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Comparative study on spoilage and pathogenic bacteria in selected commercial marine and freshwater fishes

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Abstract

Microorganisms are the major cause of spoilage of most seafood products. Fishes are more perishable than other protein foods and thus more prone to bacterial contamination. Based on above perspectives, a bacterial invasion in commercially important fresh and spoiled marine *(Lates calcarifer, Lutjanus sanguineus)* and freshwater fish *(Pangasius pangasius)* were analyzed using API 20E kit. Out of 25 isolates obtained from fresh water fish, only 6 isolates were characterized as Gram-positive bacteria and the rest were Gram negative strains (19 isolates). The most dominant genera were *Vibrio, Enterobacter, Serratia,* and *Aeromonas*. All these bacteria were found in both fresh fish and spoiled fish sample while *Erwinia* spp. and *Kluyvera* spp. were identified only in fresh fish samples. Out of four (4) strains of *Staphylococcus* spp., *S. xylosus* was detected exclusively from spoiled fish. The higher number of bacterial micro flora in the spoiled fish gut indirectly indicated increased microbial degradation in the fish gut during spoilage process. Notably, almost all the isolates were lactose degraders, positive oxidizers and carbohydrate fermenters. *Vibrio fluvialis, Proteus mirabilis, Proteus vulgaris, Brucella* sp. and *Ochrabactrum anthropi* were the human pathogenic bacteria found in marine fish *Lates calcarifer* (Sea perch). While *Vibrio fluvialis, Proteus mirabilis* and *Proteus vulgaris* were detected in *Lutjanus sanguineus* (Red snapper). The study portrays that the existing post-harvest handling techniques could be a vital factor for degrading hygienic conditions of fish in local fish markets. Nevertheless, a long term monitoring is an urgently needed for sustaining the quality flesh of fish towards the betterment of the consumer's health.

Introduction

Fishes by reasons of their habitat are continually bathed in aqueous suspension of microbes; their external surface therefore is in constant contact with these organisms. Some of these organisms may colonize the surface of the fish becoming part of the resident microflora. The presence of these microflora inhibits the arrival and subsequent colonization by other organisms that may be pathogenic to the fish. Bacteria inhabits other parts of the fish such as gill, mouth and gut (Jalal *et al*., 2010), while, a large stable population of bacteria inhabits the fish gut (Hamid *et al.*, 1979). These populations are able to survive the harsh conditions of the gastrointestinal tract. The microbial flora of freshly caught fish and other aquatic specimens is largely a reflection of the microbial quality of the waters from where they are harvested or stored. Of particular significance is whether the water is sewage polluted in which case the fresh water food is potentially capable of transmitting various pathogenic microorganisms (Pelczar *et al*., 1998).

Microorganisms are the major cause of spoilage of most seafood products. However, only a few members of the microbial community, the specific spoilage organisms (SSOs), give rise to the offensive off-flavors associated with seafood spoilage. In food industry, microbial degradation may manifest itself as spoilage that is changes in the sensory properties of a food product rendering it unsuitable for human consumption (Gram and Dalgaard, 2002). When the

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food stuffs are spoiled by bacteria, the opportunistic human pathogenic bacteria enter the food stuffs and leads to various food-borne illnesses in human such as meningitis, bacillary dysentery, typhoid, and food poisoning (Garbutt, 1997). Hence the association of bacteria in food is always being an interesting field of study (Gram and Dalgaard, 2002).

Fishes are more perishable than other protein foods and thus more prone to bacterial contamination Therefore, many studies have been conducted for the improvement in the handling of fresh fish (Derrick *et al*., 1982). A study on the effect of delayed icing on tropical fish has been conducted to determine the presence of bacteria at various time intervals. From the study, the bacterial count is still increasing even in immediate iced fish. Meanwhile, the delayed iced fish shows tremendous number of bacteria (Jayasekaran *et al*., 1991). However, the bacteriological counts could not be proper indicators of spoilage. This is because, not all bacteria species isolated from fish are associated with fish spoilage. It was also reported that the bacterial flora of fish depends solely upon the fish's recent intake of food and the degree of contamination in the food (Mizraji, 2017). Similar results were observed from the fishes caught from the marine environment, and both authors concluded that coliform bacteria are not usually associated with the normal intestinal flora of fish (Jorgel *et al*., 2016). Moreover, the number of spoilage organisms as a proportion of the total bacterial population changes as spoilage proceeds. Fish carry a variety of microorganisms from both aquatic and terrestrial sources. Apart from spoilage microorganism, fish may also contain various potential human pathogens. The difficulties in maintaining the quality is always being an issue because of the considerable distance between consumers and harvesting areas, which provides opportunities for microbial growth and recontamination (Fraser and Sumar, 2005).

Microbiological, chemical and organoleptic analyses are useful when assessing the quality of fish since these changes are associated with deterioration of fish quality during handling and storage. In Malaysia, the study on bacterial contamination in fresh and water fishes is still scanty. Hence, Malaysian government is giving priority on the importance of fish safety and nutritional quality of commercial fishes. Thereby, this study was mainly aimed to detect the presence of spoilage and pathogenic bacteria in different parts of commercially important marine (*Lates calcarifer* and *Lutjanus sanguineus*) and freshwater fish *(Pangasius pangasius)*.

Materials and methods

Sample collection and preparation

Two species of marine fishes namely *Lutjanus sanguineus* [n=15] (Cuvier, 1829) and *Lates calcarifer* [n=18] (Bloch, 1790) and a commercially important freshwater fish *Pangasius pangasius* [n=25] (Hamilton, 1822) were collected from the central fish market, Kuantan, Pahang, Malaysia and transported in iced condition to the laboratory prior to analysis. Fishes were further divided into two groups (fresh ice-chilled fish and 24 hours ice chilled fish) for bacterial analysis. Individual fish were placed in separate polyethylene bags containing 4 to 5 liters of filtered fresh seawater and incubated for the desired time at 38°C in a water bath. Sensory analyses were carried out to determine the organoleptic properties of fish by observing the deteriorating condition of its different body parts (Nielsen *et al*., 2002). Using sterile cotton tips, the fish samples were swabbed at four different parts; skin, gill and gut under laminar flow.

Skin samples [as thin as (1×2 mm)] were excised from a 10-cm^2 of skin surface from the central area of the fish by using a sterile scalpel and then placed in a sterile Petri dish. Gill samples were excised by first sterilizing the surface with a red-hot knife blade and then removing 5 g of gill tissue in a clean Petri dishes. The intestinal tract of each fish was removed aseptically with sterile scalpel and forceps. The individual samples were then directly placed in nutrient agar plates and incubated for 24hrs at 37^0C to observe bacterial growth.

Isolation of pathogenic and spoilage bacteria in fishes

Bacterial analysis was performed according to the method proposed by Buller (2004). Spoilage bacteria were isolated from different body parts (skin, gill and gut). Duplicate sets of Trypticase soy agar plates were inoculated and incubated at 38°C under aerobic and anaerobic conditions. Isolates of the spoilage bacteria were obtained from each set by sub-culturing all of the colonies on an aerobic and an anaerobic plate from identical dilutions of the fish sample. Aerobic isolates were gram-positive cocci and were identified by the catalase test and carbohydrate fermentations (Kumar *et al*., 2017) and from colony morphology and various biochemical tests (Society of American Bacteriologists, 1957). Facultatively anaerobic isolates were gram-negative rods that consisted of oxidase-negative Enterobacteriaceae and were identified by differential media and carbohydrate tests (Edwards and Ewing, 1972). Stock cultures of aerobic

Table 1. Sensory analysis for both fresh and spoiled fresh water fish *(Pangasius pangasius)* is represented

Quality parameter	Character	Condition	Fresh fish sample			Spoiled fish sample (After 24hrs)		
General appearance	Skin	Bright, shining	P1	P2	P3	P1	P2	P3
		Bright	√	√	√			
		Dull				√	√	√
	Bloodspot on gill Cover	None	√	√	√			
		Small				√	√	√
		Big						
		Very big						
	Stiffness	Stiff, in *rigor mortis*	√	√	√			
		Elastic						
		Firm				√		
		Soft					√	√
	Belly	Firm	√	√	√			
		Soft					√	
		Belly burst				√		√
	Smell	Fresh, seaweed/metallic	√	√	√			
		Neutral						
		Musty/sour				√	√	√
		Stale meat/rancid						
Eyes	Clarity	Clear	√	√	√			
		Cloudy						
	Shape	Normal	√	√	√			
		Plain				√	√	√
		Sunken						
Gills	Colour	red	√	√	√			
		Faded						
		discoloured				√	√	√
	Smell	Fresh, seaweed/metallic	√	√	√			
		Neutral						
		Sweaty/slightly rancid				√	√	√
		Sour stink/stale, rancid						

Note: Body temperature was noted for fresh fish sample (24^0C-25^0C) and spoiled fish sample (26^0C- 27^0C). Three replicate of fishes (P1, P2 and P3) were observed for organoleptic analyses.

and facultatively anaerobic isolates were maintained on Trypticase soy agar slants and subcultured periodically to ensure viability. Obligately anaerobic isolates were maintained in cooked meat medium under a Vaspar anaerobic seal. In order to maintain the viability of pure colonies, they were preserved in 15% glycerol solution and stored at -20°C (Giraffa and Rossetti, 2004).

Bacterial identification

The Gram staining was performed based on a method proposed by American Society of Clinical Pathology (ASCP, 2004) with some modifications. The slides were readily observed under the light microscope to determine the cell shape and gram positive and negativity of bacteria.

API® 20E test

A tube containing 5 ml of sterile saline was prepared for each bacterial isolate and then using a sterile pipette a single isolated colony from pure culture were transferred into the tube containing sterile saline and homogenized. The homogenized bacterial suspension was distributed into the 21 microtubes of the API® 20E strip and incubated at 37°C for 18 to 24 hours. Following the incubation, the results of the reactions on the strip were red by referring API 20E kit standard manual. All the positive and negative reactions were recorded and results were analyzed by using Apiweb™ software for the bacterial identification.

Results

Organoleptic properties of fresh and spoiled fishes were analyzed. After 24 hours of fish spoilage, fishes showed dull skin, small bloodspot on gill cover and musty or sour smell. They had no longer stiff flesh and the eyes were cloudy and sunken. The gills were discoloured and have slightly rancid smell (Table 1).

The results showed that the isolated bacterial microflora in fresh and spoiled Pangasius pangasius was dominated with gram negative strains. Out of 25 isolates obtained, only 6 isolates were characterized as Gram-positive bacteria and the rest which is 19 isolates were Gram negative bacteria. The observed possible isolates from 9 genera were identified from the skin, gill, and gut of both fresh and spoiled fish samples. The most dominant genera were Vibrio, *Enterobacter, Serratia,* and *Aeromonas*. All these bacteria were found in both fresh fish and spoiled fish sample while *Erwinia* spp. and *Kluyvera* spp. were identified only in fresh fish samples (Table 2).

Three isolates of *Staphylococcus* spp. (*Staphylococcus intermedius, Staphylococcus cohnii* and *Staphylococcus lentus*) were identified in fresh fish while *Staphylococcus xylosus* was detected from spoiled fish. 7 genus of bacteria microflora were

Table 2. Bacteria microflora identified in fresh and spoiled fresh water fish *(Pangasius pangasius)* using API 20E kit.

Body parts	Species of Bacteria Identified in fresh sample	Species of Bacteria Identified in spoiled fish (After 24hrs of spoilage)
SKIN	*Enterobacter cloacae*	*Serratia odorifera 1*
	Brucella spp.	*Serratia odorifera 2*
	Providencia rettgeri	*Klebsiella ornithinolytica*
	Vibrio parahaemolyticus	*Staphylococcus xylosus*
	Vibrio fluvialis	*Staphylococcus lentus*
	Aeromonas hydrophilla Group 1	
	Aeromonas hydrophilla Group 2	
	Yersinia enterocolitica	
	Staphylococcus intermedius	
	Staphylococcus cohnii	
GILL	*Erwinia spp.*	*Vibrio fluvialis*
	Enterobacter cloacae	*Aeromonas hydrophilla Group 1*
	Enterobacter sakazakii	*Aeromonas hydrophilla Group 2*
	Klebsiella ornithinolytica	*Klebsiella ornithinolytica*
	Kluyvera spp.	*Serratia odorifera 1*
	Serratia odorifera 1	*Enterobacter sakazakii*
GUT	*Enterobacter cloacae*	*Vibrio fluvialis*
	Klebsiella ornithinolytica	*Vibrio cholerae*
	Enterobacter aerogenes	*Enterobacter aerogenes*
		Enterobacter cloacae
		Klebsiella terrigena
		Aeromonas hydrophilla Group 1
		Aermonas hydrophilla Group 2
		Serratia odorifera 2

identified in skin of fresh fish samples and 5 from gill and 2 from gut regions. Whereas 3, 5 and 5 genus were identified in skin, gill and gut respectively from spoiled fish samples indicating that the bacterial degradation increases in the gut region during spoilage process. It was also evident from the result that there were 15 bacterial isolates observed in gut region of spoiled part followed by skin (11 isolates) and gill (8 isolates) of fresh fish samples (Figure 1). Among the identified bacteria *Enterobacter* spp. was dominant in fresh sample and *Serratia* spp. and *Aeromonas* spp. were dominant in spoiled fish. *Kluyvera* spp. *Erwinia* spp. *Yersinia* sp. *Providencia* sp. and *Brucella* spp. were only identified in fresh fish samples (Figure 2). It was also noted that almost all the isolates were Lactose degraders, positive oxidizers and carbohydrate fermenters.

Among the two (2) selected marine fishes, *L.calcarifer* had higher number of bacterial microflora in different parts of fishes. The study revealed that *Vibrio fluvialis, Proteus mirabilis, Proteus vulgaris, Brucella* sp. and *Ochrabactrum anthropi* were the human pathogenic bacteria found in sea perch while *Vibrio fluvialis, Proteus mirabilis* and *Proteus vulgaris* were detected in red snapper. *Vibrio fluvialis* was the fish spoilage bacteria found in both fishes while *Shewanella putrefaciens* was detected specifically in sea perch and *Photobacterium damsala* was detected in red snapper (Figure 3).

As It was observed in fresh water fish *(Pangasius pangasius)*, bacterial load in gut region of spoiled marine fishes were also high (42 isolates) compare to fresh sample (12 isolates). There were least differences observed in number of bacterial isolates in other body parts (gill, scale and flesh) of marine fish (Figure 4).

Discussion

The spoilage of fish and fish products are always being associated with the chemical and biological changes happens during postharvest techniques including handling and storage. These changes are responsible in causing spoilage of the food by changing the odor, taste, texture and its appearance. The live and healthy fish is free from pathogenic microbes, but right after death, microorganisms start to grow. Typically, only one or two bacterial species would be responsible for the production of metabolites associated with the off-flavours and off-odours of spoilage (Fraser and Sumar, 2005). Similar result was observed in the present study. Considerable physical changes in organoleptic properties of spoiled fishes showed the role of certain microbial secondary metabolites. It was reported that microbial metabolites such as amines, sulfides, alcohols, aldehydes, ketones and organic acids leads to unpleasant and unacceptable off-flavours and off-odours. On the other hand, carbohydrates and fatty acid can be broken down to give particularly lactic acid and fatty acid respectively which exhibit the smell of rancidity (Gram and Dalgaard, 2002). It is also to be noted that fresh sea foods are highly susceptible to spoilage from post-mortem microbial growth, chemical reaction and continuing activity of endogenous enzymes. As reported by previous

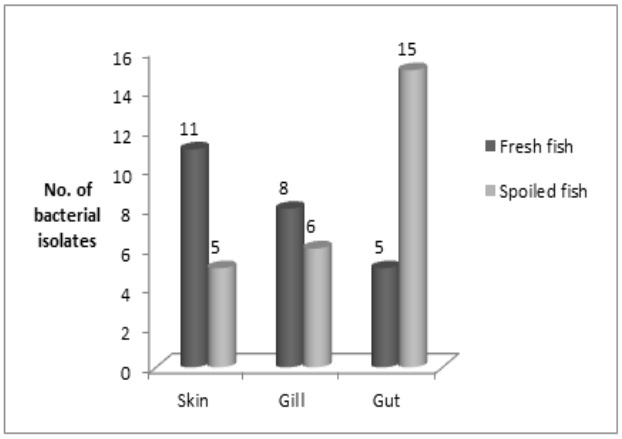

Figure 1. Number of Bacterial isolates observed in different parts of fresh and spoiled fresh water fish *(Pangasius pangasius)*

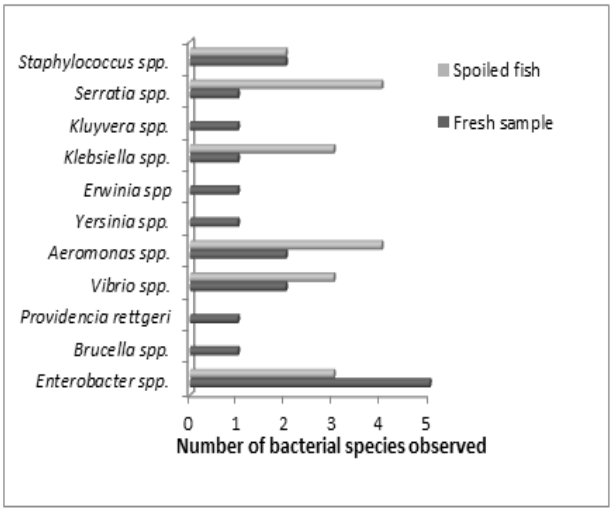

Figure 2. Number of bacterial species corresponds to their related genus observed in fresh and spoiled fresh water fish *(Pangasius pangasius)*

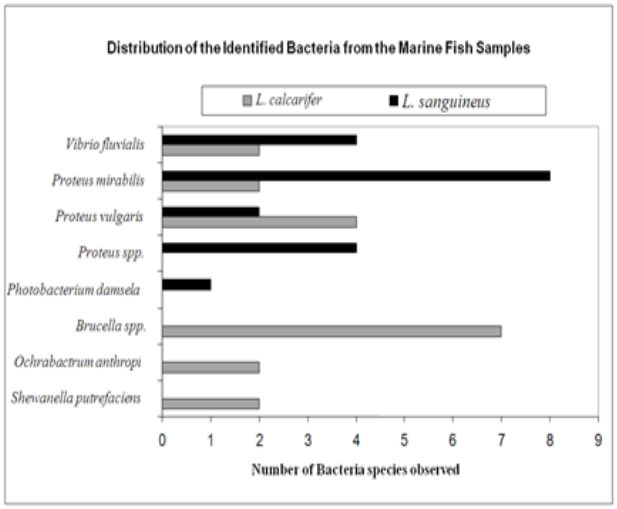

Figure 3. Number of bacterial species corresponds to their related genus observed in marine fishes (*Lates calcarifer* and *Lutjanus sanguineus*)

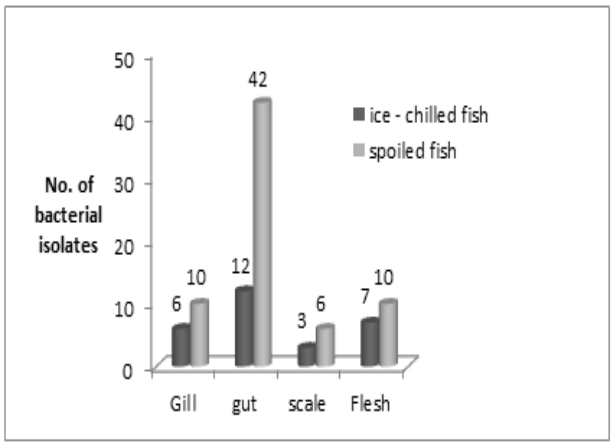

Figure 4. Number of bacterial isolates observed in different parts of fresh and spoiled marine fish *(Lates calcarifer)*

studies, fish spoilage is primarily due to (i) Autolysis. (ii) Bacterial growth and metabolism resulting in the formation of off-flavor compounds and (iii) Chemical oxidation of lipids. Among these reasons, microbial activity is by far the most important factor influencing the fish quality (Ezquerra-Brauer *et al*., 2016; El-shemy *et al*., 2016; Zahra *et al*., 2016). However, not all microorganisms in sea food are equally important for quality change. Fish feeding habits, geographical location, season, sea temperature, type of fish, place in which the fishes were harvested and storage conditions, determine the spoilage domains of specific spoilage organism (SSO) (Comai *et al*., 1982; Drosinos and Board 1995). Hence it is important to be noted that microbial flora of fresh fish on the fishing depends on the environment in which it was caught (Ali, 2010).

Bacteriological examination of various organs of freshwater fish *(Pangasius pangasius)* has successfully isolated 25 isolates of bacteria. Both gram negative (19 isolates) and gram positive (6 isolates) bacteria were found and each of them may affect differently (Kasing *et al*., 1999). The major spoilage bacteria commonly found in fish consists primarily of members of the genera *Pseudomonas* and *Achromobacter* spp. (Nielsen *et al*., 2017). *Pseudomonas* spp. is psychrotrophic bacteria that are usually found in iced or refrigerated fish. Meanwhile, the major pathogenic bacteria mostly found are from Enterobacteriaceae family usually consisting of *Shewanella, Salmonella* and *Escherichia* spp. (Scheu *et al*., 1998). In Europe, enterobacteriaceae has been widely used for years as indicators of food quality and as indices of food safety (Frances and Keith, 2001). An attempt was made in 1970s differentiate between the use of *E. coli,* Enterobacteriaceae and coliforms as a marker or the index of the potential presence of pathogens (i.e., food safety) and the use of these organisms as an indicators of overall food quality (Mossel, 1978). These observations were well

corresponded with our findings where *Enterobacter* spp. was the dominant species in fresh samples and its count increased over a period of spoilage. Ramos and Lyon (2000) also reported that Fresh catfish fillets are known to be contaminated with a large number of spoilage and pathogenic bacteria.

Microflora in marine fish samples revealed that the presence of human pathogenic bacteria that are capable of producing heat stable protein that could cause various ill effects on normal functioning of human body when it is consumed. Among the bacterial microflora observed *Vibrio fluvialis, Proteus mirabilis* and *Proteus vulgaris* were the human pathogens present in both the marine fishes whereas only *V. fluvialis* was observed in fresh water fish (Peerbooms *et al*., 1985; Economopoulou *et al*., 2016; Younes *et al*., 2016).

Fishes are more susceptible to bacterial invasion from the external environment during normal instance and in particularly during spoilage process. In fact poor hygienic of fish handling practices and improper fish storage conditions have been observed to be the potential contamination sources of these bacteria. The present findings might be instrumental to aid both the food safety regulatory bodies and the Hazard Analysis Critical Control Point System (CCPS) in setting up new standards and guidelines for the awareness on post-harvest fish handling practices in the public fish market and sea food restaurants in Malaysia.

Conclusion

In conclusion, commercially important fishes sampled in this study were contaminated either with water born human pathogenic bacteria *Vibrio* spp. and terrestrial born *Staphylococcus* spp. The post harvesting handling procedure has significant role in hygiene maintenance whereby avoiding wounds in fish flesh would help in mitigating airborne bacterial contamination. Constant inspections and long term monitoring in local markets by authorities would pave a way in minimizing the spread of bacterial pathogenicity in human and thereby help in consumer's health support.

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References

Ali, A. 2010. A review on pseudomonas in marine fish. World Journal of Fish Marine Sciences 2(4): 291-296.

American Society for Clinical Pathology (ASCP). 2004. Educational commentary-Gram staining. Retrieved on August 27, 2007 from ASCP website: *http://www.api-pt.com/pdfs/2004Bmicro.pdf*

Buller, N., 2004. Bacteria from Fish and other Aquatic Animals: A Practical Identification Manual. Australia: CABI Publishing.

Comai, L., Surico, G. and Kosuge, T. 1982. Relation of plasmid DNA to indolacetic acid production in different strains of Pseudomonas syringae pv. Savasatoni. Journal of General Microbiology 128: 2157-2163.

Derrick, H., Yoshinaga, H.A. and Frank. 1982. Histamine-producing bacteria in decomposing skipjack tuna *(Katsuwonus pelamis)*. Applied and Environmental Microbiology 44: 447-452

Downes, F.P. and Itō, K. 2001. Compendium of methods for the microbiological examination of foods. 4th ed. Washington: American Public Health Association.

Drosinos, E.H. and Board, R.H. 1995. Microbial and physicochemical attributes of minced lamb: source of contamination with Pseudomonas. Food Microbiology 12: 189-197.

Economopoulou, A., Chochlakis, D., Almpan, M.A., Sandalakis, V., Maraki, S., Tselentis, Y. and Psaroulaki, A. 2016. Environmental investigation for the presence of *Vibrio* species following a case of severe gastroenteritis in a touristic island. Environmental Science and Pollution Research: 1-6.

Edwards, P.R. and Ewing, W.H. 1972. Identification of Enterobacteriaceae. 3rd ed. Minneapolis: Burgess Publishing Co.

El-shemy, M.G.Y., Yasin, N.M., Gadallah, M.G.E. and Hanafi, E.K. 2016. Microbiological Quality and Enzymes Activity of Refrigerated Bolti Fish *(Tilapia nilotica)* Pretreated with Organic Acids. Journal of Agricultural and Veterinary Sciences 9(1): 57-70.

Ezquerra-Brauer, J.M., Miranda, J.M., Cepeda, A., Barros-Velázquez, J. and Aubourg, S.P. 2016. Effect of jumbo squid *(Dosidicus gigas)* skin extract on the microbial activity in chilled mackerel *(Scomber scombrus)*. LWT-Food Science and Technology 72: 134-140

Fraser, O.P. and Sumar, S. 2005. Compositional changes and spoilage in fish (part II) – microbiological induced deterioration. Journal of Nutrition and Food Science 98: 325-329.

Garbutt, J. 1997. Essentials of Food Microbiology. London: Arnold Publisher.

Giraffa, G. and Rossetti, L. 2004. Monitoring of the bacterial composition of dairy starter cultures by RAPD-PCR. FEMS Microbiology Letters 237: 133-138.

Gram, L. and Dalgaard, P. 2002. Fish spoilage bacteria--problems and solutions. Current Opinion in Biotechnology 13(3): 262-266.

Hamid, A., Sakata, T. and Kakimoto, D. 1979. Microflora

in the alimentary tract of grey mullet. 4. Estimation of enzymic activity of the intestinal bacteria. Bulletin of the Japanese Society for the Science of Fish 45: 99-106.

Jalal, K.C.A., Fatin, Mardiana, Akbar John, B., Kamaruzzaman, Y.B. and Mohd. Nor. 2010. Antibiotic Resistance Microbes in Tropical Mangrove Sediments, East Coast Peninsular Malaysia. African Journal of Microbiology Research 4(8): 640-645.

Jayasekaran, G. and Saralaya, K.V. 1991. Influence of fish chilling methods on the quality of white sardine. Fish Technology 28(1): 55 - 58.

Jorgel, R.J., Redondo, P.N. and Dosta, M.M. 2016. Bacterial load comparison of marine fish collected and commercially obtained for human consumption in western region of Yucatan Peninsula, Mexico. International Journal of Aquatic Science 7(1): 6-12.

Kasing, A., Asiah, M.Y. and Kumbang, J. 1999. Distribution of bacteria in tropical freshwater fish and ponds. International Journal of Environmental Health Research 9(4): 285 – 292.

Kumar, M.P. and Ramulu, K.S. 2017. Presumptive and definitive identification of Pseudomonas from infected Pangasius hypophthalmus in culture ponds of West Godavari and Krishna districts of Andhra Pradesh. Journal of Microbiology and Biotechnology Research 3(3): 41-45.

Mizraji, R., Ahrendt, C., Perez-Venegas, D., Vargas, J., Pulgar, J., Aldana, M. and Galbán-Malagón, C. 2017. Is the feeding type related with the content of microplastics in intertidal fish gut?. Marine Pollution Bulletin [In press].

Mossel, D.A.A. 1978. Index and Indicator organisms- a current assessment of their usefulness and significance. Food Technology in Australia 30: 212–219.

Nielsen, J., Hydlig, G. and Larsen, E. 2002. Eating quality of fish- A review. Journal of Aquatic Food Technology 11(3/4): 125-141.

Nielsen, S.M., Nørskov-Lauritsen, N., Bjarnsholt, T. and Meyer, R.L. 2016. *Achromobacter* Species Isolated from Cystic Fibrosis Patients Reveal Distinctly Different Biofilm Morphotypes. Microorganisms 4(3): 33.

Peerboomas, P.G.H. Verweij, M.J.J. and Maclaren. D.M. 1985. Uropathogenic Properties of *Proteus mirabilis* and Proteus vulgaris. Journal of Medical Microbiology 19(1): 55-60.

Pelczar, M.J., Chan, E.C.S., Krieg, N.R. and Pelczar, M.F. 1998. Microbiology. 5th ed. New York: Mc Graw-Hill Book Co.

Ramos, M. and Lyon, W.J. 2000. Reduction of endogenous bacteria associated with catfish fillets using the Grovac process. Journal of Food Protection 63(9): 1231-1239.

Scheu, P.M. Berghof, K. and Stahl, U. 1998. Detection of pathogenic and spoilage micro-organisms in food with the polymerase chain reaction. Food Microbiology 15: 13-31.

Society of American Bacteriologists. 1957. Manual of microbiological methods. New York: McGraw-Hill Book Co.

Younes, A.M., Fares, M.O., Gaafar, A.Y. and Mohamed, L.A. 2016. Isolation of *Vibrio alginolyticus* and *Vibrio vulnificus* Strains from Cultured Oreochromis niloticus Around Qarun Lake, Egypt. Global Veterinaria 16(1): 1-5.

Zahra, S.A., Butt, Y.N., Nasar, S., Akram, S., Fatima, Q. and Ikram, J. 2016. Food Packaging in Perspective of Microbial Activity: A Review. The Journal of Microbiology, Biotechnology and Food Sciences 6(2): 752.