## Original Article

# Anisakis spp. Parasites and Staphylococcus aureus, Bacillus cereus in Sushi and sashimi from Thammasat University (Rangsit Campus) Area Restaurants

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# Abstract Introduction: Consuming sushi and sashimi is very popular in the Thai society as they can be easily bought in general. The prices may will vary depending on the locations. Sushi and sashimi were a diet that does not pass heat. There is a unique method in the process of preparing raw fish and molding rice. In addition, the production process requires the use of hands for cooking, which may cause parasitic contamination and pathogenic microbes in food that are harmful to consumers. Objective: To determine the contamination of helminth Anisakis spp. and bacterial contamination S. aureus, B. cereus in ready-to-eat food samples, sushi and sashimi.

Methods: Total sample 160 sample in total, comprising 125 sushi and 35 sashimi samples, were randomly sampled from the Janpanes restaurants Thammasat University. Using the histological characteristics of helminth *Anisakis* spp. through microscopic was performed using Baird-Parker Egg Yolk Tellulite Agar (BPEY) and Mannital egg Yolk phenol red Polymyxin Agar (MYPA) which are selective media.

**Results:** The sample analyzed from 17 of the approximately restaurants sushi. The restaurants were sampled up to three times over a 3-month period. The results showed no contamination of the *Anisakis* spp. Contamination of *S.aureus* 75/160 (46.88%) specimens found in sushi 69/125 (55.20%) samples and sashimi 12/35 (34.29%) samples of *B.cereus* there were 6/160 (3.75%).

**Conclusion:** This research shows that ready-to-eat foods such as sushi and sashimi are contaminated with microbes that exceed the standards. This indicates that these food can be a super spread source of the disease.

Key words: Anisakis spp., Staphylococcus aureus, Bacillus cereus, Sushi, Sashimi

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### Introduction

Sushi and sashimi is a raw seafood originating in Japan that Thai people know can easily buy everywhere such as local restaurant and shop along on street in Thailand. That cooking process goes through the cook's hand inevitably. Consumption of raw seafood and undercooked seafood is a risk factor, pathogenic bacteria or parasites.

Approximately 20,000 anisakiasis cases has been reported annually in the world, and over 90% of cases were reported from Japan.<sup>1</sup> According to the statistics on food poisoning, there were 294 cases of Anisakis food poisoning with 301 infected individuals mackerel being the most common source of the infection 62 cases (21%). This found on Anisakis contamination of vinegar-marinated mackerels that were used as part of a sushi topping. This found that 7 of 40 sushi topping samples obtained were contaminated with anisakid larvae.<sup>2</sup> The Hong Kong Food and Environmental Department reported that sushi and sashimi had contributed to 3% of the Hong Kong outbreaks involving V.parahaemolyticus, B.cereus and S.aureus.<sup>3</sup> Australia reported 10 outbreaks of foodborne illness associated with the consumption of sushi affecting 87 people.<sup>4</sup> At this moment in Thailand, there is no documented outbreak of foodborne disease due to sushi consumption. The information about microbiota in sushi preparation using raw fish, such as sashimi, finger-sushi, nigirisuhsi and chirashi-sushi is uncertained making the estimation of the potential risk of sushi consumption more complex. A preliminary study was published in which of salmon sushi were analyzed for anisakis and for B.cereus and S.aureus. B.cereus was commonly present in rice and other grains and can form a toxin hazardous to humans, while S.aureus was of interest as a contaminant of the cooked rice through insanitary handling and storage.<sup>5</sup>

It was the purpose of this study to determine the safety of sushi in regards to exposure to both parasitic and bacterial agents through the analysis of the actual product available to the consumer. The aim investigation of the microbiological quality of sushi and sashimi available in the area of Thammasat Hospital market, and Japanese restaurants inside and outside Thammasat University, Rangsit Center.

### Methods

### Sample collection

The stores were selected packaged sushi samples (raw fish sushi, n = 80; ebiko sushi, n = 16; fried tofu sushi, n = 5; california roll, n = 18; wakame sushi, n = 6). Total of 125 sample. Packaged sashimi (salmon fish, n = 14; Tuna fish, n = 10; buri fish, n = 7; saba fish, n = 4) were collected between August and October 2019. Figure 1. Shows the various types of samples used in this study. Sushi samples were made of raw fish (Figure. 1a) ebiko sushi (Figure. 1b), wakame sushi (Figure. 1c), fried tofu (Figure. 1d) and california roll samples included normal. The restaurants could provide the types of sushi noted above, but of those types available, collections generally consisted of 5 to 6 pieces of each. Restaurants were visited up to three times at widely disparate dates in a 3-month period The personnel at the restaurants were unaware of the study in progress. Collections of sashimi were also acquired from specialty grocery stores that provide restaurants with fish and provide consumers with fish fillets cut for use as sushi and sashimi. Samples were transported to the laboratory at ambient temperature, duplicating consumer practices and analysis was initiated within 2 h of collection. Analysis were placed in sterile stomacher bags.



Figure 1 Examples of popular Thailand ready-to-eat foods; Sushi: (A) Raw fish sushi, (B) ebiko sushi, (C) Wakame sushi, (D) fried tofu sushi, (E) california rolls, and (F) Sashimi; Salmon, Tuna, Saba

### Taxonomic identification Anisakia spp.

Analyses for parasites the sample were placed in individual bags, they were carried out under a stereoscopic microscope. Morphological characteristics measured were body width, esophageal length, ventricular length, tail length, body length/ body width, body length/esophageal length, body length/ventricular length, and body length/tail length.<sup>6</sup>

### Quantitative microbiological analysis

Twenty-five grams of aseptically transferred into the stomacher bags min in stomacher bagfilter. Samples were homogenized with 225 ml of peptone water buffer saline using a stomacher at speed 260 rpm./min 2 min. Homogenized samples were serially dilution methods. Total colony-count and total psychrotrophic microorganisms were enumerated in plate count agar media incubated at 35°C for 48 h. *S.gureus* identification

Homogenized samples were serially of dilute 10<sup>-1</sup> level and then continued to dilute until dilution level 10<sup>-3</sup> once the desired dilution is achieved, the pipette sample on Baird-Parker Egg Yolk Tellulite Agar (BPEY) incubater at 35<sup>o</sup>C 48 h. *S.aureus* was

characterized by the formation which has a round of black colonies which are shiny convex, smooth edge and surrounded by clear zones of 2 to 5 mm in diameter resulting from proteolysis. The randomly picked and transferred to tubes containing 0.2 ml of brain heart infusion broth. A sample was identified as *S.aureus* positive only when the results of biochemical tests were positive in more than three reactions. The isolates were subjected to further testing using API STAPH IDENT 32 Staph

### B. cereus identification

*B.cereus* counts were assayed in the examined products according to the colony count technique. The homogenized sample the level of dilute 10<sup>-1</sup> and until dilution level 10<sup>-3</sup> once the desired dilution is achieved, inoculate duplicate on Mannital egg yolk phenol red polymyxin agar (MYPA) incubate plates 18-24 h at 30°C and observe for colonies surrounded by precipitate zone, which indicates lecithinase is production. *B.cereus* colonies are usually a color pink on MYPA. Pick at least 5 presumptive positive colonies from the MYPA plates and transfer one colony to BHI with 0.1% glucose

for enterotoxin studies and a nutrient agar slant for storage. Typical colonies grown MYPA must be confirmed by grams taining, catalase reaction, shape and position of spores and for their ability to lyse red blood cells on the medium supplemented with 5% sheep blood (Oxoid) and biochemical testing as described testing using API 50 kits.

### pH of homogenized samples

The pH of homogenized samples was measured with a pH meter with a penetration tip for semisolid food all measurements were done in triplicate.

### Statistical evaluation of data

Correlation coefficients were calculated with linear regression statistics for pH versus APC; versus *B. cereus* counts; and versus *S. aureus* counts. Significance was determined at P = 0.95.

### Results

### Parasites

Parasites Sampled from the stores that are sold at the flea market Japanese Restaurant and near Thammasat University (Rangsit campus) which each type of sushi and sashimi were purchased and the total number of pieces analyzed are given in Table 1. In testing for contamination of helminthes Anisakis spp. Samples of sushi and sashimi food were collected by separating fresh fish meat samples into a petri dish and examined by physical methods by observing the color of the fresh and odorless and analyzed by microscopy method characteristics of Anisakis spp. parasites. The larvae are classified using various academic documents.<sup>6</sup> Out of a total of 160 samples. Based on the diagnosis, food samples that have not been contaminated with helminthis Anisakids spp. in Table 1.

### Qualitative microbiological analysis

A representative sample of the ready to eat total 160 sample table 1. Evaluation to determine the microbiological quality were as follows: Microbiological quality criteria for ready–to-consume food from the Department of Medical Sciences 2017 satisfactory aerobic amount (APC) <10<sup>5</sup> CFU/g., for S. *aureus* and APC <10<sup>1</sup> CFU/g. for *B. cereus* 

Analysis results of 160 microbial contamination in 125 sushi and 35 sashimi samples. Of these samples were sushi 60/125 (48%) of sashimi 2/35 (5.71%). APC total bacterial contamination exceeded the quality criteria in microbiology. Among these samples can be found in topping raw fish sushi samples 46/125 (36.80%) total of 80 sample the samples exceeded the microbiological quality criteria of most food followed by california roll 10/125 (8.00%) total of 18 sample, wakame sushi 2/125 (1.60%) of total 6 sample and 2/125 (1.60%) ebiko sushi total 16 sample respectively. In the example fried tofu sushi the number of micro-organisms that did not pass the quality criteria was zero of sashimi found 2/35 (5.71%) that most through. Microbiological quality criteria of food in Table 1.

And from the results of the analysis of the total number of microbes found that in the example of ready-to-eat food sushi the average amount of CFU/g. is  $5.80 \times 10^6$  the average sashimi is  $3.8 \times 10^5$ . In the example of raw fish sushi, the highest microbial value is  $9.82 \times 10^6$  CFU/g. followed by ebiko sushi 6.72  $\times 10^6$  CFU/g. california roll 7.49  $\times 10^5$  CFU/g. wakame sushi  $3.25 \times 10^5$  CFU/g. and the fried tofu sushi 1.76  $\times 10^2$  CFU/g. which is considered the only sushi that meets microbiological quality criteria. Example of sashimi food Found in salmon, the highest of microorganisms was  $6.3 \times 10^5$  CFU/g. in Table 2.

 Table 1 Number of dishes according to the microbial quality criteria for the total number of bacteria and contamination Anisakis spp.

Type of produce	criteria Micro				
of sample	No.	Not pass	Pass the	- Anisakis spp	
	the quality standards	quality criteria			
Sushi					
Raw fish sushi	80	46 (36.80)	34 (27.20)	NF*	
Ebiko sushi	16	2 (1.60)	14 (11.20)	NF*	
Fried tofu sushi	5	0 (0.00)	5 (4.00)	NF*	
California roll	18	10 (8.00)	8 (6.40)	NF*	
Wakame sushi	6	2 (1.60)	4 (3.20)	NF*	
Total (sushi)	125 (100.00)	60 (48.00)	65 (52.00)		
Sashimi					
Salmon	14	2 (5.70)	12 (34.29)	NF*	
Tuna	10	0 (0.00)	10 (28.57)	NF*	
Buri	7	0 (0.00)	7 (20.00)	NF*	
Saba	4	0 (0.00)	4 (11.43)	NF*	
[Total (sashimi)	35 (100.00)	2 (5.71)	33 (94.29)	NF*]	
Total (sushi + sashimi)	) 160 (100.00)	62 (38.75)	98 (61.25)	NF*	

NF\* = No contamination was detected Anisakis spp.

Table 2 Prevalence and Counts of Aerobic plate counts, S. sureus, B. cereus

Tupo of products	Maan nu	Mean APC	Mean S.aureus	Mean B.cereus
Type of products	Mean pH	(CFU/g)	(CFU/g)	(CFU/g)
Sushi				
Raw fish sushi	4.5 – 5.5	$9.82 \times 10^{6}$	$7.59 \times 10^{3}$	$2.53 \times 10^{1}$
Ebiko sushi	4.2 - 5.4	$6.72 \times 10^{6}$	$2.51 \times 10^{2}$	0.0
Fried tofu sushi	4.6 - 5.6	$1.76 \times 10^{2}$	0.0	0.0
California roll	4.6 - 5.3	$7.49 \times 10^{5}$	$5.32 \times 10^{3}$	0.0
Wakame sushi	4.8 - 5.4	3.25 × 10⁵	$4.20 \times 10^{2}$	0.0
Total	4.5 – 5.4	$5.80 \times 10^{6}$	$4.41 \times 10^{3}$	$2.53 \times 10^{1}$
Sashimi				
Salmon	5.8 - 6.0	6.3 × 10 <sup>5</sup>	$0.86 \times 10^{1}$	0.0
Tuna	5.6 - 6.0	$1.2 \times 10^{2}$	$8.23 \times 10^{2}$	0.0
Buri	6.0 - 6.3	$2.3 \times 10^{3}$	$1.24 \times 10^{2}$	0.0
Saba	6.2 - 6.5	$5.4 \times 10^{2}$	$5.3 \times 10^{2}$	0.0
Total 35	5.9 - 6.2	$3.8 \times 10^{5}$	$3.90 \times 10^2$	0.0
Total 160 (sushi + sashimi)	5.1 - 5.7	4.75 × 10⁵	$4.16 \times 10^3$	2.53 x 10 <sup>1</sup>

### Prevalence of S. aureus

From the sampling of analysis *S.aureus* was separated from 160 ready-to-eat foods. From the study found *S.aureus* contamination in sushi samples 69/125 (55.20%) found that 41/125 (32.80%) samples were contaminated exceeds the microbiological quality criteria of ready-to-consume. In this amount raw fish sushi found contamination of *S. aureus* 50/125 (40.00%) samples there were 37/125 (29.60%) samples and *S.aureus* contamination found in sashimi 12/35 (34.29%) samples were 3/35 (8.57%) that exceed the microbiological quality criteria. Examples were tuna buri, saba, each sample exceeds the quality criteria of food products in salmon contamination was detected but not exceeding the criteria. When comparing the above criteria It was found that sushi and sashimi samples in this study found the amount of *S.aureus* in the sample with an average of  $4.16 \times 10^3$ . The most found in raw fish sushi samples is  $4.41 \times 10^3$  and in

sashimi samples the amount of *S.aureus* was an average of  $3.90 \times 10^2$  in the salmon samples in only one salmon sample not exceeding the criteria in Table 2 and 3.

### Prevalence of *B. cereus*

From sampling sushi and sashimi total of 160 samples found contamination of *B.cereus* 6/125 (4.80%) samples. Most of the sushi that was examined contained *B.cereus* below the standard but there were 2/125 (1.60%) samples of raw fish sushi that have a tendency to find *B.cereus* in table 2. Exceeding the specified criteria since detected as equal to  $2.53 \times 10^1$  CFU/g in table 2 and 3. For the detection of *B.cereus* in sushi, it may be due to in making sushi there is a high risk of contamination. Since having to use hands in molding and having rice as the main ingredient which bacteria may be in rice or may be contaminated for many reasons.

	No. (%) Sample positive for <i>S.aureu</i>		or S.aureus	No. (%) Sample positive for <i>B.cereus</i>			
Type of products	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
i)pe of produces	Sample	Not pass the	Pass the	Sample	Not pass the	Pass the	
	positive	quality	quality	positive	quality	quality	
		standards	criteria		standards	criteria	
Sushi							
Raw fish sushi	50 (40.00)	37 (29.60)	13 (10.40)	6 (4.80)	2 (1.60)	4 (3.20)	
Ebiko sushi	7 (5.60)	1 (0.80)	6 (4.80)	ND**	ND**	ND**	
Fried tofu sushi	ND*	ND*	ND*	ND**	ND**	ND**	
California roll	8 (6.40)	1 (0.80)	7 (5.60)	ND**	ND**	ND**	
Wakame sushi	4 (3.20)	2 (1.60)	2 (1.60)	ND**	ND**	ND**	
Total 125	69 (55.20)	41 (32.80)	28 (22.40)	6 (4.80)	2 (1.60)	4 (3.20)	
Sasimi							
Salmon	4 (11.43)	0 (0.00)	4(11.43)	ND**	ND**	ND**	
Tuna	3 (8.57)	1 (2.86)	2 (5.71)	ND**	ND**	ND**	
Buri	3 (8.57)	1 (2.86)	2 (5.71)	ND**	ND**	ND**	
Saba	2 (5.71)	1 (2.86)	1 (2.86)	ND**	ND**	ND**	
Total 35	12 (34.29)	3 (8.57)	9 (25.71)	ND**	ND**	ND**	
Total 160							
(sushi + sashimi)	75 (46.88)	44 (27.50)	37 (23.13)	6 (3.75)	2 (1.25)	4 (2.50)	

 Table 3
 Prevalence of the microbiological in sushi and sashimi sample

ND\* = No contamination was detected *S.aureus*.

ND\*\* = No contamination was detected *B.cereus* 

### Discussion

Fresh dining menus like sushi and sashimi are considered to be potential foods as there are no preheat cooking before consumption. Base on revewing literceture for food poisoing from Japanese fresh menus, we focused on the contamination of *Anisakis* spp. and bacterial contamination. In the example of sushi and sashimi which found *S.aureus* and *B.cereus* contamination at 46.88% and 3.75%, respectively. According to survey of the Department of Medical Sciences in the survey on the quality and safety of salmon and sashimi sold in the country, the results showed that no *Anisakis* spp. However, relevant previous research on the detection of *Anisakis*  spp. parasites found 11 species of *Anisakis* spp. in short Mackerel *Rastrelliger brachysoma* (3.67%).<sup>7</sup> Out of all fish detected, *Anisakis* simplex is a parasite found in tropical and tropical fish in Thailand. This parasite is found in all 14 marine fish in the gulf of Thailand. Sold in shopping malls in Bangkok, Samut Sakhon and Chon Buri. Found parasites in up to 9 species of fresh fish, such as silver fish, otters, bigeye fish, blue fish, mackerel, Kula fish, banana fish and crate fish etc. The absence of parasites in sushi and sashimi may be the result of collecting a small sample, but it can be difficult to buy into sushi. The same specialty shop from sashimi bar with seafood. The detection of anisakids is still available in this variety of sushi.

From the above analysis, it is found that it is consistent with the report found *S.aureus* in rice used to make sushi 31 percent, but the amount found is still in the criteria acceptable for the detection of S.aureus contamination in food indicates the lack of control of cleanliness and hygiene.<sup>8</sup> Therefore, those who cook or who are exposed to food does not wash hands or cook utensils thoroughly bacteria that stay on the surface of the container when in contact with food will have extreme risk of cross-contamination or may be directly contaminated from the hand of a cook is possible.<sup>9</sup> Although the rice is cooked and the topping of some sushi is cooked, the manipulation for shaping the sushi and placing sashimi may cause unhygienic procedures. After disinfection the food that has been heated must hurry to cool down quickly or leave at room temperature not over 30 minutes, otherwise *S.aureus* may increase in number which is consistent with the report of Yang et al.<sup>10, 11</sup> Although the pH of sushi is slightly acidic value (pH 4.5 - 5.4) but *S.aureus* can grow well in the range of pH 4.0 - 9.8. Therefore, pH at this level does not affect the inhibits the growth of *S.aureus*.<sup>12</sup>

The contamination of *B.cereus* in foods higher than 10<sup>6</sup> CFU/g. to cause food disease can be toxic. The amount *B.cereus* of found in this sushi is lower than the value that can cause disease.<sup>13</sup> For the seasoning of rice used to make sushi with vinegar may also be one factor. It was found that the pH of < 5.0can inhibit *B.cereus* growth which corresponds to the report of Valero et al., (2003).<sup>14, 15</sup> Found that only a small amount of acid (pH 5.0) helps to inhibit growth of *B.cereus* in vegetable substrates at least 60 days and in accordance with Martinez et al., (2007)<sup>16</sup> that says reducing the pH value in the liquid media can reduce the rate of grow and increase the resting phase of B.cereus. Among sashimi no contamination was found *B.cereus*. It is possible that there were simple components to make. There is no need to mold the rice and reduce the process using hands.

From the above analysis results found that the amount of bacteria that exceeded the standard is quite high. The possible that in the example of ready-to-eat food in the category of, considered to be at high risk the quantity of these microbes indicates excessive contamination of raw materials or during preparation including inadequate cleaning and sanitation conditions. The condition of the readyto-eat food is a major problem because no additional cooking such as improper handling, may affect food dangerous microbes are caused by (i) Most of the ingredients being served cold and eaten raw preheat before serving (ii) Storage temperature. (iii) Preparation involves touch with bare hands.

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พยาธิ Anisakis spp., เชื้อ Staphylococcus aureus, Bacillus cereus ในซูชิ และซาซิมิ จากร้านอาหารในพื้นที่มหาวิทยาลัย ธรรมศาสตร์(ศูนย์รังสิต)

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ບทนำ:	การบริโภคซูชิ และซาซิมิ เป็นที่นิยมอย่างมากในสังคมไทยซึ่งสามารถหาซื้อได้ง่าย โดยทั่วไปราคาจะแตกต่าง กันไปขึ้นอยู่กับตามสถานที่จำหน่ายซูชิ และซาซิมิ เป็นอาหารที่ไม่ผ่านความร้อน มีวิธีเฉพาะในขั้นตอนในการเต รียมปลาดิบ การปั้นข้าว นอกจากนี้ขบวนการผลิตต้องมีการใช้มือในการประกอบอาหารซึ่งอาจก่อให้เกิดการ ปนเปื้อนของพยาธิ และเชื้อจุลินทรีย์ที่ก่อโรคในอาหารซึ่งเป็นอันตรายต่อผู้บริโภคได้
วัตถุประสงค์:	เพื่อตรวจสอบการปนเปื้อนของพยาธิ Anisakis spp. และการปนเปื้อเชื้อแบคทีเรีย S. aureus, B. cereus ใน
	ตัวอย่างอาหารพร้อมบริโภคซูชิ และซาซิมิ
วีธีการศึกษา:	จากการเก็บตัวอย่าง ซูชิ จำนวน 125 ตัวอย่าง และซาซิมิ จำนวน 35 ตัวอย่าง รวมทั้งหมด 160 ตัวอย่าง
	โดยการสุ่มเก็บตัวอย่างจาก ร้านอาหารญี่ปุ่น ร้านค้าตลาดนัดภายในมหาลัยวิทยาลัยธรรมศาสตร์ (วิทยาเขต
	รังสิต) เป็นการศึกษาลักษณะตามสันฐานวิทยาของพยาธิ <i>Anisakis</i> spp. ผ่านกล้องจุลทรรศน์ และการจำแนก
	เชื้อแบคทีเรียโดยใช้ Baird-Parker Egg Yolk Tellulite Agar (BPEY) และ Mannital egg yolk phenol red
	polymyxin agar (MYPA) ซึ่งเป็น selective media ใช้ในการแยกลักษณะเฉพาะของแบคทีเรีย
ผลการศึกษา:	กลุ่มตัวอย่างวิเคราะห์จาก 17 แห่งในร้านอาหารโดยประมาณ ร้านอาหารได้รับการสุ่มตัวอย่างสามครั้งในช่วง
	3 เดือน ผลการทดลองไม่พบการปนเปื้อนของ <i>Anisakis</i> spp. การปนเปื้อนของตัวอย่าง <i>S.aureus</i> 75/160
	(46.88%) ที่พบในตัวอย่างซูชิ 69/125 (55.20%) และ sashimi 12/35 (34.29%) ตัวอย่างของ <i>B.cereus</i> มี
	6/160 (3.75%)
สรุปผลการศึกษา:	การวิจัยครั้งนี้แสดงให้เห็นว่าอาหารพร้อมบริโภคประเภทซูชิและซาซิมิที่ขายนั้นมีการปนเปื้อนเชื้อจุลินทรีย์ที่
	เกินมาตรฐานและสามารถเป็นแหล่งแพร่กระจายของโรคได้ ข้อมูลที่ได้จากงานวิจัยครั้งนี้เพื่อเป็นแนวทางการ
	ป้องกันโรคอาหารเป็นพิษ และใช้เป็นการตัดสินใจในการเลือกซื้อหรือหลีกเลี่ยงซูชิที่ไม่ได้มาตรฐานและเมื่อซื้อ
	มาแล้วควรรับประทานทันทีเพื่อลดเวลาการเพิ่มจำนวนจุลินทรีย์
คำสำคัญ: Anisaki	is spp., Staphylococcus aureus, Bacillus cereus, ซูชิ, ซาซิมิ