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Isolation and Identification of *Staphylococcus* sp. from Korean Fermented Fish Products

UM, MI-NA AND CHERL-HO LEE*

Center for Advanced Food Science and Technology(CAFST),
The Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea

In order to find out if staphylococci occur in significant numbers in Korean fermented fish products, a total of 40 different fermented fish products were collected from different markets in Korea and analyzed for their physico-chemical and microbiological states. The pH, salt concentration and water activity of the products were measured and the total viable cell count and the number of *Staphylococcus* grown on mannitol salt agar were determined. The identification of the strains of *Staphylococcus* were made by API Staph Strip and MIS identification kits, and the physiological properties of the identified strains were further characterized by different conventional methods. The pH, salt content and water activity of fermented fish samples varied widely from 4.8 to 7.1, 7.4-28.7% and 0.77-0.84, repectively, depending on the type of product. The total viable cell count varied from 10^4-10^9 cfu/ml, and most of the samples had 10^5-10^6 cfu/ml. No correlation was found between the viable cell count and the pH, NaCl concentration and water activity of the samples. Among the 35 colonies identified as *Staphylococcus* strains by the identification kits, *S. xylosus* was the most frequently occurring strain marking 17, and *S. warneri* was 8, *S. epidermidis* 4 and *S. cohnii* 2. *S. hominis*, *S. saprophyticus*, *S. haemolyticus* and *S. aureus* were also identified once each. In some samples (K-3, P-6, K-8, G-5 and G-10), 2-3 different species of *Staphylococcus* were found. Considering the region of sampling, among the 10 samples from Kunsan 5 were identified as *S. warneri*, while in the other regions *S. xylosus* was predominant. Although the physiological characteristics of the identified strains were generally consistent with those in Bergey's Manual, some discrepances were also observed. All the strains were highly salt tolerant, growing in the media containing over 18% NaCl. All the strains except *S. aureus* (G-11) showed negative in hemolysis activity, plasma coagulation and DNase tests. All the strains including *S. aureus* (G-11) showed negative in enterotoxin test.

Fermentation of fish and marine products is a traditional method of processing food in Korea. Various types of fish, shell fish and crustacean are fermented. Whole fish, parts of the body, viscera and roe are used to make a variety of products (7). Products are divided into two groups according to the type of fermentation which is determined by the salt concentration and the use of added carbohydrate; high-salt joetkal and low-salt sikhae with cooked cereals added. Sikhae is made by primarily lactic acid fermentation involving *Leuconostoc* and *Lactobacillus* species (8). On the other hand, joetkal is made by mixing fish with salt only, adding about 25% of the weight of fish, and ageing. Low-salt jeotkal, containing salt to less than 10% of total weight, is made today using refrigerated systems (6).

*Corresponding author
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The fermentation of jeotkal depends on the action of endogenous enzymes as well as microorganisms spontaneously involved in the system. Although the relative importance of endogenous enzymes and spontaneous microorganisms to the fermentation largely depends on the type of product, it is evident that microorganisms play an important role in the flavor formation of fermented jeotkal (11).

The spontaneous microbial flora in fermented jeotkal have been reported by some investigators. In case of Myeolchi-jeot(salt-fermented anchovy), *Acromobacter*, *Bacillus* and *Flavobacterium* appear during the first month of fermentation and disappear at the second month, where *Pediococcus*, *Halobacterium*, *Micrococcus* and *Sarcina* become the dominant flora. The yeasts, *Saccharomyces* and *Torulopsis*, become dominant at the third month of ageing, where rapid decomposition of fish meat occurrs (9). In case of fermented sardines, the number of

viable cells decreased gradually during the first month of fermentation and then increased to reach the maximum by 60 days of fermentation. *Brevibacterium*, *Bacillus*, *Pseudomonas* and *Flavobacterium* disappeared during the first 10 days of salt ageing, while *Micrococcus*, *Halobacterium* and *Pediococcus* grew during the 80 day fermentation period (4). These studies indicated the appearance of *Micrococcus* in the salt-fermented fish products, but did not fully identify the microbial species involved in the products.

Tanasupawat *et al.* (14) isolated gram-positive and catalase-positive cocci from fermented fish and soysauce mash, and studied them systematically on the basis of their phenotypic and chemotaxonomic characteristics and identified them as *Staphylococcus carnosus* and then confirmed the identification by the DNA-probe method. Tanasupawat *et al.* (16) also isolated a new coagulase-negative *Staphylococci* from fermented fish in Thailand. It was identified as *Staphylococcus pisciferm*entans sp. nov. on the basis of its DNA relatedness and biochemical characteristics.

The involvement of micrococci in sausage fermentation is well documented, and it was recently recognized that many of these organisms belong to the genus *Staphylococcus* (13). The present study focused on the identification of *Staphylococcus* in Korean fermented fish products, in order to isolate food grade strains useful as the starter microorganism for European fermented meat sausages.

MATERIALS AND METHODS

Test Samples

Salt-fermented fish products sold in different markets in Korea were collected. Table 1 shows the location of sampling sites and the type of fermented fish products bought. A total of 40 samples were collected; 10 samples from Kunsan, a city on the west coast, 10 samples from the south coast city Pusan, 14 samples from a market (Garakdong) in Seoul, 2 samples from the southern island of Chejudo, 2 samples from Sokcho on the east coast, and 2 samples from Suwon, a city in the middle of the country. Equally there were various products, 6 samples were made from anchovy, 5 from squid, 3 from small shrimp, 3 from sea arrow, 3 from clam, 4 from Alaskan pollack roe and cod roe, 3 from big eyed herring, 6 from pollack tripe and cod gill, 3 were fish sauces and five were micellaneous products. The samples were stored at 4°C in a refrigerator during the test periods.

Physico-chemical Measurements

Some important physico-chemical properties of the fermented fish samples were measured. pH was measured with a Corning Ion Analyzer 250 (U.S.A.), the concentration of sodium chloride by Mohr's mothod (1), and the water activity by a Hygromeasuring System (Defensor Novasima MSI, Switzerland).

Table 1. Location of sampling sites and number of samples.

Location	Name (raw material)	Code number
Kunsan	Saewoo-jeot (small shrimp)	K-1
(West coat)	Myeolchi-jeot (anchovy)	K-2
	O-jeot (small shrimp)	K-3
	Hwangsai-gi-jeot (hwangangdali)	K-4
	Goldoo-gi-jeot (sea arrow)	K-5
	Kalchi-Naijang-jeot (hair-tail viscera)	K-6
	Bendengi-jeot (big eyed herring)	K-7
	Jogai-jeot (clam)	K-8
	Myungran-jeot (Alaska pollack roe)	K-9
	Yuk-jeot (fishsauce)	K-10
Pusan	Daigu-agami-jeot (cod gill)	P-1
(South coast)	Changran-jeot (pollack tripe)	P-2
	Jogai-jeot (clam)	P-3
	Daigu-al-jeot (cod roe)	P-4
	Juneo-jeot (shad)	P-5
	Sawoo-jeot (small shrimp)	P-6
	Myungran-jeot (Alaska pollack roe)	P-7
	Goldoogi-jeot (sea arrow)	P-8
	Ojingo-jeot (squid)	P-9
	Myeolchi-jeot (anchovy)	P-10
Seoul	Gonjeng-i-jeot (mysis)	G-1
(market in	Myelochi-jeot (anchovy)	G-2
Garakdong)	Jogai-jeot (clam)	G-3
	Saewoo-jeot (small shrimp)	G-4
	Bendeng-i-jeot (big eyed herring)	G-5
	Bendeng-i-jeot (big eyed herring)*	G-6
	Changran-jeot (pollack tripe)*	G-7
	Agami-jeot (gill)*	G-8
	Golodoogi-jeot (sea arrow)*	G-9
	Ojingo-jeot (squid)*	G-10
	Myungran-jeot (Alaska pollack roe)*	G-11
	Myeolchi-jeot (anchovy)	G-12
	Myeolchi-jeot (anchovy)	G-13
	Yuk-jeot (fish sauce)	G-14
Cheju	Jari-jeot (damsel fish)	J-1
(Southern island)	Myeolchi-jeot (anchovy)	J-2
Sokcho	Ojingo-jeot (squid)*	S-1
(East coast)	Myungran-jeot (Alaska pollack roe)	S-2
Suwon	Ojingo-jeot (squid)*	Su-1
(Middle land)	Yuk-jeot (fish sauce)	Su-2

* seasoned.

Microbial Tests

Total viable cell counts were made by plate count agar (PCA) with 3% NaCl added. The number of colonies that appeared after 48 h at 37°C was counted. The number of *Staphylococcus* in the sample was analyzed by counting the colonies that appeared on the mannitol salt agar (MSA) inoculated with the sample, homogenized and diluted in phosphate buffer saline (PBS), and incubated at 37°C for 48

h. The *Staphylococcus* species grown in tryptic soy broth (TSB) medium containing 10% NaCl were isolated by inoculating on mannitol salt agar. Different types and colors of colonies were taken for isolation and identification.

Identification of *Staphylococcus* Species

The classification of the strains of *Staphylococcus* were made according to Bergey's Manual of Systematic Bacteriology (12). The methods used in the tests for the identification were adopted from the Bacteriological Analytical Manual (3) and the Biochemical Tests for the Identification of Medical Bacteria (10). The following tests were carried out:

1) Gram staining and optical microscopic observation
2) Tellurite reducing power test
3) Catalse and oxidase tests (Ewing-Johnson method)
4) API STAPH Strip and MIS identification (bio-Merieeux sa, France)
5) Sugar fermentation test
6) Paraffin oil oxidation test
7) Urease test
8) Nitrate reductase test
9) Voges-Proskauer test
10) Citrate utilization test
11) Gelatinase test
12) NH_3 from Arginine
13) Salt tolerance test
14) Hemolysis test
15) Coagulase test
16) DNase test
17) Enterotoxin test (SET-RPLA: *Staphylococcus Enterotoxin* A,B,C,D Detection Kit by Reserved Passive Latex Agglutination, Denka, Japan)

RESULTS AND DISCUSSION

pH of Fermented Fish Samples

The pH of fermented fish samples varied from 4.8 to 7.1, as shown in Table 2. Most of the salt-fermented fish products made from anchovy, squid, sea arrow, clam, Alaskan pollack roe, big eyed herring and fish sauce were in the range of pH 5.0-5.5. However, seasoned products made from the same sort of fish showed relatively lower pH values, ranging from 4.8-4.9. This may be due to the added acidulants or to the lactic acid fermentation of the carbohydrates incorporated with added seasonings. The pH of fermented small shrimp was relatively high, ranging from 6.6-7.1. This may be due to the calcium ions dissociated from the shell during fermentation. Fermented viscera and gills, fermented damsel fish and fermented mysis had also slightly high pH, ranging from 5.6-6.6.

NaCl Concentration of Fermented Fish Samples

The concentration of salt in fermented fish products

Table 2. Total viable cell count and staphylococcal count and some characteristics of fermented fish products collected in Korea.

Sample	pH	NaCl (%)	Aw	Total viable count (CFU/ml)	staphylococcal count (CFU/ml)
Anchovy					
K-2	5.3	20.8	0.81	1.6×10^6	–
P-10	5.2	21.3	0.83	1.9×10^5	1.5×10^2
G-2	5.2	24.3	0.83	7.5×10^5	–
G-12	5.1	29.1	0.80	2.1×10^6	4.8×10^3
G-13	5.2	25.8	0.79	2.8×10^8	5.2×10^4
Mean	5.2	24.2	0.81		
Anchovy (Seasoned)					
J-2	4.8	8.7	0.87	1.2×10^7	–
Squid					
P-9	5.5	28.7	0.77	1.9×10^6	–
S-1	5.4	20.3	0.77	4.2×10^8	3.7×10^4
S-2	5.4	20.8	0.77	3.7×10^5	2.1×10^2
Mean	5.4	23.3	0.77		
Squid (seasoned)					
G-10	4.9	12.3	0.87	1.6×10^4	3.6×10^2
SU-1	4.9	9.3	0.89	5.2×10^7	6.4×10^3
Mean	4.9	10.8	0.88		
Small shrimp					
K-1	7.1	28.8	0.77	6.2×10^6	–
P-6	6.2	29.7	0.77	4.2×10^6	1.0×10^2
G-4	6.6	25.4	0.77	6.4×10^5	–
Mean	6.6	28.0	0.77		
Sea arrow					
K-5	4.9	24.5	0.77	1.8×10^4	2.1×10^2
P-8	5.2	25.5	0.79	2.5×10^5	–
Mean	5.1	25.0	0.78		
Sea arrow (Seasoned)					
G-9	5.1	7.4	0.90	1.7×10^7	4.5×10^2
Clam					
K-8	4.8	16.6	0.88	3.0×10^6	8.6×10^2
P-3	5.5	19.2	0.85	4.8×10^6	8.6×10^2
G-3	5.1	22.5	0.83	1.4×10^7	–
Mean	5.2	19.4	0.85		
Alaska pollack roe and cod roe					
K-9	5.3	16.4	0.87	1.2×10^7	4.8×10^3
P-7	5.8	9.6	0.92	2.4×10^8	6.8×10^3
P-4	5.3	8.9	0.94	6.4×10^9	4.0×10^5
Mean	5.5	11.6	0.91		
Alaska pollack (Seasoned)					
G-11	8.9	10.5	0.90	1.9×10^5	1.5×10^2
Big eyed herring					
K-7	5.5	21.1	0.82	8.0×10^5	3.3×10^2
G-5	5.5	25.3	0.77	2.6×10^5	1.3×10^2
Mean	5.5	23.2	0.79		
Big eyed herring (Seasoned)					
G-6	5.4	22.8	0.88	4.0×10^5	–

Table 2. continued.

Sample	pH	NaCl (%)	Aw	Total viable count (CFU/ml)	staphylococcal count (CFU/ml)
Pollack tripe and hair tail viscera and cod gill					
K-6	5.8	22.7	0.88	4.5×10^5	–
P-2	5.6	21.0	0.85	5.3×10^6	9.6×10^3
P-1	6.1	16.0	0.88	3.4×10^5	3.2×10^3
Mean	5.8	19.9	0.87		
Pollack tripe and cod gill (seasoned)					
G-7	4.8	7.9	0.88	3.6×10^7	7.6×10^2
G-8	4.9	9.4	0.88	2.6×10^8	2.9×10^2
Mean	4.9	8.7	0.88		
Fish sauce					
K-10	5.2	26.3	0.78	7.1×10^7	7.4×10^2
Su-2	5.2	26.1	0.79	4.5×10^6	5.2×10^2
G-14	5.2	25.7	0.79	3.6×10^7	2.4×10^3
Mean	5.2	26.0	0.79		
Others					
P-5	5.2	27.4	0.81	1.5×10^5	–
K-4	5.7	23.8	0.79	2.1×10^5	4.3×10
J-1	6.2	17.5	0.85	5.9×10^5	1.8×10^2
G-1	6.6	18.9	0.84	5.2×10^7	9.4×10^2
K-3	6.6	26.6	0.77	3.0×10^6	1.0×10^2
Mean	6.1	22.8	0.81		

–: Not detected.

varied widely depending on the type of products but also within the same type of product, as shown in Table 2. The seasoned products, often mixed with lactic acid, sorbitol and alcohol, generally contained low levels of salt ranging from 7.4% to 12.3%. Most of the salt fermented products contained 20-25% salt. Fermented small shrimp had relatively high salt content, 25.4-29.7%, while fermented Alaskan pollack roe and cod roe had low salt content, 8.9-16.4%. There were no significant differences in the salt content in relation to where the sample was purchased. No significant correlations between the salt content and pH of the products were observed.

Water Activity of Fermented Fish Samples

The water activity of the products varied from 0.77-0.94, as shown in Table 2. In most cases the water activity varied in accordance with the salt concentration of the product, but some exceptions were also observed. Seasoned products and fermented fish roe showed high water activity ranging from 0.87-0.94. On the other hand, most of the salt fermented fish and fish sauces had sufficiently low water activity, 0.77-0.85, to prevent the growth of putrefactive and hazardous microorganisms. A close correlation between salt concentration and water activity of the fermented fish products was observed, as shown in Fig. 1.

Total Cell Count and Number of *Staphylococcus* in the Samples

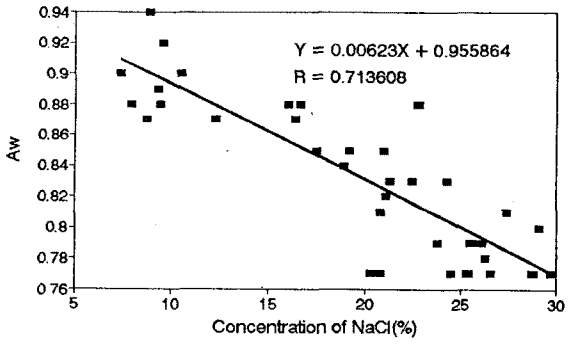

Fig. 1. Correlation between salt content and water activity of fermented fish products.

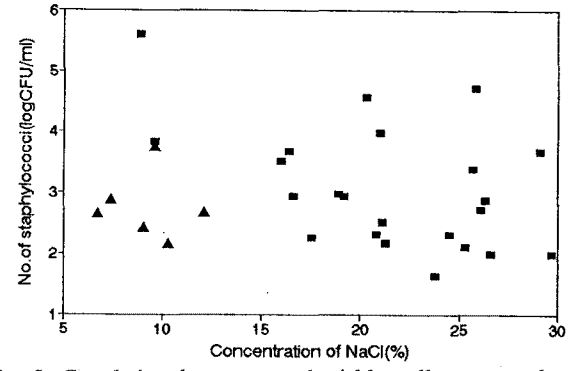

Fig. 2. Correlation between total viable cell count and salt content of fermented fish products.
■, High-salted; ▲, Seasoned.

As shown in Table 2, the total cell count of the products varied from 10^4-10^9 cfu/ml, and most of the products had 10^5-10^6 cfu/ml. Among the 40 samples tested, 29 samples were appeared to contain *Staphylococcus* by forming colonies on mannitol salt agar. No significant correlation between total viable count and pH, NaCl concentration or water activity was found (Fig. 2). The same was true with the number of *Staphylococcus* in the sample (Fig. 3). This implies that salt concentration up to 25% was not effective in restricting the growth of halophilic bacteria in fermented fish products.

Isolation and Identification of the Genus of Gram-Positive Cocci

The gram-positive and catalase-positive cocci were isolated from 120 colonies grown on the mannitol salt agar of 40 fermented fish samples. A total of 80 colonies were selected as gram-positive and found to be cluster forming cocci under microscopic observation. They were catalase positive and oxidase positive, and did not show mobility. They could grow well on Baird Parker Agar and formed black colonies by reducing potassium tellurite. These properties were consistent with the characteristics of the genus *Staphylococcus* and *Micrococcus* according to

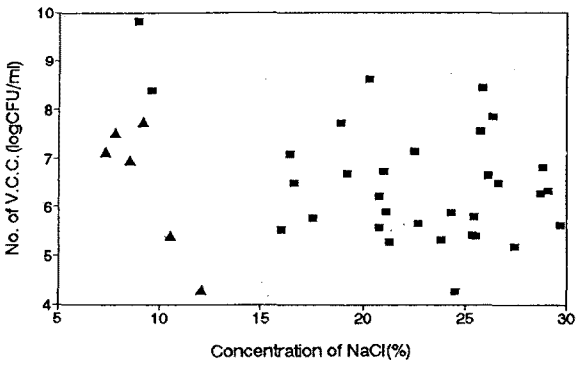

Fig. 3. Correlation between number of Staphylococci and salt content of fermented fish products.
■, High-salted; ▲, Seasoned.

Bergey's Manual.

Identification of *Staphylococcus* Species by Using API Staph and MIS Kits

The identification of *Staphylococcus* species of the isolated gram-positive cocci was investigated by using API Staph Strip and MIS Kits. Table 3 shows the species identified from the colonies from different fish products. Among the 35 colonies identified as *Staphylococcus*, *S. xylosus* was most frequent marking 17 samples, and *S. waneri* was 8, *S. epidermidis* 4 and *S. cohnii* 2. *S. hominis*, *S. saprophyticus*, *S. haemolyticus* and *S. aureus* were also identified once each in the test.

In case of O-jeot (K-3,fermented small shrimp) three different species, *S. waneri*, *S. xylosus* and *S. haemolyticus*, were found in one sample of fermented fish product. Two different species in one sample were found in cases of fermented small shrimp from Pusan (P-6), fermented clams from Kunsan (K-8), fermented big eyed herring from Seoul (G-5), and fermented and seasoned squid from Seoul (G-10), as shown in Table 3. Takai *et al.* (14) reported that in fermented squid containing 10% salt, 98% of the gram-positive microorganisms were *Micrococcus* species when the squid was mixed with salt, but the *Staphylococcus* species increased gradually, and after 4 days of fermentation 60% was *S. warneri* and no gram-positive rods were observed. Later *S. xylosus* became dominant making up 80% of the gram-positive cocci during the 7-15 days of fermentation, while *S. epidermidis* became the dominant flora during the later stage of fermentation. Cha *et al.* (5) identified *S. saprophyticus*, which had strong proteolytic activity, from a low-salt fermented anchovy.

Reviewing the data by region, among the 10 samples from Kunsan 5 were identified as *S. warneri*, while in other regions *S. xylosus* was predominant.

Sugar Utilization of the Identified *Staphylococcus*

All the identified strains could utilize glucose and fructose without gas formation, but could not utilize rhamnose, cellobiose, sorbitol and raffinose. The use of mannose, xylose, arabinose, sucrose, lactose, trehalose, mannitol, melobiose and saccharose varied with the strains. Discrepancies from Bergey's Manual were also observed. One strain of *S. warneri* (K-3-a) and *S. aureus* (G-11) could utilize mannose, but *S. saprophyticus* could not. Some strains of *S. xylosus* (K-3-a, K-8-b, P-2, P-3, P-7 and G-1) could not utilize arabinose, and *S. cohnii* could not utilize sucrose.

Table 3. Identification of *Staphylococcus* species isolated from fermented fish products at API STAPH and MIS.

Code number	Identified as
K-3-a	API STAPH 6332113 *S. warneri*, MIS: 73565060010, *S. warneri*
K-3-b	MIS 734750700300 *Staphylococcus xylosus*
K-3-c	API STAPH 6632111 *Staphylococcus haemolyticus*
K-4	API STAPH 6212112 *Staphylococcus hominis*
K-5	API STAPH 6332113 *Staphylococcus warneri*
K-7	API STAPH 6706112 *Staphylococcus epidermidis*
K-8-a	API STAPH 6232113 *Staphylococcus warneri*
K-8-b	API STAPH 6732652 *Staphylococcus xylosus*
K-9	API STAPH 6232113 *Staphylococcus warneri*
K-10	API STAPH 6232113 *Staphylococcus warneri*
P-1	API STAPH 6232113 *Staphylococcus Warneri*
P-2	API STAPH 6732452 *Staphylococcus xylosus*
P-3	API STAPH 6732452 *Staphylococcus xylosus*
P-4	API STAPH 6374140 *Staphylococcus cohnii*
P-6-a	API STAPH 6374142 *Staphylococcus cohnii*
P-6-b	API STAPH 6336452 *Staphylococcus xylosus*
P-7	API STAPH 6772152 *Staphylococcus xylosus*
P-10	API STAPH 6706112 *Staphylococcus epidermidis*
G-1	MIS: 73475070030 *Staphylococcus xylosus*
G-5-a	API STAPH 6334112 *Staphylococcus warneri*
G-5-b	API STAPH 6602013 *Staphylococcus epidermidis*
G-7	API STAPH 6232113 *Staphylococcus warneri*
G-8	API STAPH 6270150 *Staphylococcus saprophyticus*
G-9	API STAPH 6732452 *Staphylococcus xylosus*
G-10-a	API STAPH 6736452 *Staphylococcus xylosus*
G-10-b	API STAPH 6702112 *Staphylococcus epidermidis*
G-11	API STAPH 6736152 *Staphylococcus aureus*
G-12	API STAPH 6776452 *Staphylococcus xylosus*
G-13	API STAPH 6776452 *Staphylococcus xylosus*
G-14	API STAPH 6776452 *Staphylococcus xylosus*
J-1	API STAPH 6776452 *Staphylococcus xylosus*
S-1	MIS: 77475170470 *Staphylococcus xylosus*
S-2	API STAPH 6776452 *Staphylococcus xylosus*
SU-1	API STAPH 6736452 *Staphylococcus xylosus*
SU-2	API STAPH 6776452 *Staphylococcus xylosus*

Other Physiological Characteristics of the Identified Strains

Table 4 shows the physiological characteristics of the *Staphylococcus* species identified with API kits. All the strains showed positive in the fermentation and oxidation of paraffin oil. All strains except *S. hemolyticus*, *S. cohnii* and *S. Saprophyticus* showed positive in the urea

Table 4. Physiological characteristics of *Staphylococcus* species isolated from fermented fish products collected in Korea.

Code number	fermentation	oxidation	urease	nitrate reduction	VP test	citrate utilization	gelatin	NH_3 from arginine
K-3-a	+	+(w)	+	+	+	−	+	+
K-3-b	+	+(w)	+	+	+	−	−	−
K-3-c	+	+	−	+	+	−	−	+
K-4	+	+	+	+	+	−	−	−
K-5	+	+(w)	+	+	+	−	+	+
K-7	+	+	+	+	+	−	+	−
K-8-a	+	+	+	+	+	−	+	+
K-8-b	+	+(w)	+	+	+	−	−	−
K-9	+	+	+	+	+	−	−	+
K-10	+	+	+	+	+	−	−	+
P-1	+	+	+	+	+	−	+	+
P-2	+	+	+	+	+	−	−	−
P-3	+	+	+	+	+	−	−	−
P-4	+	+	−	−	+	−	+	−
P-6-a	+	+	−	+	+	−	−	−
P-6-b	+	+	+	+	+	−	+	−
P-7	+	+	+	+	+	−	+	−
P-10	+	+	+	+	+	−	−	−
G-1	+	+	+	+	+	−	+	−
G-5-a	+	+	+	−	+	−	−	−
G-5-b	+	+	+	+	+	−	+	−
G-7	+	+	+	+	+	−	−	+
G-8	+	+(w)	−	−	+	−	−	+
G-9	+	+(w)	+	+	+	−	−	−
G-10-a	+	+(w)	+	+	−	−	−	−
G-10-b	+	+	+	+	+	−	−	−
G-11	+	+	+	+	+	−	+	−
G-12	+	+	+	+	+	−	−	−
G-13	+	+	+	+	+	−	−	−
G-14	+	+	+	+	+	−	−	−
J-1	+	+	+	+	+	−	−	−
S-1	+	+	+	+	+	−	−	−
S-2	+	+	+	+	+	−	−	−
SU-1	+	+	+	+	+	−	−	−
SU-2	+	+	+	+	+	−	−	−

W: Weekly.

test. In the Voges-Proskauer test, all strains except some strains of *S. epidermidis* (G-5-b, G-10-b) produced acetoin. No strain in the list could utilize citrate. *S. cohnii*, *S. aureus* and some strains of *S. warneri* (K-3-a, K-5,G-5-b), *xylosus* (P-7, G-1) and *epidermidis* (K-7) could liquify gelatin, and all strains except for P-4, G-5-a and G-8 could reduce nitrate to nitrite. Some strains of *S. warneri* (K-3-a, K-5), *hemolyticus* (K-3-c) and *saprophyticus* produced ammonia from arginine. The physiological characteristics of the identified strains were generally consistent with those of Bergey's Manual.

The salt tolerance test showed that all the strains identified could grow well in media containing up to 18% salt, and some strains (K-3-a, K-4, K-5, K-7, P-6-b, P-10, G-5-b and G-10-a) could grow at 23% salt concentration. Some strains of *S. warneri* (K-5), *S. epidermidis* (K-7) and *S. xylosus* (P-6-b) could grow at 25% salt concentration. The same strains identified by using API Staph Strip and MIS Kits showed different salt tolerances, indicating the influence of the habitat environment. The salt tolerance of the strains of *Staphylococcus* exhibited in this study is much higher than those listed in Bergey's Manual and other reports (15).

All the strains except *S. aureus* (G-11) showed negative in hemolysis activity, plasma coagulation and DNase test. However, all the strains including *S. aureus* (G-11) showed negative in the enterotoxin test using SET-RPLA (*Staphylococcus enterotoxin* A,B,C,D detection kit by reserved passive latex agglutination), blood hemolysis test, plasma coagulase test and DNase test. Bautista *et al.* (2) reported that *S. cohnii*, *S. epidermidis*, *S. hemolyticus*, *S. xylosus* isolated from sheep milk were

coagulase negative but showed a positive reaction in enterotoxin production. The enterotoxin negative nature of the high salt tolerant *Staphylococcus* strains in fermented fish products was noted in this study. The mechanisms for these physiological changes need to be studied further.

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