Hazard evaluation of *Listeria monocytogenes* in the process of cold-smoked salmon

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Abstract

Listeria monocytogenes can grow well even in the vacuum packaged frozen fishery products stored at 4℃. Due to the increasing consumption of frozen fishery products, it is important to establish microbiological standards for those products to prevent food-borne illness associated with *L. monocytogenes*. Thus, this study was performed to identify risk factors for *L. monocytogenes* contamination during processing of cold-smoked salmon. Based on the study, appropriate guidelines for frozen meat and fishery processing plants will be established. For the microbiological analyses of coliform and *L. monocytogenes* strains, sampling of *L. monocytogenes* was carried out in cold-smoked salmon processing plants. A total of 280 samples were collected from the raw materials, processing units, working environment, working utensils and workers in the processing plants for 6 months. The swab and food samples were examined according to the Food Code (2008). The isolated *L. monocytogenes* was confirmed by PCR and serotyping method. As a result, aerobic bacteria and coliform counts were exceeded the safety level in most of the samples taken from the cold-smoked salmon processing plants except for water and workers. *L. monocytogenes* was isolated from 12 of these samples and then identified. In the serotype test, 9 of the 12 isolated samples were serotype 1/2a (75%) while only 3 were serotype 1/2b (25%). These results suggest that cold-smoked salmon can be exposed to *L. monocytogenes* during the processing of raw materials. It is important to identify the factors in order to control the growth of *L. monocytogenes* in frozen smoked salmon products. Therefore microbiological standards are definitely needed to control prevalence of *L. monocytogenes.*

1. Introduction

The consumption of frozen food and fishery products has been increased worldwide due to the cold chain system[1]. Accordingly, hygiene standards for frozen fish and meat products are required. Some of frozen food products can be eaten without heating or cooking process[2]. In that case, there are possibilities of food spoilage and decay by pathogenic bacteria due to raw material contamination or cross-contamination during the manufacturing distribution. It may cause food hygiene matters.

In general, while processing frozen fish foods, hard-frozen raw material has to thaw for the secondary processing[3]. Then thawed fish go through a simple process such as removing the guts depending on fish species, cutting (fish head), and peeling and slicing[4]. After the simple process, they have to be frozen again. Thus, the microbial contamination in the frozen food products can be occurred during this process. In addition, the fish processing plant sanitation is poor. Most of the processings have to be done by hand due to the lack of automated processing facilities, which raises the possibility of exposure to the pathogenic microorganisms. In particular, psychrotrophic pathogens can survive and grow well at refrigerator temperature and psychrotrophic spoilage microoraganisms can cause various kinds of diseases through the food stored in refrigerators[5]. It is the main factor of food decay[6]. Moreover, *Listeria monocytogenes* is one of the typical low temperature pathogens. It has been recognized as new food poisoning bacteria due to the increasing consumption of frozen fishery products[7]. It is necessary to establish microbiological standards for those products to prevent food-borne illness caused by *L. monocytogenes* and clarify the causes of the disease. The main route of acquisition of *L. monocytogenes* is contaminated food. To block the *L. monocytogenes* infection, investigations on level of food contamination is essential. Up to now, it has been reported that the foods causing Listeriosis are dairy products, meat, processed meat products, seafood and vegetable salad and so on. According to the report, investigations on level of contamination associated with those products have been conducted.

Recently, research trends seem to focus on identification and serotyping of *L. monocytogenes.* For instance, the study conducted by Thimothe et al. (2004) showed that among 553 environmental samples, *L.monocytogenes* was found in 23.7% of the samples collected from the drain and 4.8% of the samples taken from food contact surfaces[8]. Furthermore, it was reported that the main strains causing epidemic outbreak in USA, Europe and so on were serotype 4b, whereas serotype commonly isolated from foods was 1/2a.

Therefore, this study was carried out to evaluate the safety and sanitation of the cold-smoked salmon products by microbiological method. To analyze the risk factors of the production process, *L. monocytogenes* was isolated from the samples and then identified. Data of the study can be used to establish microbiological standards for frozen food products having potential to cause food borne

illness associated with *L.* monocytogenes and find alternative way to block the *L. monocytogenes* infection

2. Material and methods

This study was carried out for the microbiolological analysis of the samples obtained from water, working utensils, working environments and processings used in the cold-smoked salmon processing plants. The sampling was performed over 5 times in the cold-smoked processing plants located in Incheon Metropolitan city from January to June in 2009.

2.1. Sampling and Pretreatment

The cold-smoked salmon samples were collected from the 14 step production process (Fig.1). Each 500g of the samples were put into the sterilized packs (whirl pack, Nasco Co., U.S.A.) and kept in the ice boxes. All the samples were immediately transferred to the laboratory and then stored at 4℃ until the test end. The test was performed within 24hrs. after sampling. For test solution, 25g of the each sample was added into 225ml of 0.85% sterilized NaCl solution and then homogenized for 2mins using a stomacher (Bagmixer 400, Interscience, France). The 1L of water sample was obtained from the cold-smoked salmon processing plants. For the test, 250ml of the water sample was filtrated by sterilized microfiltration (0.45ul, Advantech MFS Inc., Japan). The samples of the workers' hands were collected from the working utensils and the working environments after washing or sterilizing. According to FSIS sample collection guidelines[9], the samples of the workers' hands were collected from 100cm^2 surfaces area with the e-swab™ (3M Co., U.S.A.) The microbiological analysis of the total plate counts, coliform and *Listeria monocytogenes* known as hygiene indicator bacteria were carried out according to the method in Food code 2008[10].

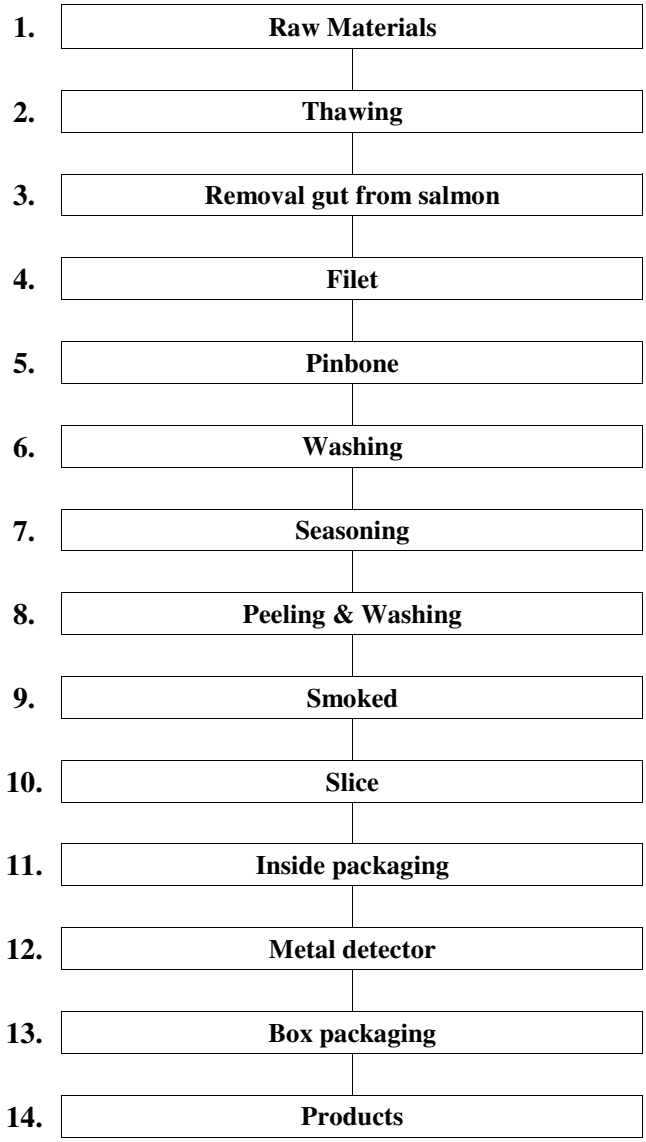

Fig. 1. Flow diagram for the production of cold smoked salmon

2.2. Microbiological analysis of Total plate count and Coliform count

For the total plate count, the samples were cultivated in plate count agar (Difico Co., U.S.A) for 48hrs. at 35℃. Then the colony counts were calculated. For the coliform count, the samples were cultivated in desoxycholate lactose agar (Oxoid Co., England) for 48hr. at 35℃ according to the quantitative determination method. Then the colony count in dark red color and suspicious colony were calculated.

2.3. Isolation of *Listeria monocytogenes*

The food samples should be cultivated as follows. 25g of the test samples was added into 225ml of *Listeria* selective enrichment broth (BD Diagnosics, U.S.A). Then it was cultured with enrichment for 24~48 hrs at 30℃. The culture enrichment solution was inoculated into palcom agar (Oxoid Co., England) using a sterilized cotton swab band and then it was cultivated for 24~48 hrs. at 30℃. When the suspicious colony was confirmed, the colony was inoculated into the tryptic soy agar contained 0.6% yeast extract and confirmed by the API Listeria (BioMerieux, France) or PCR analysis. For the environ- mental samples test, 1ml of the sample was added into 9ml of Listeria selective enrichment broth to make 10ml of test solution. Then it was cultured with enrichment for 24~48 hrs at 30℃. The culture enrichment solution was inoculated into palcom agar (Oxoid Co. England) using a sterilized cotton swab band and then it was cultivated for 24~48 hrs. at 30℃. In the investigation on the pathogenicity of *L. monocytogens* isolates, the strain obtained from API test was confirmed by β-Hemolysis test.

The presumed *Listeria* strains could be confirmed whether hemolysin formation was created in 5% of blood agar according to β-Hemolysis test method.

2.4. PCR analysis and Serotyping

The strain was confirmed by PCR analysis using the *Listeria monocytogenes* detection kit (Kogene Biotech.Co., Korea). The conditions of PCR were as follows: 35 cycles of amplification, denaturation for 30 sec. at 94℃, annealing for 30sec. at 60℃, extension for 30sec. at 72℃ and final extension for 5min. at 72℃. The obtained PCR products were separated by electrophoresis in 2% agarose gel (TAE buffer). The isolated strains were *Listeria* OⅠ/Ⅱ, OⅠ,OⅣ, OⅤ/Ⅵ, OⅥ, OⅦ, OⅧ, OⅨ and HA, HAB, HC, HD antiserum (Denka Seiken, Japan). They were confirmed by Serum tube agglutination test and then serotypes of the positive strains were classified.[11].

3. Results

3.1. Microbiological hazard analysis of water

The microbiological test result on the water used in the cold-smoked salmon processing plants is shown in Table 2. Total plate counts and coliform counts were not detected in those four samples(waterworks, processing tables washing water, thawing water, after disinfection water). In addition, *L. monocytogene* caused by the cross contamination from raw materials was not detected over the entire period. Thus, the water directly contacting with raw materials and water tanks used in the processing plants seem to be well- managed.

Table 2 . Microbiological hazard analysis of water

(CFU/ml)

Samples		1st	2nd	3rd	4th	5th
Waterworks	Total Plate count	ND	ND	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Processing tables washing water	Total Plate count	ND	ND	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Thawing water	Total Plate count	ND	ND	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
After disinfection water	Total Plate count	ND	ND	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	-

ND ; Not detected, – ; Negative

3.2. Microbiological hazard analysis of the working utensils

The result of the microbiological test on the samples obtained from working utensils in the cold-smoked salmon processing plants is shown as Table 3. For evaluating the microbial levels for the working utensils, Harrigan and McCance (1976)[12] suggested that if the total plate count is less than 5 units per cm^2, it can be satisfactory. However, if it is 5 to 25 units per cm^2, it should be corrected. If it is greater than 25 units, the immediate action should be taken against this matter. They also insisted that coliform should be less 10 units per $100cm^2$ and should not be isolated from any of samples in

order to receive satisfactory. According to the standards suggested by Harrigan and MaCance, in evaluation of total plate counts, some samples showed the satisfied level but most of them were required to be corrected or take an immediate action. The total plate counts and coliform counts of the samples collected from the working utensils such as tools used for removing guts (4^{th}, 5^{th}) and chopping boards (3^{rd}) seemed to extremely high. Those tools are mainly used for handling of raw materials. Thus, considering the result, the hygienic status of the cold-smoked salmon processing plants were very poor. So an immediate action should be taken to improve hygienic matter. Furthermore, the total plate counts of thawing boxes used for primary storage of raw materials and the thawing knives for handling the raw materials were not satisfactory. They were required to be corrected. The result of the coliform test also showed unsatisfactory level. This suggested that there was a great possibility of cross contamination of the working utensils associated with raw materials. Moreover, the seasoning boxes and materials used for seasoning smoked salmon were stored at room temperature without a proper management. The sanitation of those boxes and materials were unsatisfactory. However, the seasoning shovels were sanitized regularly and well-managed. Due to this, it seemed to be kept in hygienically safe condition. The results of biochemical identification of *L. monocytogenes* isolated from the working utensils showed that 5^{th} samples collected from the preprocessing knives and the dewatering brushes were found to be positive for *L. monocytogenes.* It confirmed microbiological risk of *L. monocytogenes.*

Table 3. Microbiological hazard analysis of working utensils

(CFU/100cm^2)

Samples		1st	2nd	3rd	4th	5th
Raw material storage box	Total Plate count	ND	ND	ND	8.0×10^3	7.2×10^3
	Coliforms	ND	ND	8.0×10^2	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Thawing box	Total Plate count	ND	3.0×10^2	ND	6.0×10^2	4.1×10^2
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Thawing knife	Total Plate count	ND	7.0×10^2	3.0×10^2	3.0×10^2	5.2×10^2
	Coliforms	ND	ND	2.0×10^2	ND	4.5×10^2
	Listeria monocytogenes	–	–	–	–	–
Gut removal container	Total Plate count	ND	ND	9.5×10^4	ND	4.0×10^2
	Coliforms	ND	1.4×10^2	ND	ND	2.0×10^2
	Listeria monocytogenes	–	–	–	–	–
Gut removal tool	Total Plate count	ND	3.0×10^2	ND	2.5×10^4	9.0×10^4
	Coliforms	ND	ND	ND	3.0×10	5.0×10^2
	Listeria monocytogenes	–	–	–	–	–

ND ; Not detected, – ; Negative, + ; Positive

Gut removal knife	Total Plate count	ND	7.0×10^2	ND	1.0×10^2	6.0×10^2
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Gut removal chopping board	Total Plate count	ND	2.0×10^2	1.0×10^3	6.3×10^2	4.5×10^2
	Coliforms	ND	ND	3.5×10^2	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Pre-process operation knife	Total Plate count	ND	ND	ND	2.0×10^4	9.0×10^4
	Coliforms	ND	1.6×10^2	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	+
Whetstone	Total Plate count	ND	1.6×10^2	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Dewatering Brush	Total Plate count	ND	ND	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	+
Seasoning box	Total Plate count	ND	8.5×10^4	5.5×10^5	2.9×10^2	3.0×10^2
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Seasoning fabric	Total Plate count	ND	6.0×10^3	1.0×10^2	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Seasoning shovel	Total Plate count	ND	ND	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Peeler	Total Plate count	ND	ND	ND	ND	3.5×10^2
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Slicer knife	Total Plate count	ND	5.0×10^2	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Slicer brush	Total Plate count	ND	ND	ND	ND	7.0×10^2
	Coliforms	ND	ND	ND	ND	1.0×10^2
	Listeria monocytogenes	–	–	–	–	–
Slice room disinfection tank	Total Plate count	ND	7.0×10	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–

ND ; Not detected, – ; Negative, + ; Positive

3.3. Microbiological evaluation of the working environments

The microbiological test result on the working environments in the cold-smoked salmon processing plants is shown as Table 4. According to the standards suggested by Harrigan and MaCance (1976)[12], the total plate counts were found to be high in the second disinfecting chambers

(4^{th}, 5^{th}), gut removal tables (4^{th}, 5^{th}), preprocessing tables, smokers, peelers and packing benches except for injectors and injection horses used for seasoning salmon. Thus, the working environments were in extremely bad hygiene condition. So an immediate action was required to resolve the problems. Particularly, the total plate counts of half carcass tables and preprocessing tables were found to be high. The table management was desperately needed. In addition, the total plate counts and coliform were detected in the third samples obtained from the peeler. Hygienic conditions of the peelers were unsatisfactory. Even the total plate counts in the packing benches were high. Thus, the results showed the overall hygienic status of the working facilities were found to be in bad condition. So, special hygienic care and microbiological management were required. Regarding the surrounding equipment and facilities, *L. monocytogenes* was found to be positive in the 5^{th} samples taken from the filet machines, gut removal tables and preprocessing tables. It proved that there was microbiological risk of *L. monocytogenes.* Hence, for the risk management, it requires improvement of sanitizers and the development of proper ways to sterilize the working environments using disinfectants after washing. Furthermore, to proper hygienic management, outsiders should not be allowed in the workplace. The ventilators and drainages and personal hygiene should be observed thoroughly.

Table 4. Microbilological evaluation of working environments

(CFU/100cm^2)

Samples		1st	2nd	3rd	4th	5th
Preprocessing table	Total Plate count	9.5×10^2	6.0×10^2	ND	2.5×10^2	4.5×10^2
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	+
Carcass table	Total Plate count	5.0×10^2	3.2×10^2	ND	9.5×10^2	4.8×10^2
	Coliforms	ND	2.0×10	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Gut removal table	Total Plate count	ND	ND	ND	6.3×10^2	3.0×10^2
	Coliforms	ND	ND	ND	7.0×10	ND
	Listeria monocytogenes	–	–	–	–	–
Filet machine	Total Plate count	ND	ND	2.0×10^2	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Secondary disinfecting chamber	Total Plate count	ND	ND	ND	5.2×10^2	8.0×10^2
	Coliforms	ND	ND	ND	6.0×10	5.0×10
	Listeria monocytogenes	–	–	–	–	–
Injector	Total Plate count	ND	9.0×10	ND	ND	1.0×10
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Injection hose	Total Plate count	ND	ND	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–

Smoking net drain	Total Plate count	ND	3.4×10^2	ND	ND	ND
	Coliforms	ND	ND	ND	ND	2.0×10
	Listeria monocytogenes	–	–	–	–	–
Smokehouse	Total Plate count	4.2×10^2	2.5×10^2	ND	ND	ND
	Coliforms	ND	1.0×10	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Vacuumizer	Total Plate count	ND	3.0×10	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Package bench	Total Plate count	3.0×10^2	1.6×10^2	6.0×10^3	ND	ND
	Coliforms	ND	ND	4.2×10^2	ND	ND
	Listeria monocytogenes	–	–	–	–	–

ND ; Not detected, – ; Negative, + ; Positive

3.4. Microbiological hazard analysis of the workers

Table 5 showed the results of the microbiological test for the workers in the cold-smoked salmon processing plants. In the case of the worker in charge of fishbone removal, the total plate counts were found to be high in workers' hands due to the cross contamination of preprocessing task. Even on packers' aprons, the total plate counts were high. It is concerned to be contaminated again during the final stage due to use of infected aprons. Therefore hand hygiene education for workers and worker management are required to control the microbiological hazardous factors. In addition, considering the major factors of food born diseases are associated with the working utensil and workers' hands, the risk management should focus on the working utensils, container s and workers' hands[13].

Table 5 . Microbiological hazard analysis of the workers

(CFU/100cm^2)

Samples		1st	2nd	3rd	4th	5th
Gut removal worker	Total Plate count	ND	5.0×10	8.0×10	6.2×10^2	7.0×10
	Coliforms	ND	2.0×10	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Slicer worker	Total Plate count	ND	ND	1.2×10^2	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–
Packer's apron	Total Plate count	ND	8.5×10^2	5.0×10^2	7.0×10^2	9.0×10^2
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	–	–	–	–	–

ND ; Not detected, – ; Negative

3.5. Microbiological hazard analysis of the processings

The microbiological hazard analysis results of the samples obtained from the cold-smoked salmon plants are shown as Table 6. The overall manufacturing process goes through the following procedures such as stock of raw materials, thawing, removal of guts, filleting , removal of pinbone, washing, seasoning, peeling, smoking, drying and internal and external packing. The total plate counts and the coliform counts were gradually increased while the frozen smoked salmon was thawed. It could be caused by the cross contamination from the thawing water and boxes. The total plate counts and the coliform counts were increased more and more as the process progressed. In addition, the total plate counts and coliform counts in the samples collected during the filleting and fishbone removal were high. So it was required to be corrected. Furthermore, the total plate counts and the coliform counts in samples collected while seasoning were much higher. So an immediate action should be taken to resolve this matter. Even in the final package process, the total plate counts were detected from the internal and external packages. According to the identification of *L. monocytogene* isolated from the samples in each process, *L. monocytogenes* was detected in the samples taken from each process such as thawing (5^{th}), filet (3^{rd}), Pinbone (3^{rd}, 5^{th}), before washing (2^{nd}, 3^{rd}, 5^{th}), seasoning (5^{th}), peeling and washing (5^{th}). *L. monocytogenes* is a cryophilic bacterium that has caused food borne disease associated with the foods stored in refrigerator since the 1980's[14]. Due to this, governments, organizations and processing companies all around world have been concerned about mess outbreaks of food-borne illnesses related to *L. monocytogenes.* In order to prevent the food borne diseases caused by *L. monocytogenes,* appropriate measures should be prepared.

Table 6. Microbiological hazard analysis of the processings

(CFU/g)

Samples		1st	2nd	3rd	4th	5th
Raw materials	Total Plate count	2.0×10	6.0×10	ND	ND	ND
	Coliforms	ND	5.0×10	ND	ND	ND
	Listeria monocytogenes	−	−	−	−	−
Thawing	Total Plate count	5.0×10	4.0×10	3.0×10	2.8×10^3	8.0×10
	Coliforms	4.0×10	3.0×10	ND	5.0×10	ND
	Listeria monocytogenes	−	−	−	−	+
Removal gut from salmon	Total Plate count	3.8×10^3	9.0×10^3	1.5×10^3	6.5×10^3	2.3×10^2
	Coliforms	8.0×10	ND	ND	9.0×10	ND
	Listeria monocytogenes	−	−	−	−	−
Filet	Total Plate count	5.5×10^3	7.0×10^3	5.0×10	7.6×10^3	4.1×10^2
	Coliforms	9.0×10	ND	4.0×10	1.7×10^3	3.0×10
	Listeria monocytogenes	−	−	+	−	−
Pinbone	Total Plate count	6.4×10^3	1.0×10^3	3.0×10	8.2×10^3	3.0×10
	Coliforms	2.3×10^3	2.0×10	3.0×10	6.8×10^3	ND
	Listeria monocytogenes	−	−	+	−	+

Washing	Total Plate count	3.8×10^3	8.0×10	4.0×10^3	9.0×10^3	5.0×10
	Coliforms	5.0×10^3	ND	1.2×10^3	8.0×10^3	ND
	Listeria monocytogenes	−	+	+	−	+
Seasoning	Total Plate count	8.7×10^3	7.8×10^3	9.5×10^3	5.6×10^3	5.0×10^5
	Coliforms	2.6×10^3	3.1×10^2	ND	ND	9.0×10
	Listeria monocytogenes	−	−	−	−	+
Smoked	Total Plate count	7.0×10	ND	2.3×10^3	4.0×10	8.0×10^2
	Coliforms	ND	ND	ND	ND	1.0×10
	Listeria monocytogenes	−	−	−	−	−
Slice	Total Plate count	5.0×10	ND	3.0×10^2	ND	1.3×10^2
	Coliforms	ND	ND	ND	ND	ND
	Listeria monocytogenes	−	−	−	−	−
Inside packaging	Total Plate count	5.5×10^3	3.0×10^2	1.7×10^3	1.6×10^3	9.0×10^2
	Coliforms	ND	6.0×10	ND	ND	1.0×10
	Listeria monocytogenes	−	−	−	−	−
Box packaging	Total Plate count	5.5×10^3	3.0×10^2	1.7×10^3	1.6×10^3	9.0×10^2
	Coliforms	ND	6.0×10	ND	ND	1.0×10
	Listeria monocytogenes	−	−	−	−	−
Products	Total Plate count	6.3×10^3	5.2×10^3	5.4×10^3	3.0×10^3	4.0×10^3
	Coliforms	ND	8.0×10	ND	ND	1.0×10
	Listeria monocytogenes	−	−	−	−	−

ND ; Not detected, − ; Negative, + ; Positive

3.6. PCR analysis and Serotypes of *L. monocytogenes isolates*

L. monocytogenes were detected from the raw materials, the working utensils, the working environments and the processings used in the cold-smoked salmon processing plants by Polymerase chain reaction (PCR) and the results were shown as Fig.2.

L. monocytogenes were isolated from the preprocessing tables (5^{th}) and brushes (5^{th}) included in working utensils as well as processing tables (5^{th}) in the working environment. It was also isolated from the thawing (5^{th}), fileting (3^{rd}), Pinbone (3^{rd}, 5^{th}), before washing (2^{nd}, 3^{rd}, 5^{th}), seasoning(5^{th}) and peeling(5^{th}) in the processing stage. The PCR analysis showed the isolated strains were located in a band at 454 bp, which was equivalent to standard strain of *L. monocytogenes* (ATCC13932). Thus, the isolated strains were proven to be positive for *L. monocytogenes*. The serotyping test was conducted by the slide agglutination method. 9 out of 12 samples were serotype 1/2a (75%) and 3 of them were serotype 1/2b (25%)(Table 7).

Table 7. Distribution of serotype of *L.monocytogenes* isolated from samples

Sample	Number	Isolates	Serotypes		
			1/2a	1/2b	1/2c
Water	20	0	-	-	-
Working utensils	105	2	-	2	-
Working environments	80	1	-	1	-
Workers	15	0	-	-	-
Processings	60	9	9	-	-
Total	280	12	9	3	-

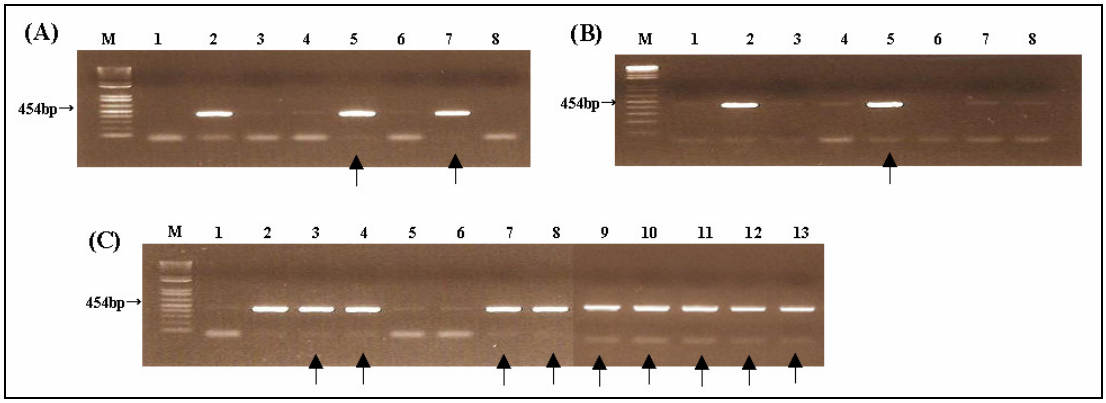

Fig. 2. Agarose gel electrophresis PCR amplified products of isolated *L. monocytogenes* strains. (→: indicate *Listeria monocytogenes* detection)

(A) Working utensils

Lane M ; DNA marker, Lane 1 ; No template control, Lane 2 ; Positive control, Lane 3 ; Thawing knife(5^{th}), Lane4 ; Thawing box(4^{th}), Lane 5 ; Preprocessing knife(5^{th}), Lane 6 ; Gut removial tool(5^{th}),, Lane 7 ; Dewatering Brush(5^{th}), Lane 8 ; Slicer brush (5^{th})

(B) Working environments

Lane M ; DNA marker, Lane 1 ; No template control, Lane 2 ; Positive control, Lane 3 ; Gut removal table(5^{th}), Lane4 ; Filet machine(5^{th}), Lane 5 ; Preprocessing table(5^{th}), Lane 6 ; Smoke house(1^{st}), Lane 7 ; Vacuumizer, Lane 8 ; Package worktable(3^{rd})

(C) Processings

Lane M ; DNA marker, Lane 1 ; No template control, Lane 2 ; Positive control, Lane 3 ; Thawing(5^{th}), Lane 4 ; Filet(3^{rd}), Lane 5 ; Smoking(5^{th}), Lane 6 ; Slicing(5^{th}), Lane 7 ; Pinbone(3^{rd}), Lane 8 ; Pinbone(5^{th}), Lane 9 : Washing(3^{rd}), Lane 10 : Washing(4^{th}), Lane 11 : Washing(5^{th}), Lane 12 : Seasoning(5^{th}), Lane 13 : Peel&Washing(5^{th})

4. Discussion

Listeria monocytogenes is a bacterium that causes the food borne illness listeriosis. It has been isolated from meat, dairy products, and vegetable and so on. Since the consumption of ready-to-eat foods such as dairy products, processed meat or fish products and vegetable salad has been rapidly increasing, guidelines for the management of *L. monocytogenes* is required[15~18]. Farber and Peterkin (1991) reported that *L. monocytogenes* could survive in frozen, surface drying and cooling conditions[19]. In addition, Guyer and Jemmi (1991) emphasized importance of preventing *L. monocytogenes* infection before and after processing[20]. They also insisted that special care should be taken to control *L. monocytogenes* because risk of *L. monocytogenes* infection was increased due to long-term storage. Therefore, this study was carried out to identify risk factors for contamination with *L. monocytogenes* during the processing of cold-smoked salmon. A total of 280 samples were collected from cold-smoked salmon processing plants. Of these samples, *L. monocytogenes* were isolated from 12 samples taken from processing units, working utensils, working environment. Specifically, 9 samples taken from processing knives, brushes (working utensils) and tables (working environment) were found to be positive for *L. monocytogenes*. In the study performed by Thimothe et al. (2004), among 553 environ mental samples, *L.monocytogenes* was found in 23.7% of the samples collected from the drain and 4.8% of the samples taken from food contact surfaces[8]. Those results suggest that hygiene and safe management for working utensils and working environments is needed because contamination of working utensils and working environments can lead to contamination of raw materials.

In this study, a number of bacteria, coliform bacteria and *L. monocytogenes* were isolated from the processing knives and brushes. This result indicates that hot water disinfection or heat sterilization for knives, knife grinding tools and chopping boards are not performed periodically. In addition, those utensils are only washed, not sterilized before and after the processing. That is one of the main reasons for bacteria infection. In the McCarthy (1991) study, pathogenicity of *L.monocytogenes* was reduced after heat sterilization[21]. Due to the reduction of the pathogenicity, causative agents of listeriosis could be reduced. Furthermore, Smith (1992) reported that *L.monocytogenes* was killed after heat sterilization for 10~20 seconds at 80℃[22]. He also suggested high temperature sterilization would be effective for *L.monocytogenes* disinfection. Therefore, special care for knives, knife grinding tools and cutting boards frequently contacting with salmon is needed. Regular heat sterilization is highly recommended before and after the processing.

To prevent *L.monocytogenes* infection, hot water, chlorine and organic acids are commonly used as disinfectants. Those sanitizers are effective to kill *L.monocytogenes.* However, the chlorine-based disinfectants create trihalomethane and chloramine if it is used for a long period. In order to resolve the safety concerns for the chlorine-based disinfectants, it is necessary to develop new

sanitizers[23,24]. Sodium lactate can be used as an alternative sanitizer. It has great potential to be a safe and environment-friendly sanitizer. Sodium lactate is also expected to inhibit the growth of *L. monocytogenes* effectively.

The results of this study revealed that *L.monocytogenes* isolated from the samples was mainly serotype 1/2a (75%). 9 out of the 12 isolated samples were serotype 1/2a (75%) while only 3 were serotype 1/2b (25%). Currently, it has been reported that there are 14 serotypes of *L.monocytogenes.* However, clinically problematic strains are mostly 4b, 1/2a and 1/2b[25~27.] The main strains causing epidemic outbreak in USA, Europe and so on were serotype 4b, whereas serotype commonly isolated from foods was 1/2a. In addition serotype 1/2c was hardly isolated from human clinical strains while it was isolated from foods[28]. Most of human listerioses were caused by serotype 4b, 1/2a and 1/2b. These results suggest that there is a possibility of listeriosis outbreak in Korea.

In conclusion, due to the possibility of listeriosis associated with cold-smoked salmon products (eatable without heating), working utensils and environment should be disinfected completely and sanitary inspections of the processing plants should be done thoroughly. Moreover, the possibility of listeriosis outbreak should be minimized with special care for the processing.

5. References

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