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Cultivable Bacterial Community Analysis of Saeu-jeotgal, a Korean High-Salt-fermented Seafood, during Ripening

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To determine the dominant bacterial species during the Saeu-jeotgal ripening process, the cultivable bacterial population was examined over a 135-day period using six different growth media. The greatest numbers of bacteria were identified when marine agar was used for culture, with maximum cell density identified at day 65 (2.51×10^7 colony forming units/g). Over the course of 135 days, the bacterial diversity was analyzed eight times. A total of 467 isolates, comprising 87 species from 42 genera, as well as 16 isolates belonging to previously unknown species, were identified. The number of species detected decreased from 39 at day 1 to 13 at day 135. The order of dominance at the genus level was as follows: *Staphylococcus*, *Salimicrobium*, *Kocuria*, and *Psychrobacter*. *Staphylococcus* and *Salimicrobium* accounted for 2% of the diversity at day 1, and then increased to 39% and 36%, respectively, at day 135. The dominant species *Staphylococcus equorum*, *Salimicrobium salexigens*, and *Kocuria palustris* accounted for 23.6%, 16.1%, and 10.9% of all isolates, respectively. Importantly, both *St. equorum* and *Sm. salexigens* remained viable at a NaCl concentration of 21% (w/v), which indicates their strong involvement in the ripening of Saeu-jeotgal.

Keywords: **High-salt fermentation, jeotgal, *Kocuria*, *Salimicrobium*, *Staphylococcus***

Introduction

Jeotgal is the generic term given to traditional Korean high-salt seafoods. These products are used as an additive for improving the taste of other foods, or eaten alone as a side dish, providing saltiness and nutrition to steamed rice. Jeotgal is made by adding up to 30% (w/w) sea salt to various types of seafood, and becomes palatable through subsequent ripening. During the >1-year ripening period, jeotgal attains rich flavors and unique physical and chemical structures through autolytic and microbial proteolytic processes.

In the typical production of jeotgal as fish sauce in Southeast Asian countries, which may take up to 12–18 months, no control measures are implemented, which sometimes results in inconsistent product quality. Therefore, acceleration of the ripening process is a major research focus, not only to improve quality but also reduce costs. Many attempts have been made to accelerate the fish sauce ripening process by reducing salt content, lowering pH, and elevating the temperature [12, 26]. The addition of proteases from plants and fish intestines has been relatively successful in accelerating the ripening process [5, 11, 23]. However, the proteases from plants did not produce the traditional aromatic flavors, and the activity of proteases from fish intestines declined gradually during ripening because of the high salt content and acidic pH [36].

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The natural microflora found in fish sauce is an important source of proteases. Moderately halophilic bacteria, which grow well at relatively high salt concentrations (3–15% NaCl), were isolated from fish sauces, and their proteases have been studied with the aim of accelerating protein hydrolysis at high salt concentrations and subsequently reducing ripening time [15, 33-35]. Starter cultures of moderately halophilic *Virgibacillus* and *Staphylococcus* species were successfully used to accelerate fish sauce ripening in combination with commercial proteases [41]. In addition, the halophilic archaeon *Halobacterium* sp. SP1(1), which grows optimally at 25% NaCl and produces a protease that is stable at high salt concentrations, has come to the forefront as a potential starter to accelerate the fish sauce manufacturing process [2]. The halophilic lactic acid bacterium *Tetragenococcus halophilus* is also used as a starter culture, and is reported to improve the flavor of fish sauce [39].

Although these bacterial additives are somewhat successful in accelerating the ripening of fish sauce, they were selected solely on the basis of physiological characteristics such as proteolytic activity and high-salt tolerance, not on an understanding of the microbiota and population dynamics during ripening. A better understanding of the microbial ecology will enhance the performance of bacterial additives, and will contribute to the manufacturing process, safety, and standardization of products.

Starter cultures are defined as preparations containing living or resting forms of microorganisms that have a desired metabolic activity in the fermentation of a substrate. General requirements for starter cultures are technological effectiveness, safety, and economics, with probiotic features now also included [3]. To obtain starter candidates that could be used to accelerate the ripening and standardize the quality of Saeu-jeotgal, one of the most commonly consumed types of jeotgal, which is made from tiny sea shrimp (*Acetes japonicus*), we analyzed the cultivable bacterial community during ripening, and determined the dominant species based on their salt tolerance.

Materials and Methods

Saeu-jeotgal sample

Fresh tiny sea shrimp (*Acetes japonicus*) caught off the west coast of the Korean Peninsula and sea salt produced in Korea were purchased from Sorae Harbor, Incheon, Korea, in July 2011. To make Saeu-jeotgal, sea salt was added to the shrimp to a final NaCl concentration of 24% (w/w). Three kilograms of sample packed in a 5-L capacity plastic jar were stored at 15℃ and sampled at days 1, 5, 10, 15, 25, 35, 65, and 135.

Bacteria isolation

At each time point, 150 g of sample were ground using an IKA Ultra-Turrax homogenizer T-18 (IKA, Germany) and then filtered through sterilized gauze. The NaCl content was measured according to the Mohr method [4], and the pH was measured using a pH meter. The filtrates were then spread on agar plates after appropriate dilutions in saline. Six types of agar media were used in the current study: marine agar (MA), MRS agar (MRSA), and nutrient agar (NA), and then MA, MRSA, and NA supplemented with NaCl (6% (w/w) final concentration) (MAS, MRSAS, and NAS). MA, MRSA, and NA were purchased from Difco (USA). All agar plates were incubated at 30℃. Colonies on MA, MRSA, and NA were counted following 24-h incubation, and those on MAS, MRSAS, and NAS were counted after 3 days. Typically, 10 different types of colonies were collected from each plate based on differences in their morphology, growth characteristics, and the numbers of colonies per plate. The collected colonies were purified by successive transfer on their isolated agar medium.

Identification of the isolates by 16S rRNA gene sequence analysis

Genomic DNA was extracted from each of the isolates using a DNeasy tissue kit (Qiagen, Germany). The 16S rRNA gene was amplified using universal primers 27F and 1492R [19] in a T3000 Thermocycler (Biometra, Germany). The PCR mixture consisted of the template DNA, 0.5 μM of each primer, 1 U of *Taq* polymerase (Roche, Germany), 200 μM dNTPs, and 1.5 mM $MgCl_2$. Thermal cycler parameters consisted of 95℃ for 5 min, followed by 30 cycles of 95℃ for 1 min, 65℃ for 1 min, and 72℃ for 1 min. The resulting amplicons were purified using a gel and PCR purification system kit (SolGent, Korea), and sequenced by SolGent using the primer sets used for their amplification. The 16S rRNA gene sequences were identified using BLAST analysis

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against the GenBank database (http://blast.ncbi.nlm.nih.gov/). The identified 16S rRNA gene sequences were then aligned with those of bacteria from neighboring taxa based on secondary structure information using the PHYDIT program (available at http://plaza.snu.ac.kr/~jchun/phydit/). Evolutionary distances were calculated according to the Kimura two-parameter model [22], and clustering was performed using the neighbor-joining method [32]. A phylogenetic tree was generated using a treeing algorithm contained within the PHYDIT program.

Evaluation of salt tolerance

Salt tolerance of the predominant species was determined by examining growth on MA and NA supplemented with NaCl at concentrations up to 24% (w/v). Growth on NaCl-supplemented agar media was observed at 2, 6, and 10 days post-incubation at 30℃. Ten randomly selected strains from each of the species were tested, and all of the experiments were conducted three times on separate days using fresh cultures. Tolerance was defined as the maximum concentration of NaCl where more than six strains from each species formed colonies.

Results

Changes in NaCl concentration and pH during ripening of Saeu-jeotgal

In a microbiological safety assessment of 36 commercial Saeu-jeotgal products available in Korea, the mean salt concentration of the products was measured as 24% (w/w) [30]. We therefore added sea salt to the shrimp at a concentration of 24% (w/w), taking into consideration the moisture content of the raw materials. It took 15 days for the salt to completely dissolve, and a NaCl concentration of approximately 24% was maintained throughout the experimental period (Table S1). In line with other Saeu-jeotgal products in Korea [40], the pH of the fresh-made sample was approximately 7, and was maintained for the entire 135 days (Table S1).

Growth of bacteria during ripening

Average bacterial numbers across the six media types were 2.51×10^5 colony forming units (CFU)/g at day 1, and dropped to an average of 6.31×10^4 CFU/g after 5 days of storage (Fig. 1). Cell numbers recovered gradually until day 35, but did not achieve the levels observed on day 1, except on MA and MAS. The timing of maximum recovery differed according to media. The cell numbers detected on MA and MAS exceeded those of day 1 after 25 days of storage, and increased to 2.51×10^7 CFU/g at day 65, before decreasing to 1.58×10^6 CFU/g at day 135. Cell numbers on MRSA, MRSAS, NA, and NAS during ripening were similar to those determined in earlier studies [16, 25].

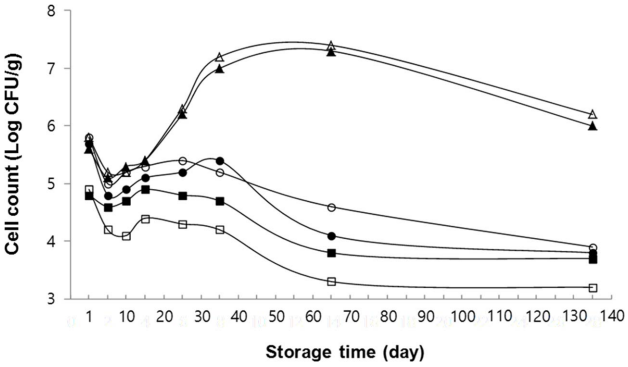

Fig. 1. Viable cell counts in Saeu-jeotgal during ripening on six types of media. Media: marine agar (△), marine agar containing 6% NaCl (▲), nutrient agar (○), nutrient agar containing 6% NaCl (●), MRS agar (□), and MRS agar containing 6% NaCl (■). The results are the average values of three replicates.

Bacterial diversity

Typically, the bacterial population present in the initial stages of production of fermented foods reflects that of the raw materials, and has a higher diversity than the later stages. We therefore used shorter sampling intervals in the initial stages than in the late stage to detect the potentially larger bacterial diversity. The isolates identified from each medium were diverse at both the species and genus level, with notable differences according to media type (Tables S2–S4). The addition of NaCl to the three base media contributed to the diversity, but did not have as much of an impact as medium type.

Using the MA medium, 80 isolates belonging to 26 species in 19 genera were identified, while 84 isolates belonging to 29 species in 21 genera were identified from the MAS medium (Table S2). In total, 80 isolates were identified from the MRSA plates, corresponding to 23 species in 16 genera. The 74 isolates identified from the MRSAS medium were identified as 19 species in 14 gen-

Table 1. Number of isolates identified from Saeu-jeotgal during ripening, summarized at the genus level.

Class	Genus	Storage time (days)								
		1	5	10	15	25	35	65	135	Total
α-Proteobacteria	*Methylobacterium*				1			1		2
	Paracoccus		1							1
γ-Proteobacteria	*Acinetobacter*	1								1
	Idiomarina		1							1
	Marinobacter					1				1
	Psychrobacter	7	6	5	6	7	1	1	5	38
	Serratia	1								1
	Vibrio	2								2
Bacilli	*Aerococcus*		1	1	2	1				5
	Alkalibacillus							1	1	2
	Bacillus		1	1				1	1	4
	Carnobacterium	1								1
	Enterococcus		1							1
	Exiguobacterium	2					2	1		5
	Jeotgalicoccus		7	3	1	1			1	13
	Granulicatella	1(1)		1(1)	2(2)	2(2)				6(6)
	Kurthia	1								1
	Leuconostoc						2			2
	Macrococcus		2							2
	Oceanobacillus		1		1			1	1	4
	Planococcus	3	2	1	1					7
	Planomicrobium	1								1
	Salimicrobium	1	1		2	9	20	22	20	75
	Salinicoccus	6	3	3	2	1	1		1	17
	Staphylococcus	1	6	14	15	27	16	16	22	117
	Streptococcus		1							1
	Vagococcus	3	3	2	3					11
	Trichococcus	1(1)	1(1)	1(1)		1(1)				4(4)
Actinobacteria	*Actinomyces*		1(1)	2(2)	1(1)					4(4)
	Agrococcus	2	1	2	2					7
	Arthrobacter	3	1							4
	Brachybacterium	2	1	4	2	4	2		1	16
	Brevibacterium	1								1
	Citricoccus	1								1
	Corynebacterium	3	2	4			1	1		11
	Dietzia	1	3	1	1					6
	Janibacter		2							2
	Kocuria	3	5	8	8	4	11	16	3	58
	Trueperella	1(1)								1(1)
	Kytococcus		2	1	1		2			6
	Leucobacter	1(1)		1	2					4(1)
	Microbacterium	1	2	2	1	3	1	1		11
	Micrococcus	2								2
	Ornithinimicrobium				1					1
	Rhodococcus				1					1
	Rothia	1	1	3						5
Total		54	59	60	56	61	59	62	56	467

The numbers of potentially novel unidentified species are indicated in parentheses (with <97% 16S rRNA gene similarities to any known species).

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Table 2. Effect of NaCl on the growth of predominant species isolated from Saeu-jeotgal.

NaCl concentration (w/v)	*Staphylococcus equorum*						*Salimicrobium salexigens*						*Kocuria palustris*					
	MA (days)			NA (days)			MA (days)			NA (days)			MA (days)			NA (days)		
	2	6	10	2	6	10	2	6	10	2	6	10	2	6	10	2	6	10
12%	+	++	++	+	+++	+++	+	++	+++	+	+++	+++	W	+	+	+	+	++
15%	+	+	++	+	++	+++	+	++	++	+	++	++	–	W	W	W	+	+
18%	W	W	+	W	+	++	+	+	++	+	+	++	–	–	–	–	–	–
21%	–	–	–	–	W	+	W	+	+	W	+	++	–	–	–	–	–	–
24%	–	–	–	–	–	–	–	W	+	W	+	+	–	–	–	–	–	–

Abbreviations: MA, marine agar; NA, nutrient agar; +, positive growth; –, negative growth; W, weak growth.
The final concentration of NaCl in each medium is indicated, and the relative size of each colony is expressed by the number of symbols (+).

era (Table S3). The 77 isolates from the NA plates belonged to 33 species in 20 genera, and 72 isolates from the NAS medium belonged to 31 species in 19 genera (Table S4). Over the course of the 135-day ripening, bacterial diversity was analyzed eight times, and a total of 467 isolates were identified. Among the isolates, 16 belonged to previously unknown species, and 87 species from 42 genera were identified (Table 1, Fig. 2).

The bacterial diversity on each medium decreased as the total cell number increased over the ripening period. After day 15, species belonging to the genus *Staphylococcus* were predominant on MRSA and MRSAS, followed by *Kocuria* species (Table S3). The highest level of species diversity was observed on NA medium, although *Staphylococcus* and *Kocuria* species were also dominant on this medium (Table S4). Most of the *Staphylococcus* and *Kocuria* isolates were identified as *St. equorum* and *K. palustris*, respectively (Fig. 2). *Psychrobacter* strains were predominantly isolated on the NAS medium. The most populous isolate on MA and MAS after day 35 was *Salimicrobium salexigens* (Table S2, Fig. 2).

Changes in the bacterial community structure of Saeu-jeotgal during ripening

Bacterial community analysis results obtained from the cultures on the six growth media were combined and summarized at the genus level (Table 1). The proportions of each genus in the populations at each sampling time are presented in Fig. 3. The genera for which less than 10 strains were isolated across the entire sampling period were designated as "other", and comprised 43% of the total number of isolates on day 1, but decreased to only 5.4% of the population at day 135. Species with similarities of <97% with the closest type strains were designated as "unidentified bacteria" and were not isolated after day 25. While 39 species were detected at day 1, only 13 species were identified at day 135. *Staphylococcus* species were dominant from day 10, and *Sm. salexigens*, the only species identified from the genus *Salimicrobium*, increased dramatically after day 25. *Staphylococcus* and *Salimicrobium* isolates accounted for 2% of the total population at day 1, but increased to 39% and 36% of the population, respectively, at day 135. Overall, bacteria belonging to the genera *Kocuria*, *Psychrobacter*, and *Staphylococcus* were isolated across the entire ripening period, and the order of dominance at the genus level was *Staphylococcus*, *Salimicrobium*, *Kocuria*, and *Psychrobacter* (Table 1, Fig. 3). The dominant species were *St. equorum*, *Sm. salexigens*, and *K. palustris*, which accounted for 23.6%, 16.1%, and 10.9% of all isolates, respectively (Fig. 2).

Salt tolerance of the predominant species

To confirm the involvement of *St. equorum*, *Sm. salexigens*, and *K. palustris* in the Saeu-jeotgal ripening process, the growth of these strains was analyzed on MA and NA medium supplemented with 12–24% NaCl (Table 2). Salt tolerance was increased in NA compared with MA. *K. palustris* exhibited growth on media containing a maximum of 15% NaCl, which does not support its contribution to Saeu-jeotgal ripening. However, *St. equorum* and *Sm. salexigens* grew in the presence of 21% and 24% NaCl, respectively.

Discussion

Fermentation is defined as the anaerobic catabolism of organic compounds, generally carbohydrates, in the

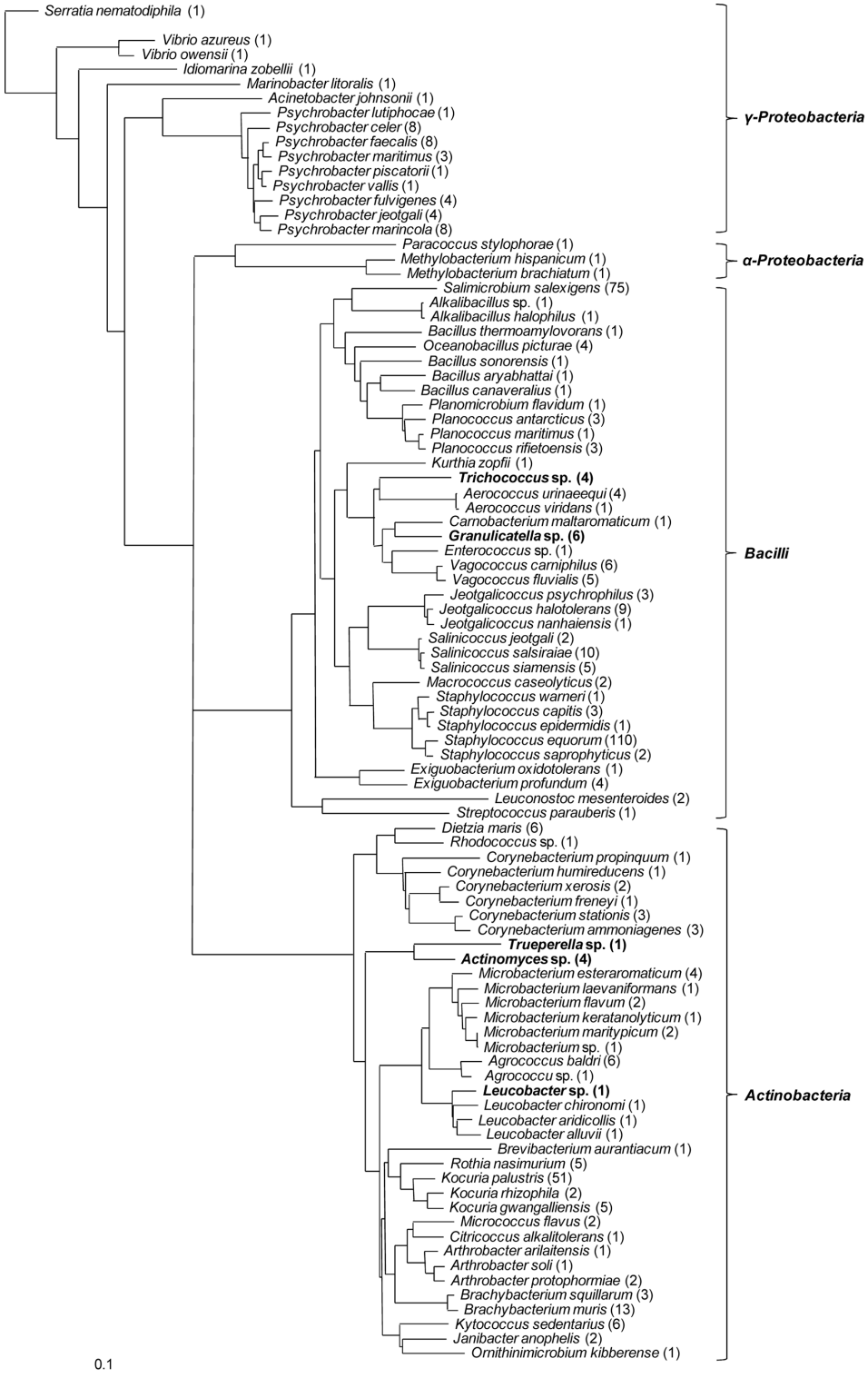

Fig. 2. Phylogenetic tree of the isolates from Saeu-jeotgal showing the relationships based on the determination of the near-complete 16S rRNA gene sequences (1407 nt). The numbers in parentheses indicate the number of isolates identified as the same species. Novel unidentified species showing similarities of <97% with the closest type strain are indicated in bold letters. The bar indicates the number of nucleotide substitutions per site.

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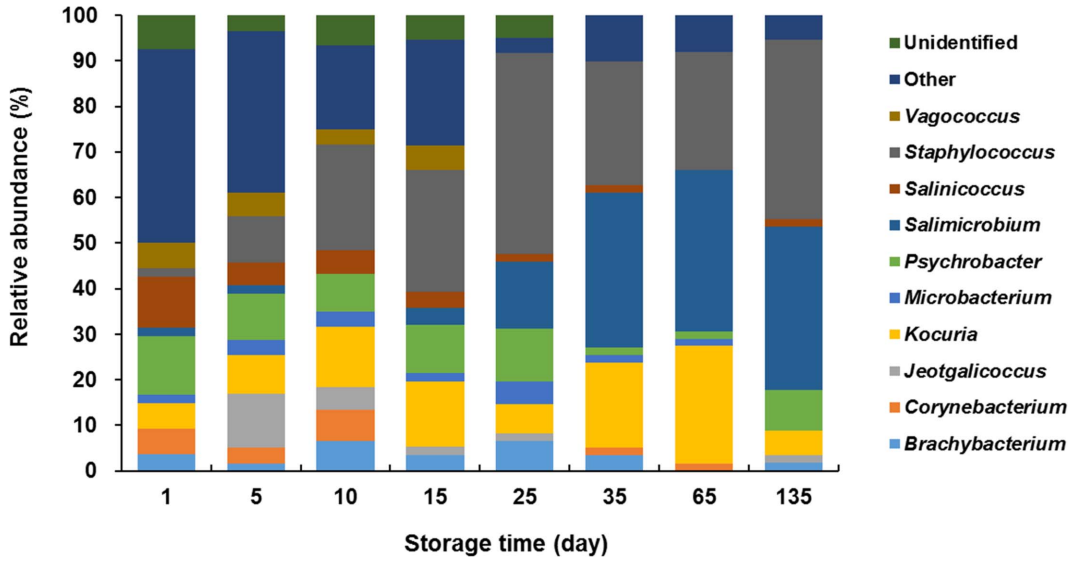

Fig. 3. Cultivable bacterial community shift during the ripening of Saeu-jeotgal.

absence of an external electron acceptor, but is also used much more broadly to refer to the bulk growth of microorganisms on a growth medium. Numerous food products owe their production and characteristics to fermentation. In the case of jeotgal, high-salt concentrations and low cell numbers (approximately 10^5 CFU/g) have raised questions about the occurrence of fermentation over the long ripening time, although physical and chemical alterations of raw materials occur during ripening. In the current study, the increase in viable cells on MA and MAS over the first 65 days suggests the involvement of bacteria in Saeu-jeotgal production (Fig. 1). As far as we know, this is the first report to identify an increase in the viable cell numbers during the ripening process which insinuates the occurrence of fermentation in broad definition. Additionally, we found that the predominant species, *St. equorum* and *Sm. salexigens*, maintained their viability at NaCl concentrations of >20% (w/v), although growth rates were very slow (Table 2). Therefore, the involvement of these bacteria in the ripening of Saeu-jeotgal is strongly implicated by the current study.

Culture-independent microbial community analysis techniques allow the examination of microbial complexity in an ecosystem, and circumvent the limitations associated with traditional culture-dependent methods. Therefore, culture-based approaches are rarely now applied in microbial community analyses. To overcome the drawbacks associated with limited culture conditions, we randomly isolated large numbers of strains using several culture media. In our previous work, amongst 295 isolates from a Saeu-jeotgal sample, we identified 53 species belonging to 30 genera [13], while in the current study we identified 87 species in 42 genera from 467 isolates (Table 1, Fig. 2). This number of identified genera was far greater than that determined by pyrosequencing [20], or by denaturing gradient gel electrophoresis (DGGE) analysis [14]. In addition, the identification of strains at the species-level was accomplished by the current study which is not practically attainable by culture-independent techniques.

The results of the current study were compared with both previous culture-independent studies of Saeu-jeotgal fermentation to identify the dominant species and bacterial succession during fermentation. Fortunately, all three studies used samples ripened at approximately 15°C, which is the average storage temperature of the high quality Saeu-jeotgal produced in the Kwangchun area [21]. At the phylum level, both culture-independent studies identified Proteobacteria and Firmicutes as the predominant phyla [14, 20]. Interestingly, neither pyrosequencing nor DGGE analysis identified Actinobacteria among the dominant phyla, while the current study identified 18 genera belonging to Actinobacteria, which outnumbered the genera belonging to Proteobacteria (eight genera) (Table 1). Among the genera of the

phylum Actinobacteria, a large number of isolates belonging to the genus *Kocuria* (58 isolates) were identified, and were isolated across all sampling points. Actinobacterial species were also identified from a commercial Saeu-jeotgal sample in our previous study [13]. Therefore, species belonging to the phylum Actinobacteria are likely to be present in Saeu-jeotgal samples.

Staphylococcus isolates were predominant in the current study, followed by *Salimicrobium* species. Although the dominance of these genera has been identified using other community analysis methods, the relative proportions of the two genera over the ripening period differs amongst the studies. In the current study, in line with the pyrosequencing analysis, *Staphylococcus* species were predominant early in the ripening process, and were then overtaken by *Salimicrobium* species in terms of relative abundance.

An anaerobic species belonging to the genus *Halanaerobium* was identified by both previous culture-independent studies of Saeu-jeotgal fermentation and prevailed after the middle stages of fermentation. *Halanaerobium* species have been isolated from hypersaline niches with NaCl concentrations of >20%, and have exhibited growth in media with NaCl concentrations of up to 30% [1, 6, 24]. The anaerobic conditions developed by static storage in a large volume container, along with the high salt concentrations, might favor the growth of this genus after the middle stages of Saeu-jeotgal fermentation.

The genus *Alkalibacillus* appeared after the sequential dominance of the genera *Staphylococcus* and *Salimicrobium* according to the pyrosequencing analysis, and then gradually decreased. This decrease coincided with the increase of the genus *Halanaerobium*, as occurred with *Staphylococcus* and *Salimicrobium*. We isolated two strains of the genus *Alkalibacillus* from the late stage of fermentation. The genus *Alkalibacillus* is phylogenetically very close to the genus *Salimicrobium* and show growth at high salt concentrations (25% NaCl (w/v)) [18, 38].

The salt tolerance of the species belonging to the genera *Staphylococcus*, *Salimicrobium*, and *Alkalibacillus* allows them to maintain their growth in the high salt environment of Saeu-jeotgal. Additionally, the maintenance of neutral pH during fermentation likely contributes to their proliferation until the late stages of fermentation. Nitrogen compounds such as amines and amino acids produced from protein degradation during ripening of Saeu-jeotgal might neutralize organic acids produced by fermentation, and calcium ions dissociated from the shrimp shell during ripening may be an additional factor in maintaining Saeu-jeotgal at neutral pH [10]. Sequential dominance of the *Staphylococcus*, *Salimicrobium*, and *Alkalibacillus* genera might reflect growth rate differences during Saeu-jeotgal ripening. For example, the low frequency of isolation of *Alkalibacillus* species using this culture method might reflect the lower growth rate of *Alkalibacillus* species on MAS medium compared with other species.

The current strategy of bacterial isolation from Saeu-jeotgal was effective in identifying a broad spectrum of bacterial species, but could not provide a good picture of the anaerobic bacterial community. In this instance, culture-independent analysis is more effective. However, the current study confirmed the presence of species in the phylum Actinobacteria, which were not identified by culture-independent studies. Culture-independent studies can introduce bias by selective extraction of nucleic acids or by selective amplification of 16S rRNA genes, which can distort the picture of the bacterial community, as is the case in culture-dependent methods. The first pyrosequencing study of Saeu-jeotgal reported the dominance of the genera *Lactobacillus* and *Weissella* [31]. Considering the salt concentration of Saeu-jeotgal, this result cannot reflect the true abundance of these species in the sample. In contrast, the dominance of the genera *Staphylococcus*, *Salimicrobium*, *Alkalibacillus*, and *Halanaerobium* is well explained by their physiological characteristics of salt tolerance and optimum growth at neutral pH.

Strains in the genera *Kocuria* and *Staphylococcus* were isolated from six media, while *Salimicrobium* strains were mainly isolated from MA and MAS. Nutritionally, *Kocuria* and *Staphylococcus* may not be more fastidious than *Salimicrobium* which may require the minerals in sea water. The lower growth rate of *Salimicrobium* on MRSA, MRSAS, NA, and NAS than *Kocuria* and *Staphylococcus* may be another reason for the low frequency isolation on the media.

Although species of facultatively anaerobic, salt-tolerant genera *Salimicrobium* and *Alkalibacillus* could be applied for use as starter cultures in Saeu-jeotgal fer-

mentation, members of the genus *Staphylococcus* are also convincing starter candidates because they have previously been used for food fermentation. The *Staphylococcus* species isolated from Saeu-jeotgal in the current study, *St. equorum*, was negative for coagulase production, and is rarely pathogenic. In addition, coagulase-negative staphylococci (CNS) are commonly isolated from naturally-fermented meat products and cheese, and have a positive impact on fermentation processes and the sensory characteristics of foods [17]. Thus, CNS have successfully been used as meat and cheese starter cultures [17, 29, 37]. The presence of *St. equorum* in fermented foods has traditionally been under-estimated because it is often confused with *Staphylococcus xylosus* by conventional identification methods [7, 28]. More recent molecular methods for the identification of CNS have confirmed the significance of *St. equorum* in fermented foods, especially meat products and cheese [7–9, 27]. A history of use in food fermentation will help to implement *St. equorum* strains in the jeotgal industry, but further proof of their safety is required.

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국문초록

배양법을 이용한 새우젓갈 숙성과정 중 박테리아상 분석

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6종류의 배지를 이용한 135일 동안 8번의 박테리아상 분석을 통하여 새우젓갈 숙성 관련 우점종을 결정하였다. Marine agar를 배양에 사용하였을 경우, 가장 많은 수의 박테리아가 검출되었고, 숙성 65일차에 그 수가 2.51×10^7 CFU/g에 달하였다. 순수분리된 총 467균주는 42속 87종으로 동정되었고, 16균주는 기존의 알려진 박테리아와 상동성을 나타내지 않았다. 1일차 분석에서 39종이 검출되었던 박테리아는 135일차에 13종으로 감소하여 우점화가 진행됨을 확인하였다. 속 수준에서의 우점은 *Staphylococcus*, *Salimicrobium*, *Kocuria*, *Psychrobacter* 순으로 나타났고, 1일차 분리균주의 2% 수준을 차지하던 *Staphylococcus*, *Salimicrobium* 속 균주는 135일차에 각각 39%와 36%에 달하였다. 우점종으로 확인된 *Staphylococcus equorum*, *Salimicrobium salexigens*, *Kocuria palustris*는 각각 전체 분리균주의 23.6%, 16.1%, 10.9%을 차지하였다. *St. equorum*와 *Sm. salexigens*는 21% (w/v) NaCl 농도에서의 생육이 확인되어 새우젓갈 숙성에 중요한 역할을 할 것으로 추정된다.

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