Original Article

Quantitative method for detecting *Staphylococcus aureus* using Bio-Theta DOX™ system

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Abstract

We developed a novel quantitative method for detecting *S. aureus* using the DOX™ system. A total of 19 strains of *S. aureus*, and 36 strains of non-*S. aureus* containing 27 species were examined. The mean positive rate of the high- (10^3 CFU/ml), medium- (10^2 CFU/ml), and low-concentration (10 CFU/ml) *S. aureus* samples was all 100%. The relationship between detection time and bacterial count of the 19 *S. aureus* had a good linear calibration curve. For the 36 non-*S. aureus* samples, the mean negative rates for the high-concentration (10^6 CFU/ml) samples and the medium-concentration (10^3 CFU/ml) samples were both 94.4% (34/36). The 2 positive non-*S. aureus* samples were *S. xylosus* ATCC 29971 and *Enterococcus faecalis* ATCC 29212. Furthermore, a recovery examination was carried out by inoculating 20 food samples with *S. aureus* isolated from rice ball and each sample was examined 2 times. *S. aureus* was detected in all samples inoculated with a low concentration of the organism (1.30-1.37 log CFU/ml), and the detection time of the positive samples was 477-807 min (9.7 hrs on average). The DOX system provided rapid results (usually within 10 hrs) and required no special techniques for measurement. Therefore, the DOX system may be a useful tool for determining the absence of *S. aureus* in food and environmental samples at food processing companies. However, more validation studies and field studies are needed.

Keywords: bacteria detecting system, DOX system, *S. aureus* count

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Introduction

According to Bergey's Manual of Systematic Bacteriology (Schleifer and Bell, 2009), *Staphylococcus aureus* contains 2 subspecies, including *aureus* and *anaerobius*. The organisms are nonmotile, nonspore-forming and Gram-stain-positive cocci, and grow well in medium containing 10% NaCl and poorly in 15% NaCl. *S. aureus* subsp. *aureus* (*S. aureus*) has been confirmed to be a major causative agent of food poisoning and nosocomial infection because many strains of the organism produce enterotoxins.

S. aureus has been found on the epidermis of animal, bird and human, and the organism can be isolated sporadically from a wide variety of environmental sources such as soil, freshwater, plant surfaces and products, feeds, meat and poultry, dairy products, and surface of cookware, furniture and clothing (Hennekinne et al., 2012). Food handlers having enterotoxin-producing *S. aureus* in their noses and/or on their hands are regarded as the main source of food contamination (Argudin et al., 2010).

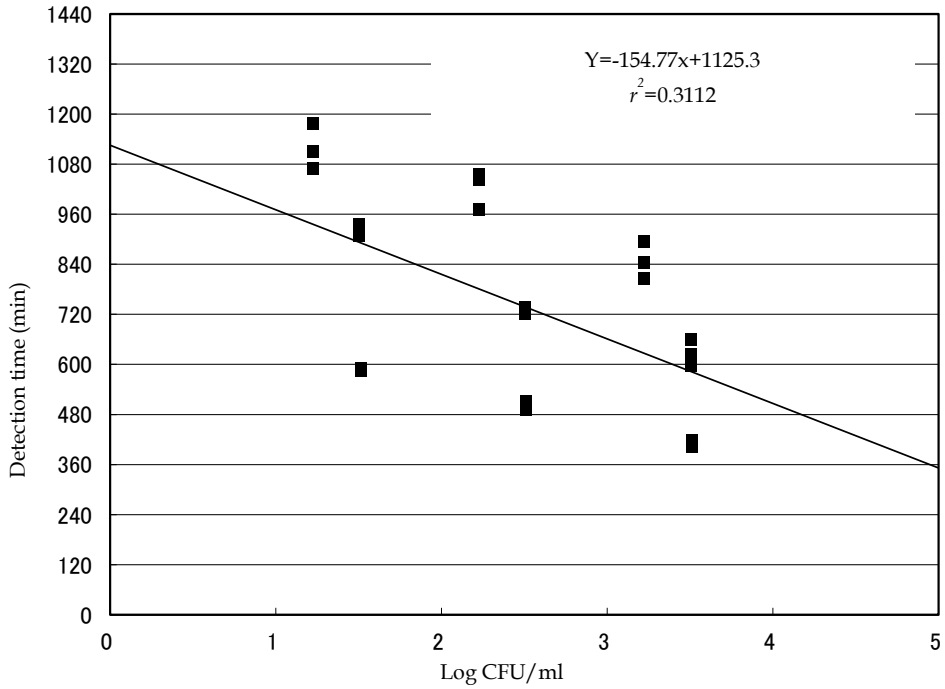
Many staphylococcal food poisoning (SFP) cases have occurred in the world. ISO 6888-1:1999 shows detection methods of *S. aureus* in food. In Japan, food hygiene law published their regulations for the growth of *S. aureus* in unheated processed meat and for the approved detection method. The standard limit in food was set at <1,000 CFU/g. In both the ISO method and the Japanese food hygiene law method, Baird Parker agar, mannitol salt agar with egg yolk (MSEY) agar, and some defined substrate technology agars shows as selection agars. Results on Baird Parker agar and MSEY agar have to be read after 48 hrs of incubation, while with many defined substrate technology agars the results are available after only 24 hrs.

The DOX™ system (Bio-Theta, Osaka, Japan) provides a quantitative method for estimating bacterial counts based on respiration rate (Amano et al., 1999; Katayama, 2000). An oxygen electrode measures the level of dissolved oxygen in a sample diluted with media. Over time, a sample with a high bacterial load will cause the level of dissolved oxygen to decrease to a given threshold value faster than will a sample with a low bacterial load. The time required to reach the threshold level correlates with the amount of bacteria in the sample. Then, by creating a standard curve for each food matrix, the level of bacterial contamination can be estimated in unknown samples. In addition, the DOX system has a special feature that enables organisms to be isolated on agar gel by using the reagents in the DOX cassette (Fig. 1a and Fig. 1b) once the organism has been detected by the DOX system. The DOX system provides rapid results and requires no special techniques for measurement. At present, Daikin Industry Ltd. staff has developed and supplied a total viable count (TVC) test kit (Amano et al., 2001), a quantitative coliform and *Escherichia coli* detection test kit (Kawasaki et al., 2003), a *Staphylococcus aureus* detection kit, a *Salmonella* detection kit, a *Listeria* spp. detection kit (Tanno et al., 2015) and a *Vibrio* spp. detection kit (Tanno et al., 2014). Furthermore, the Association of Analytical Communities International Research Institute has recognized the TVC test (Certificate No. 040801) and the quantitative coliform and *E. coli* test (Certificate No. 120801) in the DOX system.

In our DOX system, respiration rate and color change (red to yellow) were detected in the detection medium in the DOX cassette (Fig. 1c and Fig. 1d), and the DOX system only required 1 day to perform. When an inspector gets a quick result, they will be able to give a quick response, so time is of the essence. In this study, a novel quantitative method for detecting *S. aureus* using the DOX system was developed.

Figure 1 (a) Detection unit of the Bio-Theta DOX™ system
(b) The DOX cassette
(c) Start and negative color (Red) of the medium in the cassette
(d) Positive color (Yellow) of the medium in the cassette

(a) ATCC strains of *S. aureus*



(b) Field strains of *S. aureus*

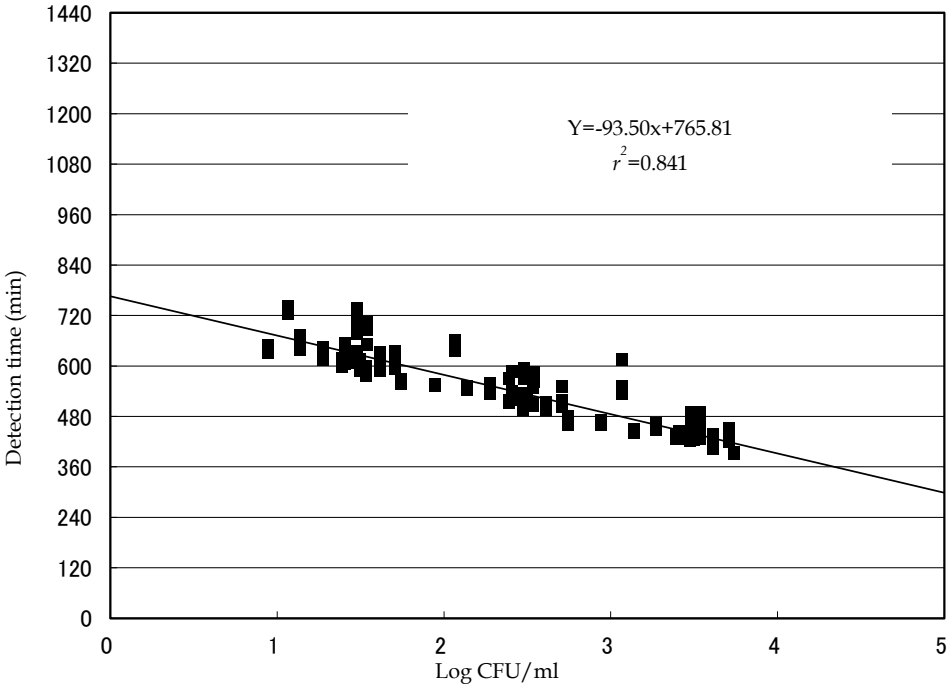


Figure 2 Relationship between detection time and bacterial count
(a) 3 ATCC strains of *S. aureus*
(b) 16 field strains of *S. aureus*

Materials and Methods

Isolates: A total of 19 strains of *S. aureus* (Table 1), and 36 strains of non-*S. aureus* containing 27 species were studied (Tables 1 and 2). In 20 strains of the non-*S. aureus*, 12 species of staphylococci were coagulase-negative. In the total of 55 strains examined in this study, twenty-three strains were supplied by the American Type Culture Collection (ATCC), 5 strains by the Japan Collection of Microorganisms (JCM), and 1 strain by the Biological Resource Center, National Institute of Technology and Evaluation (NBRC), Japan. The other 26 strains examined in this study were four strains from rice balls; two strains each from ground beef, ground pork, patients, and salmon; and 1 strain each from daily food, frozen dumpling, ground beef, egg salad, ground chicken, salmon carpaccio, dressing squid in miso sauce, hamburger patty, vegetable salad,

cabbage, scallop, hand, Spanish mackerel, smoked salmon and shrimp.

DOX system examination of isolates: All strains examined in this study were streaked on trypticase soy agar (Nissui, Tokyo, Japan) and incubated at 35°C for 16-20 hrs under aerobic conditions. The growing *S. aureus* strains were adjusted to approximately 10^3 CFU/ml (high concentration), 10^2 CFU/ml (medium concentration), and 10^1 CFU/ml (low concentration) in a physiological salt solution (PSS; pH 7.2 containing 9 g sodium chloride and 1000 ml distilled water). The non-*S. aureus* strains were adjusted to approximately 10^6 CFU/ml (high concentration) and 10^3 CFU/ml (medium concentration) in PSS. Beforehand, the concentrations of the bacteria were measured using the optical density (OD) value at 660 nm. Then, 1 ml custom diluted solution containing the test organisms and 1 ml DOX *S. aureus* media were inoculated in the DOX coliform cassette and shaken by hand for 30 s. The cassette was placed in the sample port of the DOX system and left for 24 hrs. When the DOX system showed a positive result, the medium in the cassette was verified as positive (i.e. yellow) and the total measurement time was recorded. All samples were examined in triplicate.

Recovery examination of food inoculated with S. aureus: *S. aureus* isolated from rice ball, which is sample number 4 in Table 1, was streaked on trypticase soy agar and incubated at 35°C for 16-20 hrs under aerobic conditions. The growing strain was adjusted to approximately 10^3 CFU/ml, 10^5 CFU/ml, and 10^7 CFU/ml in PSS. Twenty food samples were collected consisting of 2 samples each of rice ball and vegetable salad; and one sample each of ground beef, ground pork, ground chicken, sliced beef, sliced port, green salad, tomato salad, lettuce salad, tuna, porgy, pacific cod, yellowtail, shrimp, sushi roll, scattered sushi, and festive red rice from markets in Japan. The samples were packed in plastic containers or vinyl bags in the shops and were transferred to our laboratories in a box at 1-4°C. The samples were kept in a refrigerator at 3-5°C and analyzed within 24 hrs of collection.

Before adding *S. aureus*, *S. aureus* detection was performed by using the ISO 6888-1 method and the Japanese food hygiene law method in order to find out the naturally-contaminated volume in the examined food. For the detection of *S. aureus*, each 10 g food sample was placed in 90 ml PSS and mixed thoroughly for 1 min. Then, 1 ml custom diluted food solution (×10 for the sample solutions); 10 µl diluted approximately 10^3 CFU/ml, 10^5 CFU/ml, or 10^7 CFU/ml *S. aureus* solution; and 1 ml DOX *S. aureus* media were inoculated in the DOX coliform cassette and shaken by hand for 30 s. The cassette was placed in the sample port of the DOX system and left for 24 hrs. One ml custom diluted food solution (×10 for the sample solutions) and 1 ml DOX *S. aureus* media were also examined. When the DOX system revealed a positive result, positive (yellow) color of the medium in the cassette was checked, then total measurement time was recorded. All samples were examined in duplicate.

Results

Qualitative analysis of S. aureus strains: Results for the high-, medium-, and low-concentration *S. aureus* samples detected by the DOX system are shown in Table 3. The mean positive rate of the high- (10^3 CFU/ml), medium- (10^2 CFU/ml), and low-concentration (10 CFU/ml) *S. aureus* samples was all 100%. The detection time of the *S. aureus* samples was 392.3-848.5 min for the high-concentration samples, 471.7-1023.3 min for the medium-concentration samples, and 562.7-1118.3 min for the low-concentration samples. The rate of change of *S. aureus* samples during the detection period was 0.6-7.6% for the high-concentration samples, 0.4-6.4% for the medium-concentration samples, and 0.4-4.9% for the low-concentration samples. A linear calibration curve between the detection time and bacterial count was observed for all *S. aureus* strains examined in this study. The correlation coefficient (r) of the calibration curve was the lowest for *S. aureus* ATCC 25923 (strain no. 1; 0.9292), followed by the isolates from daily food (strain no. 5; 0.9518), and salmon (strain no. 10; 0.9683). The correlation coefficient for all other *S. aureus* strains was >0.97.

Qualitative analysis of non-S. aureus strains: For the 36 non-*S. aureus* samples, the mean negative rate for the high-concentration (10^6 CFU/ml) samples and the medium-concentration (10^3 CFU/ml) samples were both 94.4% (34/36). The results for the 2 positive non-*S. aureus* samples, *S. xylosus* ATCC 29971 (strain no. 38) and *Enterococcus faecalis* ATCC 29212 (strain no. 48), detected by the DOX system are shown in Table 4. Each 3 high-concentration samples and medium-concentration samples of the *S. xylosus* were positive, and the detection time in the high- and medium-concentration samples were 290.3 min and 732.7 min, respectively. All 3 high-concentration samples and 1 of 3 medium-concentration samples of the *E. faecalis* showed positive, and the detection time in the high- and medium-concentration samples were 685.3 min and 968 min, respectively. The positive samples of the medium in the cassette displayed yellow color (Fig. 1d).

Relationship between detection time and bacterial count in S. aureus strains: The relationship between detection time and bacterial count is shown in Fig. 2a and 2b. Of the multiple strains examined for *S. aureus*, the multiple correlation coefficient (r^2) was 0.3112 for 3 ATCC strains of *S. aureus* (strains no. 1 to 3; Fig. 2a) and 0.841 for 16 field strains of *S. aureus* (strains no. 4 to 19; Fig. 2b).

Recovery examination of food inoculated with S. aureus: No *S. aureus* was isolated from all food samples by using the ISO 6888-1 method, the Japanese food hygiene law method, and the DOX method. Therefore, twenty food samples using the recovery examination were not naturally contaminated with *S. aureus*. The results for the recovery examination of food inoculated with *S. aureus* isolated from rice ball (strain no. 4) are shown in Table 5. All 20 samples inoculated with low-concentration (1.30-1.37 log CFU/ml), medium-

concentration (3.30-3.37 log CFU/ml), and high-concentration (5.30-5.37 log CFU/ml) *S. aureus* were positive in all double examinations. The detection time for the positive samples inoculated with *S. aureus* was 477-807 min (averagely 9.7 hrs for 40 positive examinations) for the low-concentration samples, 333-530 min (averagely 6.9 hrs for 40 positive examinations) for the medium-concentration samples, and 193-317 min (averagely 4.1 hrs for 40 positive examinations) for the high-concentration samples.

Table 1 *S. aureus* strains examined in this study

Strain No.	Species	Strain or source
1	*S.aureus*	ATCC[a)] 25923
2	*S.aureus*	ATCC 6538
3	*S.aureus*	ATCC 29213
4	*S.aureus*	Rice ball
5	*S.aureus*	Rice ball
6	*S.aureus*	Rice ball
7	*S.aureus*	Rice ball
8	*S.aureus*	Ground beef
9	*S.aureus*	Ground beef
10	*S.aureus*	Ground chicken
11	*S.aureus*	Ground pork
12	*S.aureus*	Daily food
13	*S.aureus*	Dressing squid in a miso sauce
14	*S.aureus*	Egg salad
15	*S.aureus*	Frozen dumplings
16	*S.aureus*	Hamburger patty
17	*S.aureus*	Salmon
18	*S.aureus*	Salmon Carpaccio
19	*S.aureus*	Vegetable salad

a) ATCC : American Type Culture Collection.

Table 2 Non-*S. aureus* strains examined in this study

Strain No.	Species	Strain or source
20	*Staphylococcus caprae*	Clinical
21	*Staphylococcus captis*	ATCC[a)] 27840
22	*Staphylococcus cohnii*	ATCC 29974
23	*Staphylococcus epidermidis*	ATCC 12228
24	*Staphylococcus epidermidis*	ATCC 14990
25	*Staphylococcus epidermidis*	ATCC 35984
26	*Staphylococcus epidermidis*	Hand
27	*Staphylococcus hemolyticus*	ATCC 29970
28	*Staphylococcus hyicus*	JCM[b)] 2423
29	*Staphylococcus saprophyticus*	Clinical
30	*Staphylococcus saprophyticus*	Spanish mackerel
31	*Staphylococcus saprophyticus*	Smoked salmon
32	*Staphylococcus sciuri*	ATCC 29062
33	*Staphylococcus sciuri*	Ground pork
34	*Staphylococcus shleiferi*	JCM 7470
35	*Staphylococcus simnlans*	JCM 2424
36	*Staphylococcus warneri*	JCM 2415
37	*Staphylococcus warneri*	Salmon
38	*Staphylococcus xylosus*	ATCC 29971
39	*Staphylococcus xylosus*	Shrimp
40	*Acinetobacter baumannii*	ATCC 19606
41	*Aerococcus viridans*	Cabbage
42	*Aeromonas hydrophila*	JCM 1027
43	*Bacillus cereus*	NBRC[c)] 3457
44	*Bacillus subtilis*	ATCC 6633
45	*Candida albicans*	ATCC 10231
46	*Candida tropiclis*	ATCC 750
47	*Enterobacter cloacae*	ATCC 13047
48	*Enterococcus faecalis*	ATCC 29212
49	*Enterococcus faecium*	ATCC 35667
50	*Enterococcus faecium*	Scallops
51	*Escherichia coli*	ATCC 25922
52	*Klebsiella pneumoniae*	ATCC 13883
53	*Kocuria rhizophila*	ATCC 9341
54	*Pseudomonas aeruginosa*	ATCC 27853
55	*Stenotrophomonas maltophila*	ATCC 13637

a) ATCC: American Type Culture Collection
b) JCM: Japan Collection of Microorganisms
c) NBRC: Biological Resource Center, NITE

Table 3 Results for high-, medium-, and low-concentration *S. aureus* samples analyzed by the DOX system

Strain No.	Strain or source	High concentration	Medium concentration	Low concentration	Correlation coefficient (r)
1	ATCC 25923	3.23 [a]	2.23	1.23	y = -135x + 1297.1
		848.5 (5.3%) [b]	1023.3 (4.4%)	1118.3 (4.9%)	0.9292
2	ATCC 6538	3.50	2.50	1.50	y = -147.17x + 1127.1
		626.7 (5.0%)	728.3 (1.1%)	921.0 (1.4%)	0.9758
3	ATCC 29213	3.51	2.51	1.51	y = -88.667x + 722.62
		410.7 (1.8%)	500.7 (2.0%)	588.0 (0.4%)	0.9965
4	Rice ball	3.61	2.61	1.61	y = -90.333x + 739.66
		411.3 (2.9%)	507.0 (1.2%)	592.0 (0.6%)	0.9955
5	Rice ball	3.48	2.48	1.48	y = -113.83x + 871.97
		478.7 (2.7%)	584.0 (2.2%)	706.3 (4.2%)	0.9839
6	Rice ball	3.74	2.74	1.74	y = -85.167x + 709.14
		392.3 (0.6%)	471.7 (2.0%)	562.7 (0.7%)	0.9967
7	Rice ball	3.14	2.14	1.14	y = -103x + 768.41
		445.3 (0.7%)	547.3 (0.7%)	651.3 (2.9%)	0.9941
8	Ground beef	2.94	1.94	0.94	y = -86.667x + 721.41
		465.3 (1.0%)	554.7 (0.4%)	638.7 (1.3%)	0.9976
9	Ground beef	3.50	2.50	1.50	y = -87x + 734.81
		429.7 (1.3%)	518.7 (1.4%)	603.7 (2.1%)	0.9945
10	Ground chicken	3.54	2.54	1.54	y = -103.17x + 837.04
		473.3 (2.9%)	572.7 (1.9%)	679.7 (4.0%)	0.9840
11	Ground pork	3.27	2.27	1.27	y = -86.5x + 740.94
		456.3 (1.5%)	547.0 (2.3%)	629.3 (2.1%)	0.9911
12	Daily food	3.07	2.07	1.07	y = -81.5x + 816.45
		567.0 (7.6%)	646.7 (1.8%)	730.0 (1.2%)	0.9518
13	Dressing squid in a miso sauce	3.61	2.61	1.61	y = -93.167x + 755.18
		422.7 (2.9%)	503.0 (1.4%)	609.0 (3.2%)	0.9862
14	Egg salad	3.54	2.54	1.54	y = -75.333x + 706.1
		436.3 (2.3%)	522.0 (4.5%)	587.0 (1.7%)	0.9756
15	Frozen dumplings	3.71	2.71	1.71	y = -89.667x + 766.15
		433.0 (3.9%)	524.3 (4.7%)	612.3 (3.4%)	0.9738
16	Hamburger patty	3.48	2.48	1.48	y = -96.667x + 761.79
		427.3 (1.8%)	519.0 (3.9%)	620.7 (2.2%)	0.9883
17	Salmon	3.41	2.41	1.41	y = -96.833x + 770.47
		435.7 (1.1%)	546.3 (6.4%)	659.3 (3.3%)	0.9683
18	Salmon Carpaccio	3.41	2.41	1.41	y = -99.5x + 772.29
		435.7 (1.9%)	527.7 (1.8%)	634.7 (4.0%)	0.9857
19	Vegetable salad	3.39	2.39	1.39	y = -88.667x + 736.66
		431.3 (1.1%)	534.0 (5.9%)	608.7 (1.7%)	0.9735

a) Log CFU/ml of sample
b) Mean detection period (min) and rate of change (%) of triplicate examinations per sample

Table 4 Results for positive non-*S. aureus* samples by the DOX system

Strain No.	Species	High concentration			Medium concentration		
		1st	2nd	3rd	1st	2nd	3rd
		Mean (Rate of change)			Mean (Rate of change)		
38	*Staphylococcus xylosus*	295 [b]	5.88 [a]	285	752	2.88	714
			291			732	
			290.3 (1.7%) [c]			732.7 (2.6%)	
48	*Enterococcus faecalis*	662	6.43	696	968	3.43	ND
			698			ND [d]	
			685.3 (3.0%)			968	

a) Log CFU/ml
b) Detection time (min) of the 1st examination for the high concentration samples
c) Mean detection period (min) and rate of change (%) of triplicate examinations per sample
d) No detection

Table 5 Results for food samples inoculated with *S. aureus* by the DOX system

Sample No.	Foodstuff	Low concentration		Medium concentration		High concentration	
		1st	2nd	1st	2nd	1st	2nd
		Mean (Rate of change)		Mean (Rate of change)		Mean (Rate of change)	
1	Rice ball	1.37 [a)]		3.37		5.37	
		583 [b)]	551	404	407	259	269
		567.0 (4.0%) [c)]		405.5 (0.5%)		264.0 (2.7%)	
2	Rice ball	1.37		3.37		5.37	
		574	569	420	418	261	269
		571.5 (0.6%)		419.0 (0.3%)		265.0 (2.1%)	
3	Vegetable salad	1.30		3.30		5.30	
		538	536	408	389	245	254
		537.0 (0.3%)		398.5 (3.4%)		249.5 (2.6%)	
4	Vegetable salad	1.37		3.37		5.37	
		749	807	510	530	317	299
		778.0 (5.3%)		520.0 (2.7%)		308.0 (4.1%)	
5	Ground beef	1.30		3.30		5.30	
		614	575	442	436	235	242
		594.5 (4.6%)		439.0 (1.0%)		238.5 (2.1%)	
6	Ground pork	1.30		3.30		5.30	
		663	592	398	412	223	231
		627.5 (8.0%)		405.0 (2.4%)		227.0 (2.5%)	
7	Ground chicken	1.30		3.30		5.30	
		494	477	338	337	207	204
		485.5 (2.5%)		337.5 (0.2%)		205.5 (1.0%)	
8	Sliced beef	1.30		3.30		5.30	
		542	539	391	391	235	244
		540.5 (0.4%)		391.0 (0.0%)		239.5 (2.7%)	
9	Sliced pork	1.30		3.30		5.30	
		595	585	418	424	232	234
		590.0 (1.2%)		421.0 (1.0%)		233.0 (0.6%)	
10	Green salad	1.30		3.30		5.30	
		588	639	448	470	256	263
		613.5 (5.9%)		459.0 (3.4%)		259.5 (1.9%)	
11	Tomato salad	1.30		3.30		5.30	
		558	554	400	429	245	254
		556.0 (0.5%)		414.5 (4.9%)		249.5 (2.6%)	
12	Lettuce salad	1.30		3.30		5.30	
		555	619	481	407	248	257
		587.0 (7.7%)		444.0 (11.8%)		252.5 (2.5%)	
13	Tuna	1.37		3.37		5.37	
		641	671	410	354	214	227
		656.0 (3.2%)		382.0 (10.4%)		220.5 (4.2%)	
14	Porgy	1.37		3.37		5.37	
		488	555	343	333	193	201
		521.5 (9.1%)		338.0 (2.1%)		197.0 (2.9%)	
15	Pacific cod	1.37		3.37		5.37	
		558	558	399	399	251	247
		558.0 (0.0%)		399.0 (0.0%)		249.0 (1.1%)	
16	Yellowtail	1.37		3.37		5.37	
		532	494	397	380	226	200
		513.0 (5.2%)		388.5 (3.1%)		213.0 (8.6%)	
17	Shrimp	1.37		3.37		5.37	
		585	616	428	454	272	268
		600.5 (3.7%)		441.0 (4.2%)		270.0 (1.0%)	
18	Sushi roll	1.37		3.37		5.37	
		564	578	419	416	265	272
		571.0 (1.7%)		417.5 (0.5%)		268.5 (1.8%)	
19	Scattered sushi	1.37		3.37		5.37	
		585	601	425	459	266	272
		593.0 (1.9%)		442.0 (5.4%)		269.0 (1.6%)	
20	Festive red rice	1.37		3.37		5.37	
		590	636	458	452	276	273
		613.0 (5.3%)		455.0 (0.9%)		274.5 (0.8%)	

a) Log CFU/ml
b) Detection time (min) of the 1st examination for the low concentration samples
c) Mean detection period (min) and rate of change (%) of double examinations per sample

Discussion

SFP is one of the important public health problems in the world (Hennekinne et al., 2012). Enterotoxin is produced by growing *S. aureus*, and it is very important for food hygiene to detect *S. aureus* contamination in/on food. We developed a quantitative method to detect *S. aureus* rapidly using the DOX system. This system could detect all low-concentration (10 CFU/ml) *S. aureus* samples within 10 hrs, and 2 non-*S. aureus* samples as *S. xylosus* ATCC 29971 (strain no. 38) and *Enterococcus faecalis* ATCC 29212 (strain no. 48) showed positive.

S. xylosus is a Gram-positive, catalase-positive and facultative anaerobic microorganism. The organism is coagulase-negative and distributed on the skin of humans and animals and in the environment (Negase et al., 2002). *S. xylosus* is frequently isolated

from cheeses and dry fermented sausages (Coton et al., 2010), and the organism is commonly used as starter culture in meat fermentation (Talon and Leroy, 2011). *E. faecalis* is a Gram-positive, commensal bacterium inhabiting the gastrointestinal tracts of humans and other mammals (Schleifer and Bell, 2009). Enterococci contamination in salami and fresh meat is usually 2 to 5 Log CFU/g (1 to 4 Log CFU/ml), and 2 to 4 Log CFU/g (1 to 3 Log CFU/ml), respectively (Giraffa, 2002). In this examination, the detection time of *S. xylosus* strain no. 38 for the medium concentration (2.88 Log CFU/ml) was 732.7 min. This detection time is within the range of positive time for all *S. aureus* strains examined in this study. Same species but different strain of *S. xylosus* indicated different results as strain no. 38 (ATCC 29971) showed positive, whereas strain no. 39 isolated from shrimp revealed negative. All three high concentration (6.43 Log CFU/ml) samples and 1 of 3 medium concentration (3.43 Log CFU/ml) samples of *E. faecalis* (strain no. 48) were positive and the detection times were 685.3 min and 968.0 min, respectively. These detection times are also within the range of positive time for all *S. aureus* strains. In the case of *S. xylosus* and *E. faecalis* phenomena, the positive detection medium changing to yellow color may be inoculated onto isolation agars such as Baird Parker agar, MSEY agar and/or defined substrate technology agars, and be cultivated. If a component is modified and/or new antibiotics are added in our DOX *S. aureus* media, the false positive problems by the DOX system will be clear.

In all food samples, low-concentration *S. aureus* such as 1.30 to 1.37 Log CFU/ml could be detected within 10 hrs by using the DOX system, however some false positive cases as *S. xylosus* and *E. faecalis* case existed. The sensitivity of our DOX system was higher than the ISO 6888-1:1999 method and the Japanese food hygiene law method, and the detection time was short.

The number of *S. aureus* in remnant food of SFP cases is usually at least 10^5 CFU/g (Hennekinne et al., 2012). Our DOX system only required usually within 10 hrs to obtain estimates of *S. aureus* number above 100 CFU/g. On the basis of the results of the present laboratory analysis, the DOX system provides rapid results and requires no special techniques for measurement. When the DOX system gave a positive result, *S. aureus* could be isolated on the conventional isolation agars by using the reagents in the DOX cassette. The DOX system may be a useful tool for proving the absence of *S. aureus* in food and environmental samples at food processing companies. However, further consideration of a validation study between the DOX system and the many available official methods such as ISO 6888-1:1999 is needed.

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บทคัดย่อ

การตรวจหาปริมาณเชื้อ *Staphylococcus aureus* โดยใช้ระบบ Bio-Theta DOX™

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การศึกษานี้ได้พัฒนาวิธีการตรวจหาปริมาณเชื้อ *S. aureus* โดยใช้ระบบ DOX™ โดยทดลองกับเชื้อ *S. aureus* จำนวน 19 ตัวอย่าง และเชื้อ *non-S. aureus* (27 สายพันธุ์) จำนวน 36 ตัวอย่าง ระบบนี้สามารถตรวจหาปริมาณความเข้มข้นสูงสุด (10^3 CFU/ml) ความเข้มข้นระดับกลาง (10^2 CFU/ml) และความเข้มข้นระดับต่ำ (10 CFU/ml) จากเชื้อ *S. aureus* ทั้ง 19 ตัวอย่าง โดยมีอัตราการตรวจพบเป็นร้อยละ 100 ความสัมพันธ์ของเวลาที่ใช้ในการตรวจพบและจำนวนเชื้อเป็นสัดส่วนที่ดีคือ เป็น linear calibration curve ระบบนี้ยังสามารถตรวจหาอัตราผลลบของเชื้อที่มีปริมาณความเข้มข้นสูงสุด (10^6 CFU/ml) และความเข้มข้นระดับกลาง (10^3 CFU/ml) จากตัวอย่างที่ไม่ใช่เชื้อ *S. aureus* (non-*S. aureus*) ทั้ง 36 ตัวอย่าง ซึ่งคิดเป็นอัตราส่วนร้อยละ 94.4 (34/36) โดยให้ผลบวกกับ 2 ตัวอย่าง คือ *S. xylosus* ATCC 29971 จำนวน 1 ตัวอย่างและ *Enterococcus faecalis* ATCC 29212 จำนวน 1 ตัวอย่าง นอกจากนี้ ยังได้ทำการทดลองเพิ่มเติมโดยใส่เชื้อ *S. aureus* ความเข้มข้น 1.30-1.37 log CFU/ml ลงในตัวอย่างข้าวปั้นจำนวน 20 ตัวอย่าง พบว่าเวลาที่ใช้ในการตรวจพบประมาณ 477-807 นาที (9.7 ชั่วโมง) ระบบ DOX สามารถให้ผลภายใน 10 ชั่วโมงและไม่ต้องใช้เทคนิคพิเศษ สรุปได้ว่าระบบ DOX มีประโยชน์ในการตรวจหา *S. aureus* ในอาหารและสิ่งแวดล้อมในบริษัทผลิตอาหาร อย่างไรก็ดีควรมีการศึกษาในภาคสนามเพิ่มเติม

คำสำคัญ: ระบบการหาปริมาณเชื้อแบคทีเรีย ระบบ DOX ปริมาณเชื้อ *S. aureus*

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