Evaluation of Lactic Acid Bacteria, Ozone Treated Water and Ultra Violet Irradiation as Surface Decontamintant in Artificially Contaminated *Thunnus obesus* and *Clarias gariepinus*

M.N. Lani[1], Adilah Ismail[1], Nurul Ulfah Karim[2], Marzuki Ismail[3], Abdul Faiz Din Abdullah[1], Norfariha Baharin[1] and Abd. Rahman Tamuri[4]

[1] School of Food Science and Technology, Universiti Malaysia Terengganu, 20130 Kuala Terengganu, Malaysia
[2] School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, 20130 Kuala Terengganu, Malaysia
[3] School of Marine and Environmental Sciences, Universiti Malaysia Terengganu, 20130 Kuala Terengganu, Malaysia
[4] Physics Department, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia

Fishes are good sources of protein with low fat content as compared to another protein sources food such as meat and poultry. However, the problem with fish both freshwater and marine, they are associated with microbial contamination which made them perishable. In this study, Lactic Acid Bacteria (LAB), ozone treated water, and ultra-violet (UV) irradiation were used as surface decontaminant on these fishes. UV irradiation is the possible way to reduce contamination, which is commonly used for the decontamination of packaging surfaces or in food processing environments. Meanwhile, LAB have high potential as natural decontaminant against pathogens. The studies were carried out independantly on two different species of fish which were big eye tuna (*Thunnus obesus*), a marine fish and African catfish (*Clarias gariepinus*), a freshwater fish.

Big eye tuna (*Thunnus obesus*) is a marine fish that is widely consumed all around the world. The safety issues concern with tuna was contamination with *Vibrio* sp. Therefore, in this study, the fresh raw big eye tuna was artificially contaminated with 10^5 CFU/ml of *Vibrio* sp. and *Vibrio parahaemolyticus* in order to see the full potential of LAB and ozone water treatment against these organisms. The experiment was conducted by spraying of LAB cell supernatant onto fish surfaces for 4 h contact time and by soaking fish into ozone treated water with 1 and 3 ppm for 2 h contact time. The source of LAB were isolated from goat's milk and yogurt drink, which identified as *Lactobacillus plantarum* and *Pediococcus pentosaceus* by using API 50 CHL. The results showed that *L. plantarum* and *P. pentosaceus* had reduced 2-$\log_{10}$ reduction of *Vibrio* sp. and complete elimination of 10^5 CFU/ml of *Vibrio parahaemolyticus* in raw big eye tuna. As for ozone treated water, the most suitable concentration to eliminate *Vibrio parahaemolyticus* was 3 ppm. However, complete elimination of *Vibrio* sp. was not achieved with ozone treated water.

In other experiment, the role of UV irradiation and LAB as surface decontaminant in African catfish (*Clarias gariepinus*) were evaluated. This experiment was also conducted by artificially contaminated the fresh fish sample with *A. hydrophila* isolated from catfish with 10^6 CFU/ml. The contaminated fish samples were then exposed to UV radiation with 3 Watts for 0, 5, 15 and 25 minutes. The reduction of bacteria were observed significantly ($p<0.05$) in the first 0, 5 and 15 minutes of exposure. However, the result showed an increasing number of bacteria survivors afer 25 minutes of exposure against full spectrum of UV light. The increase of the counts indicated the photorepair phenomenon occured during exposure. Meanwhile, the exposure of contaminated African catfish towards *Lb. plantarum* were carried out for 3 hours of contact time and the bacterial count were checked for every hour. The bacterial count from the first three hour contact time shows significant reduction ($p<0.05$) of bacterial count from the African catfish that has 0, 1 and 2 h contact time. However, the bacterial count increases after 2 hour and 3 hour contact time. The increase of the bacterial counts suggests that the effectiveness of metabolites produced from lactic acid bacteria as surface decontaminant is time dependant.

Keywords: Lactic acid bacteria; ozone treated water; Ultra violet irradiation; *Thunnus obesus*; *Clarias gariepinus*

1. Introduction

Big eye tuna *Thunnus obesus* is a widely traded species. According to Kevan and Roland, 1999, the problem with tuna fish is vibriosis which has been associated with stress factors such as handling, high densities, and nutritional deficiencies, extremes in temperature, cuticle injuries, and elevated levels of ammonia, salinity or nitrogen. This problem concerns the consumers about the safety issues of tuna fish. In addition, recently, there are high demands from consumers for minimally processed foods with high safety level (Cleveland et al., 2001). *Aeromonas* sp. and *Vibrio* sp. are microorganisms widely distributed in nature, such as in water, soil, food and also part of normal bacterial flora of animals. However, they frequently cause problems in both feral and cultured fish (Douglas, 2007). Thus, the contamination of these organisms may responsible for heavy economic losses caused by both high mortality and deterioration of product quality (Lack, 2007).

Lactic acid bacteria have a very high potential in natural decontaminant and bio-protective agent. Their production of lactic and acetic acids, ethanol, aroma compounds, bacteriocins, exopolysaccharides and several enzymes is important in enhancing shelf life, microbial safety and improving texture (Blagojev, 2011). Until now, application of LAB have been successfully applied to the pork carcasses (Pipek et al., 2005) and raw shrimps (Nurul Ain Shohor, 2013).

Ozone is a United State Food and Drug Administration (USFDA) and United State Department of Agriculture (USDA) approved antibacterial agent that can be applied to food products. Ozone has short shelf life at ambient temperature and does not leave behind residues unlike chlorine (Chawla, 2006).

UV irradiation is the possible way to reduce contamination, which is commonly used for the decontamination of packaging surfaces or in food processing environments (Corry et al., 1995; Bolder, 1997; Dinçer and Baysal, 2004). The range of UV radiation that is considered to be germicidal against bacteria is between 220 and 300 nm (UV-C), and generally a wavelength of 254 nm is used for decontamination (Guerrero-Beltrán and Barbosa-Cánovas, 2004). Several studies have been undertaken to investigate the effect of UV irradiation on food items. Stermer et al. (1987) found that UV lowered the counts of bacteria commonly found on beef meat, mostly *Pseudomonas*, *Micrococcus*, and *Staphylococcus* spp., whereas on poultry skin and carcasses, *Salmonella* Typhimurium was effectively diminished (Wallner-Pendleton et al., 1994; Sumner et al., 1996).

Therefore in these studies, ozone treated water and UV irradiation were carried out in comparison with LAB as surfaces decontaminant on artificially contaminated *Thunnus obesus* and *Clarias gariepinus*.

2. Evaluation of ozone treated water and LAB as surface disinfectant of artificially contaminated *Thunnus obesus*

2.1 Methodology

2.1.1 Isolation and identification of *Vibrio* sp.

The fresh raw big eye tuna fish were eviscerated to separate flesh, gills and intestines parts. Then, flesh, gills and intestine of the raw big eye tuna fish were weighed into 10 g for gills and intestine while 25 g for fish flesh. After that, each part of fish was collected in a sterile stomacher bag with 90 ml and 225 ml alkaline peptone water, respectively. The serial dilution was done and 0.1 ml of each dilution from each part of fish was spread onto Thiosulfate-Citrate-Bile Salt-Sucrose Agar (TCBS) agar for isolation and detection of *Vibrio* sp. The agar was then incubated aerobically for 24 h at 37^{o}C. After 48 h, there were typical *Vibrio* sp. colonies found on TCBS such as green, yellow, and yellow-green colonies on the plate. The colonies were then checked on Gram staining and oxidase test for identification.

2.1.2 Artificial contamination of big eye tuna with *Vibrio* sp.

The fresh cultures of *Vibrio* sp. were inoculated into 500 ml Tryptic Soy Broth and incubated into 37°C for 24 hours. Then, the fresh of raw big eye tuna fish were soaked into the Tryptic Soy Broth with *Vibrio* sp. The soaking time were fixed within three different contact times on three raw big eye tuna which is 1 hour, 12 hours and 24 hours. Lastly, the three big eye tuna was evaluated by using plate count on TCBS agar.

2.1.3 Isolation and identification of Lactic acid bacteria

LAB isolated from goat's milk and yogurt drink using de Man, Rogosa and Sharpe (MRS) Agar with 0.8% $CaCO_3$ were screened by Gram staining, oxidase and catalase test. All Gram positive, oxidase negative and catalase negative strains were further identified by API 50 CHL (bioMérieux, France).

2.1.4 LAB Cell supernatant preparation

24 hours fresh colonies of LAB were inoculated in 100 ml of de Man, Rogosa and Sharpe (MRS) broth for 24 h at room temperature (28±2 °C). After that, the 50 ml of broth was centrifuged in refrigerated centrifuge (11500 rpm for 10 min). Then, the supernatant was discarded and replaced with Phosphate Buffer Saline (PBS) solution and centrifuged again at the same speed and time. The washing step was repeated. After that, the supernatant was homogenized together with the pellet by using Vortex.

2.1.5 Application of cell supernatant onto big eye tuna by spraying method

The 20 ml of the supernatant of LAB was transferred into sterile sprayer bottle. The contaminated big eye tuna fish were arranged on the sterile tray within 0, 1, 2, 3, and 4 hours respectively. Then the contaminated of raw big eye tuna fish were sprayed thoroughly within 1 minute on the surface of the fish. The setting time interval was done using a digital timer.

2.1.6 Set-up of Ozone treatment

Two close-top chambers (CTCs) were constructed and used in this study. Both of them having the same structure, fabricated from stainless steel covered with transparent polyvinylchloride (PVC) plastic as shown in Figure 1. Each CTC is 1 meter in height, 1.5 meter in diameter, 0.77m^2 in base area and 1.77m^3 in capacity. Air-conditioner was

applied as mechanical ventilation system inside each chamber in order to ensure air exchange could be done and temperature and relative humidity could be controlled. One of the CTCs was supplied with additional elevated ozone i.e. 100 part per billion (ppb) for exposure process and the other as as control; without additional elevated ozone. Microclimatic parameters i.e. temperature and relative humidity were measured both inside and outside the CTCs. Temperature and relative humidity for both chambers are similar but inside chambers were 0.7 to 5.8 ^{O}C and 4.4 to 45.8 % more than ambient condition.

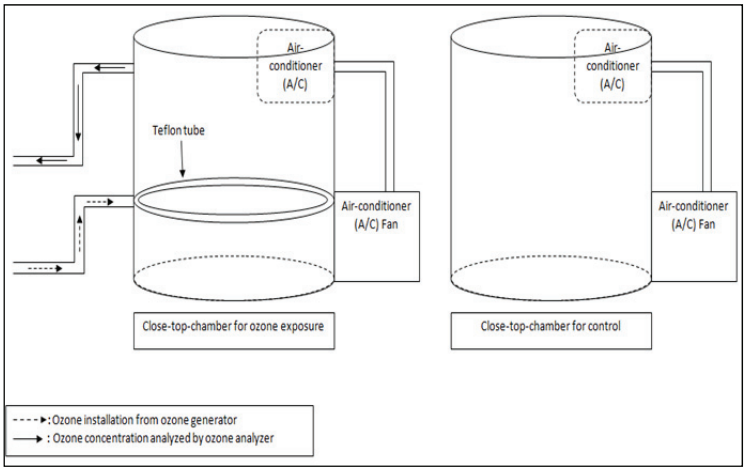

Fig. 1 Schematic diagram of ozone generator

2.1.7 Ozone treated water preparation

About 500 mL of plain water with ice cubes (40:60) was poured into a sterilized container and the icy water was treated with ozone gas. The concentration of ozone was 1.0 rpm and 3.0 rpm.

2.1.8 Application of ozone treated water against big eye tuna by soaking method

The contaminated big eye tuna fish was soaked into the ozone treated water for about 2 hours as recommended by Dehkordi (2010). After that, each sample of the big eye tuna was plated on TCBS Agar, Eosin Methylene Blue Agar and Violet Red Bile Dextrose (VRBD) Agar and incubated at 35°C for 24 h for determining the level of *Vibrio* count, coliform count and *Enterobacteriaceae* count, respectively that contained on that fish.

2.2 Results and Discussions

2.2.1 Identification of *Vibrio* spp. in raw big eye tuna

Fish are much more perishable and likewise, are better substrates for multiplication of pathogenic bacteria (Mossel et al., 1995). Bacteria can enter the fish body through the gills or skin or it can stay on the surface of the body (Douglas, 2007). In this study, *Vibrio* count, *Enterobacteriaceae* count and Coliform count were assessed in different parts in big eye tuna fish as shown in Table 1.

Table 1 The counts of *Vibrio* sp. in fresh raw big eye tuna fish

Different parts of fish	Microbial count (log_{10} CFU/g)		
	Vibrio spp. count	*Enterobacteriaceae* count	Coliform count
Gills	7.0×10^2	0	0
Intestines	0	0	0
Flesh	0	0	0

Due to the low count obtained for fresh big eye tuna fish, it is very difficult to see the full potential of ozone treated water in reducing *Vibrio sp*. contamination if the initial microbial load of *Vibrio* is too low. Therefore, artificial contamination was introduced to the fresh big eye tuna fish. This method can be used to mimic the possible risk of high microbial load of *Vibrio* in big eye tuna fish and how the application of ozone treated water and LAB supernatant could be used to reduce the *Vibrio* contamination in fish.

2.2.2 Artificially contamination with *Vibrio* spp. in raw *Thunnus obesus*

Based on the results from Figure 2, the 12-h retention time was the most suitable contact time for optimum growth of *Vibrio* spp. in the raw big eye tuna fish. This present finding was supported by Wang et al., 2010, who proved that 12 h of retention time was optimum for *Vibrio sp*. to grow in the oyster. Although oyster and fish are two different commodities, the gross chemical composition and spoilage association of these two commodities are very similar (Mossel et al., 1995).

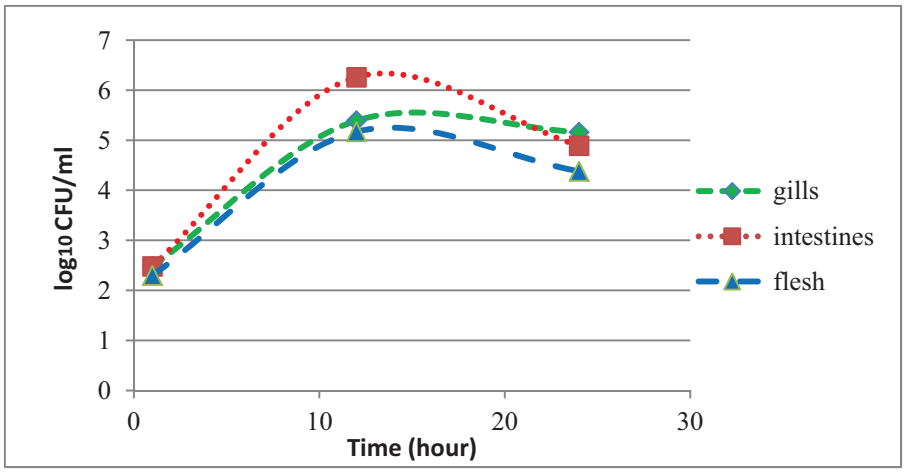

Fig. 2 The growth of *Vibrio* spp in different parts of *Thunnus obesus* after artificially contaminated.

To clarify whether the 12-h retention time was significantly different with other contact time, statistical analysis was carried out using one-way ANOVA. The treatment of soaking in artificially contaminated of intestines at 12-h contact time had the highest count of *Vibrio*. However, the *Vibrio* count in the flesh was not significantly different ($P>0.05$) at the same contact time. This is not surprising because the flesh is the most outer layer of the fish and it was easily and directly contacted with the media. Besides that, all of the contact times between samples were significantly ($P<0.05$) different as shown in Figure 3. Increasing contact time had increased the growth of *Vibrio*, however this only applicable within 12 h. Therefore, 12-h of soaking had reached the maximum growth of *Vibrio* in raw big eye tuna fish. At this point, the contamination had reached stationary phase and became saturated.

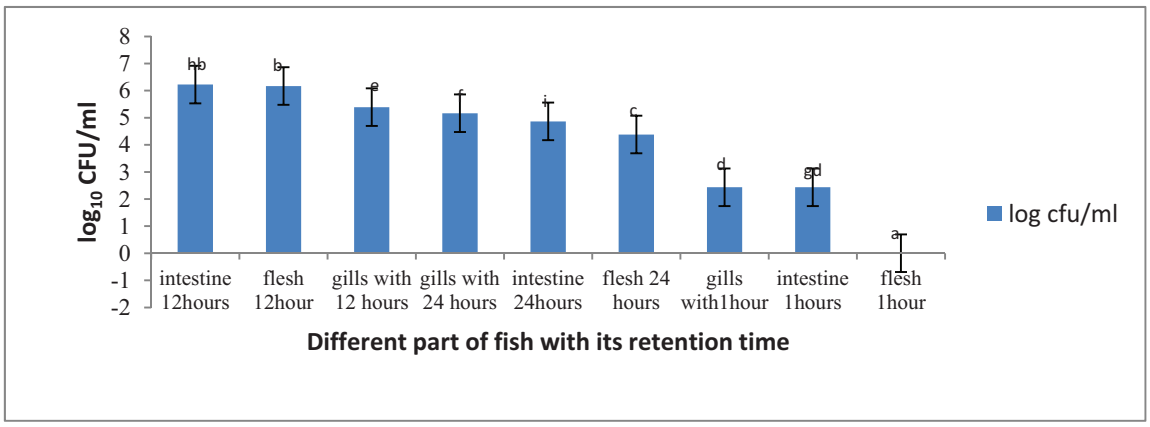

Fig. 3 The *Vibrio* count in artificially contaminated raw big eye tuna and its retention time

2.2.3 Identification of LAB strain from goat's milk and commercial yogurt

Two strains of LAB were Gram-positive bacteria with negative result for catalase and oxidase. These strains were further tested biochemically by API 50 CHL. The result obtained for strain from Goat's milk and commercial yogurt showed high probability, 99.9% and identified as *Lactobacillus plantarum* and *Pediococcus pentosaceus*, respectively.

2.2.4 Treatment of contaminated *Thunnus obesus* with LAB cell supernatant

LAB supernatant from *Lb. plantarum* was then applied to the big eye tuna fish that exposed to *Vibrio sp.* within a different contact time. LAB supernatant had the ability to reduce the pathogenic bacteria by the production of metabolites produced by LAB (Liasi et al., 2009). These metabolites consist of antimicrobial substances, such as several bacteriocins, acetic acid, propionic acid and phenyl lactic acid (Stiles, 1996). Based on the result in Figures 4 and 5, it is obvious that LAB supernatant from *Lb. plantarum* had a decontaminant effect against *Vibrio* spp.

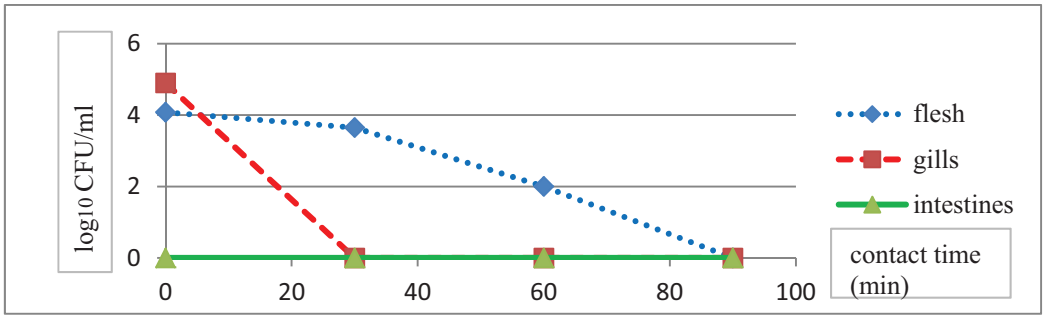

Fig. 4 The count of *Vibrio parahaemolyticus* after treated with LAB cell supernatant at different contact time.

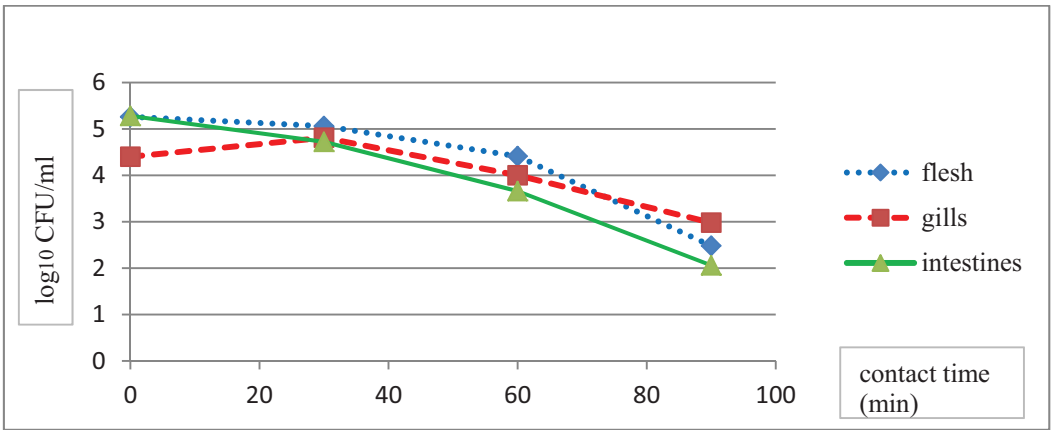

Fig. 5 The count of *Vibrio cholera* after treated with LAB cell supernatant at different contact time.

Referring to Figure 4, LAB supernatant were highly effective on reducing *V. parahaemolyticus* as the reduction of this organism was about 4-$\log_{10}$ after subjected to 90 minutes of contact time. The result had achieved greater reduction compared to previous study by Nurul Ain Shohor (2013) who reported 1-$\log_{10}$ reduction. Basically, the reduction of *Vibrio* sp. in raw big eye tuna was also increased, as the contact time between LAB supernatant with the sample increased. As for flesh, there was a complete reduction of *V. parahaemolyticus* using LAB supernatant. However, for *V. cholera*, 3-$\log_{10}$ of reduction was observed for both intestines, gills and flesh of big eye tuna samples (Figure 5).

2.2.5 Treatment of contaminated *Thunnus obesus* with ozone treated water

The ozone water treatment can directly inactivate *V. parahaemolyticus* in the raw big eye tuna fish at the concentration of 3 ppm as shown in Table 2. However, the reduction was only effective at 1 ppm for reducing contamination by *V. cholera.* For the treatment of ozone water at 3 ppm, the reduction was 2- $\log_{10}$ of reduction. The present finding results showed that the 3 ppm of ozone concentration have the ability as surface decontaminant because *V. parahaemolyticus* on flesh were completely eliminated after subjected to ozone water treatment.

Based on previous studies by Shikongo 2012, the effectiveness of ozone water can be observed at 2 ppm. Warriner et al. (1985) had reported that ozone dosage appeared to be the critical level at 3 ppm to 4 ppm. The study also suggested that 3 ppm of ozone concentration would be the highest limit due to the interaction of organic matter with higher level of ozone water treatment.

There were significant difference ($P<0.05$) between 1 ppm and 3 ppm of concentration of ozone treated water. It was shown that the 1 ppm and 3 ppm had a comparison with increased concentration and effectiveness as sanitization as supported by Chawla (2006). The highest significant reduction *of V. parahaemolyticus* was observed in intestines and flesh for 3 ppm concentration of ozone treated water. For the different part of the fish, there is no significant difference ($P>0.05$) of reduction by ozone treated water especially between intestines and flesh.

Table 2: Microbial count of *Vibrio parahaemolyticus* and *Vibrio cholera* count after subjected to different ozone concentration

Name of microorganism artificially contaminated on sample	Ozone concentration	Microbial count in different parts of fish (CFU/g)		
		gills	Intestines	flesh
V. parahaemolyticus	0	5.39 ± 0.25	6.26 ± 0.10	4.00 ± 0.21
	1	6.00 ± 0.20	6.31 ± 0.15	5.30 ± 0.18
	3	2.00 ± 0.30	0 ± 0	0 ± 0
V. cholerae	0	5.39 ± 0.12	6.26 ± 0.21	4 ± 0.14
	1	3.60 ± 0.23	3.62 ± 0.17	3.15 ± 0.16
	3	3.31 ± 0.25	4.54 ± 0.22	4.02 ± 0.19

2.3 Conclusion

In conclusion, the level of *V. parahaemolyticus* and *V. cholera* contamination in raw fresh *Thunnus obesus* was low and only present in the gills. Artificial contamination of Vibrio in raw big eye tuna, 12 hours was the most suitable contact time for the maximum growth of *Vibrio* sp. The samples were artificially contaminated at 12-h to reach maximum growth of *Vibrio* prior to treatment with ozone treated water and LAB. Results showed that ozone water treatment had a significant reduction of *V. parahaemolyticus* and *V. cholera*. A 5-$\log_{10}$ reduction was achieved to reduce *V. parahaemolyticus* with 3 ppm of ozone concentration, however, 3 ppm was not sufficient to eliminate completely of *V. cholera* in raw big eye tuna. Nevertheless, the use of cell supernatant of *Lb. plantarum* was effective to reduce *V. parahaemolyticus* completely, however, only 2-$\log_{10}$ reduction was achieved to reduce *V. cholera* using same concentration of LAB cell supernatant. It can be concluded that the effectiveness of LAB and ozone treated water to reduce *Vibrio* spp. were influenced by different part of sample and different concentration of ozone.

3. Evaluation of UV irradiation and LAB as surface decontaminant of artificially contaminated *Clarias gariepinus*

3.1 Methodology

3.1.1 Isolation and Identification of Bacteria from skin surfaces of *Clarias gariepinus*

Sample from skin surface of *Clarias gariepinus* contained the bacteria of *Aeromonas hydrophila*, *Aeromonas caviae*, *Edwardsiella tarda* and *Plesiomonas shigelloides*. All the isolates showed high probability of similarity index (%) which indicated the cultures were correctly identified. Out of 45 isolates obtained from the experiment, only 5 isolates were identified by Vitek 2 Compact System (bioMérieux, France).

3.1.2 Artificial inoculation of *A. hydrophila*

The isolated of *A. hydrophila* from catfish was chosen from 45 isolates of bacteria. The inoculum contained approximately 1.0×10^9 CFU/mL were diluted to 1.0×10^6 CFU/ml with 10-fold serial dilution. Ten times of 0.1 mL were inoculated at ten different spot of sample in ensuring the sample was homogenously contaminated with *A. hydrophila*.

3.1.3 UV Treatment as Disinfectant in *Clarias gariepinus* fillet

Four pieces of *Clarias gariepinus* fillets were contaminated artificially with *A. hydrophila*. Then, all samples were exposed to UV irradiation with 3 Watts at different time exposure (0, 5, 15 and 25 min). The UV system was developed by Universiti Teknologi Malaysia. Following the treatment, all fillets were aseptically placed into individual sealable bags and stored for 24 h at 4°C to simulate the overnight storage. The bacterial count of all samples were checked by 10-fold serial dilution.

3.1.4 Preparation of LAB Cell Supernatant

The strains of LAB (*Lb. plantarum and Lb. lactis*) were grown in 50 mL of de Man Rogosa and Sharpe (MRS) broth anaerobically for 24 h at 30°C. Then, the 50 ml broth was centrifuged in microcentrifuge (8000 rpm for 10 min). The supernatant was discarded and the 0.85% saline solution was placed into the tube and homogenized with pallet before centrifuged at the same speed and time. The washing step was repeated before it was homogenized together with the pellet by using vortex.

3.1.5 Spraying of LAB Cell Supernatant against Pathogens

Following the artificial contamination, four pieces of African catfish fillets remained at room temperature (~25°C) for 1 hour to allow the inoculum to dry. After 1 hour, 3 of the fillets were sprayed thoroughly with LAB cell supernatant. The corresponding one fillet remained untreated on the laboratory bench as the control. After spraying LAB cell supernatant on the fillets with 1x 10^6 CFU/ml, the fillets were placed into individual sealable bags within 0, 1, 2 and 3 hour contact time and stored for 24 h at 4°C to simulate the overnight storage. After 24 h, the microbiological quality of samples were determined using spread plate method on Tryptic Soy Agar.

3.2 Results and Discussion

3.2.1 Isolation and Identification of Bacteria from skin surfaces of *Clarias gariepinus*

Out of 45 isolates obtained from the experiment, only 5 isolates were identified by Vitek 2 compact system. From the results of identification, it reveals that many pathogenic bacteria were obtained from the skin; *Aeromonas hydrophila, Aeromonas caviae, Edwardsiella tarda* and *Plesiomonas shigelloides.* Many researchers have isolated different species of bacteria from the skin of the fresh water fish (catfish) including *Bacillus* species from the skin of seawater fish (Sugita et al., 1997; Shewan, 2000; Shewan and Hobbs, 1990).

3.2.2 UV Treatment as Decontaminant in artificially contaminated *Clarias gariepinus*

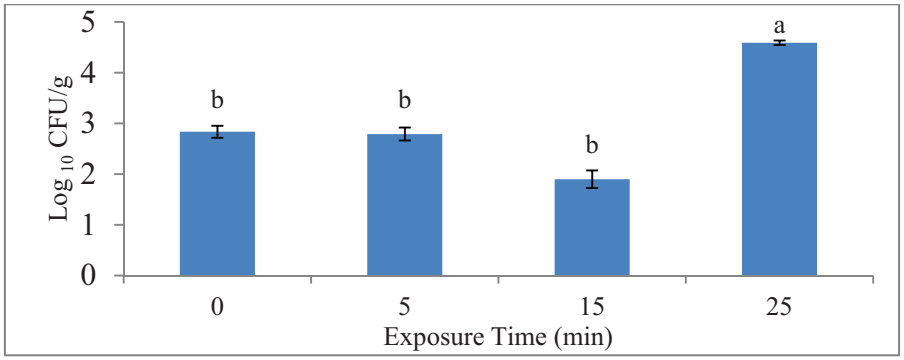

Fig. 6 The bacterial count of contaminated *Clarias gariepinus* after treated with UV light at different exposure time.

The result in the Figure 6 shows the reduction of bacterial count of contaminated *Clarias gariepinus* after the treatment with UV light for 5, 15, and 25 minutes. The reduction of bacterial count was significantly different (p<0.05) between the 0, 5 and 15 minutes compared to 25 minutes of time exposure. Results indicated that the most reduced bacteria count on TSA plate was the exposure of UV light for 15 minutes. However, the bacterial count was increased after 25 minutes time expose under UV light. The increase of bacterial count after 25 minutes showing there was photorepair phenomenon occurred during experiment (Dunn et al., 1989). The photorepair phenomenon may occur because the UV system used in this study consist of full spectrum of UV light, which cover 350-450 nm light that trigger this photorepair to occur (Lani, 2007). One possibility of the less reduction occur in this experiment probably due to insuffient exposure of the catfish fillet surfaces towards UV light. Bank et al. (1991) also proposed that the germicidal effect of a UV light was diminished when bacteria cultures were shielded from direct exposure to UV light.

3.2.3 LAB Cell Supernatant as decontaminant in artificially contaminated *Clarias gariepinus*

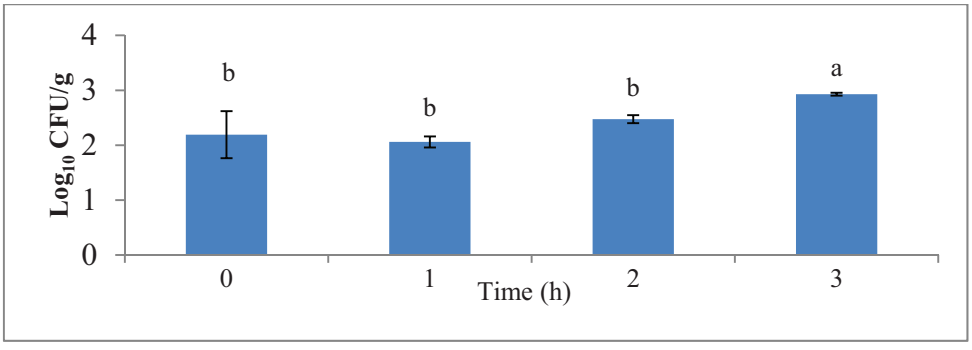

Fig. 7 Bacterial count of contaminated *Clarias gariepinus* after treated with LAB supernatant at different contact time.

Figure 7 shows the bacterial count of contaminated African catfish after treated with *Lb. plantarum* supernatant. The bacterial count from the African catfish that had 3-hour contact time showed significant reduction of bacterial count ($p<0.05$) compared to 0, 1 and 2 hour contact time. The result shows the bacterial count reduced after 1 hour contact time with *Lb. plantarum* supernatant treatment. However, the bacterial count increases at 2 hour and 3 hour contact time. The increases of the bacterial counts might be due to the metabolites of antimicrobial inhibitory substances in LAB became inactive.

The antimicrobial activity of lactic acid bacteria may be due to a number of factors. Generally, the effect of *lactobacilli* in controlling the proliferation of pathogenic bacteria is by producing a wide range of antibacterial compounds such as organic acids (e.g., lactic acid and acetic acid) and hydrogen peroxide (Makras et al., 2006). Principally, the production of lactic acid was enough to inhibit certain bacterial strains. The unionized form of lactic acid may trigger to lowering the internal pH of the cells that causes a collapse in the electrochemical proton gradient in sensitive bacteria, hence having a bacteriostatic or bactericidal effect (Klare et al., 2007). Further investigation is required to investigate the mechanism of inhibitory by LAB in raw African catfish.

3.3 Conclusion

In conclusion, the microorganisms that has been isolated and identified from African catfish using advanced identification method of VITEK2 compact system were *Aeromonas caviae, Aeromonas hydrophila, Brucella* spp, and *Edwardsiella tarda, Plesiomonas shigelloides* with highest probability of confidence. The *A. hydrophila* counts from the artificial contaminated with African catfish was reduced significantly ($p<0.05$) after exposed to UV light treatment for 15 minutes. However, the *A. hydrophila* count was increased after 25 minutes expose to UV light due to the photorepair phenomenon. The use of LAB cell supernatant on artificially contaminated with *A. hydrophila* on African catfish was only effective for 1-h contact time. Prolong contact time of LAB cell supernatant with the sample did not reduce the level of contamination of *A. hydrophila* in African catfish. It can be concluded that decontamination of raw fish with LAB and UV light system were influenced by many external factors, and further studies are required to investigate in depth of these technologies before it can be implemented in food industry.

Acknowledgements Authors thank to Ministry of Education, Malaysia for providing financial support to carry out this project through Fundamental Research Grant Scheme (FRGS), Vot. No. 59269. Special thank to Nor Atikah Mohamed Zin (UMT) for her technical advice and Institute of Marine Biotechnology, UMT for providing VITEK 2 Compact System facility.

References

[1] Bank, H.L., John, J.E., Atkins, L.M., Schmehl, M.K., and Dratch, R.J. 1991. Bactericidal action of modulated ultraviolet light on six groups of *Salmonella*. Infect. Control Hosp. Epidemiol. 12 : 486–489.

[2] Bolder, N.M. 1997. Decontamination of meat and poultry carcasses. Trends Food Sci. Technol. 8:221–227.

[3] Blagojev, N.Š. 2011. Control of mould growth and mycotoxin production by lactic acid bacteria. *Romanian Biotechnological Letters*

[4] Chawla, A. 2006. Application of Ozonated Water Technology For Improving Quality And Safety Of Peeled Shrimp Meat. A Thesis of Master, Lousiana State University.

[5] Cleveland, J.M., Montville, T.J., Nes, I.F. & Chikindas, M.L. 2001. Bacteriocins: safe, natural antimicrobials for food preservation. International Journal of Food Microbiology, 71: 1-20.

[6] Corry, J.E.L., James, C., James, S. J., and Hinton M. 1995. *Salmonella, Campylobacter* and *Escherichia coli* O157:H7 decontamination techniques for the future. Int. J. Food Microbiol. 28 : 187–196.

[7] Dehkordi, B.M., & Zokaie, N. 2010. Extension of fish shelf life by ozone treatment. International Journal of Chemical and Biological Engineering, 3:2.

[8] Dinçer, A.H., and Baysal, T. 2004. Decontamination techniques of pathogen bacteria in meat and poultry. Crit. Rev. Microbiol. 30 : 197–204.

[9] Douglas, D. (2007). Identifying fres water Aquarium fish disease. Available on line at http://fishsuite101. com/article.cfm/identifyingfishdiseases.

[10] Dunn, J.E., Clark, R.W., Asmus, J.F., Pearlman, J.S., Boyerr, K., Painchaud, F., et al. 1989. Methods for preservation of foodstuffs. US Patent 4 : 871-559.

[11] Guerrero-Beltrán, J.A., and Barbosa-Cánovas, G.V. 2004. Review: Advantages and limitations on processing foods by UV light. Food Sci. Technol. Int. 10 : 137–147.

[12] Kevan L. and Rolland L. 1999. Chapter 9 – Shrimp Health Management. Harbor Branch of Oceanographic Institution.

[13] Klare, I., Konstabel, C., Werner, G., Huys, G., Vankerckhoven, V., Kahlmeter, G., Hildebrandt, B., Müller-Bertling, S., Witte, W., and Goossens, H. 2007. Antimicrobiol susceptibilities of Lactobacillus, Pediococcus and Lactococcus human isolates and culture intended for probiotic or nutritional use, J. Antimicrobiol. Chemother. 59: 900-912.

[14] Lack, M. 2007. With An Eye To The Future: Addressing Failures In The Global Management Of Big Eye Tuna. TRAFFIC International and WWF Australia.

[15] Lani, M.N. 2007. Inactivation of *Listeria monocytogenes* by pulsed UV illumination and photorepair recovery of UV-damage cells. PhD Thesis. University of Strathclyde, Glasgow, Scotland.

[16] Liasi, S. A., Azmi, T. I., Hassan, M. D., Shuhaimi, M., Rosfarizan, M. & Ariff, A. B. 2009. Antimicrobial activity and antibiotic sensitivity of three isolates of lactic acid bacteria from fermented fish product, Budu. Malaysian Journal of Microbiology, Vol 5(1): 33-37

[17] Makras, L., Triantafyllou, V., Messaoudi, D., Adriany, T., Zoumpopoulou, G., Tsakalidou, E., Servin, A., and Vuyst, L. 2006. Kinetic analysis of the antibacterial activity of probiotic lactobacilli towards *Salmonella* enterica serovart typhimurium reveals a role for lactic acid and other inhibitory compounds, Res. Microbiol. 157 : 241-247.

[18] Mossel, D.A.A. Corry, J.E.L. Struijk, C.B. & Baird, R.M. (1995). Essentials of the microbiology of foods: a textbook for advanced studies. Chichester (England): John Wiley and Sons. 699 pp.

[19] Nurul Ain Shohor, 2013. Application of Lactic Acid Bacteria as a surface bio-disinfectant for natural presence of *Vibrio* spp. and *Vibrio parahaemolyticus* contamination in shrimps, B. Sc. Thesis, Universiti Malaysia Terengganu.

[20] Pipek P., Houska M., Hoke K., Jarmila J,, Kyhos K. & Sikulova. 2005. Decontamination of pork carcasses by steam and lactic acid. Journal of Food Engineering.

[21] Sugita, H.N., Matsuo, Y., Hirose, M., Iwato, Y. Deguchi. 1997. Vibrio species. Strain NM 10 with an inhibitory effect against pasteurella piscicida form the intensive of Japanese coastal fish. Applied Environmental Microbiology, 63, 4986 – 4989.

[22] Shewan, J.M., and Hobbs, G. 1990. The Bacteriology of fish spoilage and preservation. In progress in Industrial Microbiology. (ed., by D. J. D. Hockenhull) L Liffe books Ltd, London.

[23] Shewan, J.M. 2000. The Microbiology of sea water fish vol.1. Academic press, Newyork. pp. 487 – 560.

[24] Sumner, S.S., Wallner-Pendleton, E.A., Froning, G.W., and Stetson, L.E. 1996. Inhibition of*Salmonella* Typhimurium on agar medium and poultry skin by ultraviolet energy. J. Food Prot. 59 : 319–321.

[25] Stermer, R.A., Lasater-Smith, M., and Brasington. C.F. 1987. Ultraviolet radiation – An effective bactericide for fresh meat. J. Food Prot. 50 : 108–111.

[26] Stiles, M.E. (1996) Biopreservation by lactic acid bacteria. Antonie Van Leeuwenhoek, (2-4):331-45.

[27] Wallner-Pendleton, E.A., Sumner, S.S., Froning, G.W., and Stetson, L.E. 1994. The use of ultraviolet radiation to reduce *Salmonella* and psychotrophic bacterial contamination on poultry carcasses. Poultry Science. 73 : 1327–1333.

[28] Warriner R., Kostanbader Jr. K.D., Cliver D.O., & Ku W.C. 1985. Disinfection of Advanced Wastewater Treatment Effluent By Chlorine, Chlorine Dioxide and Ozone. Journal of Water Research. 1515-1526.l

