psychrophilic and hydrogen sulfide producing psychrophilic bacteria recovered from chilled *Mugil cephalus* According to Chai, *et al*. (1968)

Time of investigation	Psychrophilic bacteria	Hydrogen sulfide producing psychrophilic	H_2S colonies %
At the same time & and same samples	2.1 X 10^5	1.8 X 10^3	0.86% (**)

(**) = Highly significant correlation at p <0.01 (99% confidence) by using t-test (Paired system test).

Table 4: Incidence of psychrophilic and hydrogen sulfide producing psychrophilic bacterial isolates recovered from chilled *Mugil cephalus*

	Psychrophilic bacteria		Hydrogen sulfide producing psychrophilic		Total isolates	
	No.	%	No.	%	No.	%
Pseudomonas spp.	70	100.00	-------	-------	70	77.78
Pseudomonas fluorescens	------	-------	13	65.00	13	14.44
Shewanella putrefaciens	------	-------	7	35.00	7	7.78
Total	70	100.00	20	100.00	90	100.00

DISCUSSION

Hydrogen sulfide gram negative bacteria are commonly associated with the spoilage of foods. These bacteria usually constitute only a small fraction of the initial flora on newly caught fish but constitute a significant, sometimes dominant part of the microbiota during chilled storage and their numbers determine the shelf life of the product (Herbert *et al*., 1971; Jorgensen *et al*., 1988; Gram and Fonnesbech Vogel, 2000).

The obtained results in Table 1 showed that the mean values of the psychrophilic and hydrogen sulfide producing psychrophilic bacterial count of the chilled *Mugil cephalus "Mullet"* samples were 2.1 X 10^5 ± 2.9 X 10^4 and 1.8 X 10^3 ± 3.3 X 10^2 CFU/g fish respectively. These results agree with the results recorded by Hobbs (1983) and Hayes, (1992) but lower than the results recorded by Chai, *et al*. (1968) and higher than the results recorded by Fonnesbech Vogel, *et al*. (2005). The variation between our results and the results recorded by other authors may be attributed to the variation between the species of fish and the season where the counts increased in summer (Chen and Chai; 1982 and Gram and Huss; 1996) the type of the specific spoilage organisms, the storage conditions and the place in which the fish was harvested (Gram and Huss; 1996).

Regarding frequency distribution of the examined samples presented in Table 2 it is evident that most of the examined chilled *Mugil cephalus "Mullet"* (62%) had hydrogen sulfide producing psychrophilic bacteria within the range of 2 X 10^2 - $<10^3$ CFU/g while 54% had psychrophilic bacterial counts within the range of 10^5 - $<10^6$ CFU/g. On the other hand, 38% and 46% of the examined samples had hydrogen sulfide producing psychrophilic bacteria and psychrophilic bacteria within the range of 10^3- $<10^4$ CFU/g and 10^4- $<10^5$ CFU/g respectively. The lower range of most of the examined samples (62%) for hydrogen sulfide producing psychrophilic bacteria may be attributed to the lower range of the psychrophilic bacteria than the level (10^7-10^9 CFU/g) at which typically grow of hydrogen sulfide producing psychrophilic bacteria occur (Shewan 1977). Also these levels are required to produce off-odors of the food sample (Jorgensen and Huss, 1989).

The results given in Table 3 reveal that the number of H_2S producing psychrophilic bacterial colonies per gram of fish was 0.86% of the total psychrophilic flora. This result was lower than the results recorded by Chai, *et al*. (1968). The lowering of this percentage (0.86%) may be attributed to the lower of hydrogen sulfide producing psychrophilic bacterial count of our results than the results recoded by the other authors.

Statistically by using t-test (Paired system test), a highly significant relationship between the counts of each of the psychrophilic and hydrogen sulfide producing psychrophilic bacteria of the examined chilled *Mugil cephalus "Mullet"* samples (Table 3). This means that the counts of H_2S producing psychrophilic bacterial could be used as indicators of spoilage of iced fish (Kyrana and Lougovois, 2002).

Table 4 showed that the isolated strains from the examined samples were identified as *Pseudomonas spp, Pseudomonas fluorescens* and *Shewanella putrefaciens* with an incidence of 77.78, 14.44 and 7.78% respectively.

Although *Shewanella putrefaciens* play a prominent role as spoilage organisms of fish and other food products (Gram and Dalgaard, 2002), it was found in low incidence (7.78%). This may be attributed to the inhibitory activity of the isolated siderophore producing *Pseudomonas* species (Gram, 1993), through the competition inhibition of iron (Henry *et al*., 1991) or due to the antibiotic activity of the siderophore (Neilands 1981). On the other hand the high incidence of the isolated *Pseudomonas* spp. (77.78 %,) may be due to their faster growth rates at chill temperature (Miller III, *et al*., 1973), their greater affinity for oxygen and as a consequence their catabolism of glucose and lactose (Miller III, *et al*., 1973; Gill and Molin 1991).

In conclusion, to lower the number and activity of the psychrophilic and hydrogen sulfide producing psychrophilic bacterial count and prevent their spoilage effect, a good fish handling practices include: icing or rapid immersion of the catch in water chilled to -1°C, good time/temperature storage besides prevention the cross and secondary contamination, strictly hygienic measurement for prevention and removal the source of pollution from the harvested and storage sites.

REFERENCES

Adams, R.; Farber, L. and Lerke, P. (1964): Bacteriology of the spoilage of fish muscle. II. Incidence of spoilers during spoilage. Journal of Applied Microbiology. 12:277-279.

Allen, M.J. and Geldreich, E.E. (1975): Bacteriological criteria for ground water quality. Ground Water. 13:5-52

APHA "American Public Health Association" (1984): Compendium of Methods for the Microbiological Examination of Foods, 2nd Ed. American Public Health Association, Washington, D.C., p. 99-111.

Ashie, I.N.A.; Smith, J.P. and Simpson, B.K. (1996): Spoilage and shelf life extension of fresh fish/shellfish. Critical Reviews in Food Science and Nutrition. 36: 87-122.

Baumann, P. and Schubert, R. (1984): Family II. *Vibrionaceae*, p. 516-550. In N.R. Krieg and J.G. Holt (eds.), Bergey's Manual of Systemic Bacteriology, 1st ed. Williams & Wilkins Co., Baltimore/London.

BSI "British Standards Institution" (1996): Methods for the Microbiological Examination of Food and Animal Feeding Stuffs. No. BS 5763. General Laboratory Practices. London

Chai, T. and Levin, R.E. (1975): Characterization of heavily mucoid bacterial isolates from fish pen slime. Journal of Applied Microbiology. 30: 450-455.

Chai, T.; Chen, C.; Rosen, A. and Levin, R.E. (1968): Detection and incidence of specific specie of spoilage bacteria on Fish. II. Relative incidence of *Pseudomonas putrefaciens* and fluorescent pseudomonads on Haddock Fillets. Journal of Applied Microbiology. 16(11): 1738-1741.

Chen, H.C. and Chai, T.J. (1982): Microflora of Drainage from Ice in Fishing vessel Fishholds. Journal of Applied and Environmental Microbiology. 43 (6): 1360-1365.

Chen, Y.S.; Liu, Y.C.; Yen, M.Y.; Wang, J.H.; Wann, S.R. and Cheng, D.L. (1997): Skin and soft tissue manifestations of *Shewanella putrefaciens* infection. Clinical Infectious Diseases. 25:225-229

Cousin, M.A.; Jay, J.M. and Vasavada, P.C. (1992): Psychrophilic microorganisms. In: Vanderzand, C.; Splittstoesser, DF., editors. Compendium of Methods for the Microbiological Examination of Foods. 3rd ed. Washington, D.C. American Public Health Association. pp 153-168.

FDA "Food and Drug Administration" (2001): Evaluation and definition of potentially hazardous foods. Analysis of microbial hazards related to time/temperature control of food for safety. Department of Health and Human Services Food and Drug Administration. Chap. 4, pp 1-19.

Fonnesbech Vogel, B.; Venkateswaran, K.; Satomi, M. and Gram, L. (2005): Identification of *Shewanella baltica* as the most important H_2S producing species during iced storage of Danish marine fish. Journal of Applied and Environmental Microbiology. 71(11): 6689-6697.

Gennari, M. and Campanini, R. (1991): Isolamento e caratterizzazione di Shewanella putrefaciens da pesce fresco e alterato, carni fresche e alterate, prodoti lattiero-caseari, acqua e suolo. Ind. Aliment. 30: 965-976, 988.

Gennari, M. and Dragotto, F. (1992): A study of the incidence of different fluorescent *Pseudomonas* species and biovars in the microflora of fresh and spoiled meat and fish, raw milk, cheese, soil and water. Journal of Applied Bacteriology. 72(4): 281-288.

Gibson, L.F.; and Khoury, J.T. (1986): Storage and survival of bacteria by ultra-freeze. Lett. Appl. Microbiol. 3: 127-129.

Gill, C.O. and Molin, G. (1991): Modified atmospheres and vacuum packaging. p. 172-199. In N. J. Russell and G. W. Gould (ed.), Food preservative. Blackie, Glasgow, Scotland.

González-Fandos, E.; Villarino-Rodriguez, A.; Garcia-Linares, M.C.; Garcia-Arias, M.T. and Garcia-Fernández, M.C. (2005): Microbiological safety and sensory characteristics of salmon slices processed by the sous vide method. Food Control. 16: 77-85.

Gram, L. (1993): Inhibitory effect against pathogenic and spoilage bacteria of *Pseudomonas* strains isolated from spoiled and fresh fish. Journal of Applied and Environmental Microbiology. 59 (7): 2197-2203.

Gram, L. and Dalgaard, P. (2002): Fish spoilage bacteria: problem and solutions. Curr. Opin. Biotechnol. 13:262-266.

Gram, L. and Fonnesbech Vogel, B. (2000): Shewanella, p. 2008-2015. In R. Robinson, C. Batt, and P. Patel (ed.), Encyclopedia of Food Microbiology. Academic Press, Inc., San Diego, Calif.

Gram, L. and Huss, H.H. (1996): Microbiological spoilage of fish and fish products. International Journal of Food Microbiology. 33: 121-137.

Gram, L.; Trolle, G. and Huss, H.H. (1987): Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. International Journal of Food Microbiology. 4: 65-72.

Greenberg, A. and Hunt, D. (1985): Laboratory Procedures for the Examination of Seawater and Shellfish, 5th ed. The American Public Health Association, Washington D.C.

Guentzel, N. (1982): Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter and *Proteus*. Chap. 26. In Medical Microbiology, 4th ed. Online. Samuel baron, M.D. (Ed.). University of Texas Medical Branch. Washington D.C.

Harrigan, W.F. and McCance, M.E. (1966): Laboratory Methods in Microbiology, 3rd ed. Academic Press, London, United Kingdom.

Haugen, J.E. and Undeland, I. (2003): Lipid oxidation in herring fillets *(Clupea harengus)* during ice storage measured by a commercial hybrid gas-sensor array system. Journal of Agriculture and Food Chemistry. 51: 752-759.

Hayes, P.R. (1992): Food Microbiology and Hygiene. 2nd ed. Elsevier Science Publication.

Henry, M.B.; Lynch, J.M. and Femor, T.R. (1991): Role of siderophores in the biocontrol of *Pseudomonas tolaasii* by fluorescent *pseudomonad* antagonist. Journal of Applied Bacteriology. 70: 104-106.

Herbert, R.A.; Hendrie, M.S.; Gibson, D.M. and Shewan, J.M. (1971): Bacteria active in spoilage of certain seafoods. Journal of Applied Bacteriology. 34: 41-45.

Hobbs, G. (1983): Microbial spoilage of fish. In Food Advanced and Prospects. Reberts, T. A. and Skinner, F. A. (Eds.). Society for Applied Bacteriology Symposium Series No. 11, Academic Press London, 217.

Hozbor, M.C.; Saiz, A.I.; Yeannes, M.I. and Fritz, R. (2006): Microbiological changes and its correlation with quality indices during aerobic iced storage of sea salmon (*Pseudopercis semifasciata*). Lwt-Food Science and Technology. 39:99-104.

ISO/DIS 6887-1 (1997): Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1: No. 6887-1. General rules for the preparation of the initial suspension and decimal dilutions.

Jorgensen, B.R. and Huss, H.H. (1989): Growth and activity of *Shewanella putrefaciens* isolated from spoiling fish. International Journal of Food Microbiology. 9: 51-62.

Jorgensen, B.R.; Gibson, D.M. and Huss, H.H. (1988): Microbiological quality and shelf-life prediction of chilled fish. International Journal of Food Microbiology. 6: 295-307.

Krieg, N. (1984): Bergey's Manual of Systemic Bacteriology, Volume 1. Baltimore: Williams& Wilkins. ISBN 0683041088.

Krsnik, I.; Arribalzaga, K. and Romanyk, J. (2002): Shewanella alga bacteremia and associated cellulitis in a patient with multiple myeloma. Haematologia (Budapest) 32:79-80.

Kyrana, V.R. and Lougovois, V.P. (2002): Sensory, chemical and microbiological assessment of farm-raised European sea bass (*Dicentrarchus labrax*) stored in melting ice. International Journal of Food Science and Technology. 37:319-328.

Leroi, F.; Joffraud, J.J.; Chevalier, F. and Cardinal, M. (1998): Study of the microbial ecology of cold smoked salmon during storage at 8°C. International Journal of Food Microbiology. 39: 111-121.

Miller, III.A.; Scanlan, R.A.; Lee, J.S. and Libbey, L.M. (1973): Volatile compounds produced in sterile fish muscle *(Sebastes melanops)* by *Pseudomonas putrefaciens*, *Pseudomonas fluorescens* and an *Achromobacter* species. Journal of Applied Microbiology. 26:18-21.

Morais, P.V.; Mesquita, C.; Andrade, J.L. and Da Costa, M.S. (1997): Investigation of persistent colonization by *Pseudomonas aeruginosa*-like strain in a spring water bottling plant. Journal of Applied Environmental and Microbiology. 63: 851-856

Morris, J. (2003): Cholera and other types of *Vibriosis*: a story of human pandemics and oysters on the half shell. Clinical Infectious Diseases. 37: 272-280.

Murray, P.R.; Baron, E.J.; Jorgensen, J.H.; Pfaller, M.A. and Yolken, R.H. (2003): Manual of Clinical Microbiology. 8thed. American Society for microbiology, Washington, D. C.

Neilands, J.B. (1981): Microbial iron compounds. Annu. Rev. Biochem. 50:715-731.

NMKL "The Nordic Committee on Food Analysis" (2006): Aerobic count and specific spoilage organisms in fish and fish products. NMKL methods No. 189.

Olafsdottir, G.; Jonsdottir, R.; Lauzon, H.L.; Luten, J. and Kristbergsson, K. (2005): Characterization of volatile compounds in chilled cod *(Gadus morhua)* fillets by gas chromatography and detection of quality indicators by an electronic nose. Journal of Agriculture and Food Chemistry. 53: 10140-10147

Otsuka, T.; Noda, T.A.; Noguchi, A.; Nakamura, H.; Ibaraki, K. and Yakaoka, K. (2007): Shewanella infection in decompensating liver disease: a septic case. Gastroenterology 42: 87-90.

Palumbo, S.C.; Abeyta and Stelma, G. (1992): Aeromonas hydrophila group. In Compendium of Methods for the Microbiological Examination of Foods (Eds Vanderzat C. and Splittstoesser, D. F.) pp. 497-515. Washington. D.C. American Public Health Association.

Pererra, P.P.; Mathan, S.M.; Albert, P. and Baker, J. (1977): Aetiology of acute gastroenteritis in infancy and early childhood in southern India. Arch. Dis. Child. 52 (6) 482.

Popoff, M. (1984): Genus II. Aeromonas. Kluyver and Van Niel, 1936, 398^{AL}, p 545-548. In Krieg, N. R. and Holt, J. G, eds. Bergey's Manual of Systemic Bacteriology Vol. I. The Williams & Wilkins, Baltimore, London, Los Angelus, Sydney.

Satomi, M.; Vogel, F.B.; Gram, L. and Venkateswaran, K. (2006): *Shewanella hafniensis* sp. nov. and *Shewanella* morhuae sp. nov., isolated from marine fish of the Baltic Sea. International Journal of Systemic and Evolutionary Microbiology.56: 243-249.

Shewan, J.M. (1961): The Microbiology of Sea Water Fish. In. G, Borgstrom (ed.), Fish as food. Vol. 1. Academic Press, Inc., New York.

Shewan, J.M. (1977): The Bacteriology of Fresh and Spoiling Fish and the Biochemical Changes Induced by Bacterial Action, p. 51-60. In Proceeding of the Conference on Handling, Processing and Marketing of Tropical Fish. Tropical Products Institute, London, United Kingdom.

Skerman, V.B.D. (1967): A Guide to the Identification of the Genera of Bacteria. 2nd ed. The Williams and Wilkins Co., Baltimore.

Skjerdal, O.T.; Lorentzen, G.; Tryland, I. and Berg, J.D. (2004): New method for rapid and sensitive quantification of sulfide-producing bacteria in fish from arctic and temperate waters. International Journal of Food Microbiology. 15; 93 (3): 325-333.

Sneath, P.H.A.; Mair, N.S.; Sharpe, M.E. and Holt, J.G. (1986): Bergey's Manual of Systematic Bacteriology, Vol. 2. Baltimore, USA, Williams and Wilkins.

Yeung, P. and Boor, K. (2004): Epidemiology pathogenesis and prevention of foodborne *Vibrio parahaemolyticus* infections. Foodborne Pathogenic Disease. 1 (2): 74-88

Ziemke, F.; Höfle, M.G.; Lalucat, J. and Rosselló-Mora, R. (1999): Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. Nov. International Journal of Systemic Bacteriology. 48: 179-186.