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Minireview

Diversity of Halophilic Archaea in Fermented Foods and Human Intestines and Their Application

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Archaea are prokaryotic organisms distinct from bacteria in the structural and molecular biological sense, and these microorganisms are known to thrive mostly at extreme environments. In particular, most studies on halophilic archaea have been focused on environmental and ecological researches. However, new species of halophilic archaea are being isolated and identified from high salt-fermented foods consumed by humans, and it has been found that various types of halophilic archaea exist in food products by culture-independent molecular biological methods. In addition, even if the numbers are not quite high, DNAs of various halophilic archaea are being detected in human intestines and much interest is given to their possible roles. This review aims to summarize the types and characteristics of halophilic archaea reported to be present in foods and human intestines and to discuss their application as well.

Keywords: Halophilic archaea, fermented foods, microbiome, human intestine, *Halorubrum*

Introduction

Archaea refer to prokaryotes that used to be categorized as archaeabacteria, a type of bacteria, in the past. After Carl Woose's pioneering classification by phylogenetic taxonomy of the 16S ribosomal RNA [63], they are considered as an independent domain rather than being categorized as bacteria because the metabolism of archaea related to central dogma is closer to that of eukaryotes and the structure of the cell wall is significantly different from bacteria. Until now, archaea have been classified into 5 phyla (*Crenarchaeota*, *Euryarchaeota*, *Korarchaeota*, *Nanoarchaeota*, and *Thaumarchaeota*) based on the phylogenetic method [10], and they are sometimes categorized as thermophilic, halophilic, and methanogenic archaea according to the growth properties of the strains. Archaea are sometimes referred to as extremophiles because an "extreme" environment is needed for the growth of these strains or because such optimal growth often takes place in an extreme environment. However, not all archaea are extremophiles, and there are other species of bacteria or eukaryotes that grow in an extreme environment as well.

Depending on the optimal salt concentration needed for the growth of strains, halophilic microorganisms can be classified as halotolerant (~0.3 M), halophilic (0.2~2.0 M), and highly halophilic (3.0~5.0 M) [29], and most of halophilic archaea belong to the highly halophilic category. Among the highly halophilic archaea, those that especially need a minimum salt concentration of 1.5 M (9% w/v) to 2.5 M (15% w/v) are referred to as extremely halophilic archaea or haloarchaea, in general [11].

Halophilic archaea have been classified into a single family of the *Halobacteriaceae* in which 40 genera and more than 150 species are included [35]. Most of them are known to live in environments like salt lakes or salterns with very high salt concentrations. However, as new halophilic archaeal strains have been continuously identified by screening methods utilizing several new compositions of culture media, it has recently been reported that halophilic archaea exist in salted food products or fermented foods as well. In addition, people have become aware that halophilic archaea exist much closer to the surrounding human environments, as metagenomic analyzing methods are developed. Moreover, some new techniques that are able to detect very small amounts of DNA have also brought awareness about the existence of halophilic archaea in the intestines of animals. Accordingly, this study aims to understand the types and characteristics of halophilic archaea that have been reported to be present in food products and animal intestines, which are gradually receiving more attention, and also to discuss their applications.

Halophilic Archaea in Foods Identified by Culture-Dependent Methods

Diverse types of salted foods or high salt-fermented foods exist worldwide because salt has been used for a very long time to preserve food. Although salt had been known only to restrain the growth of food-poisoning bacteria and to promote the growth of fermentating bacteria such as yeast and lactic acid bacteria, new types of halophilic archaea are recently being identified in fermented food products with high salt concentration (Table 1). The existence of halophilic archaea in salted foods may be due to salterns being one of the important environments for their growth, so salt produced from these salterns would contain haloarchaea [23, 53, 55]. Among fermented foods, new types of halophilic archaea have been discovered in fermented fish and fish sauces that require a large amount of salt, and such studies are being conducted mostly in Korea, Japan, and Southeast Asian countries, despite the fact that various types of fermented fish and shellfish products are present worldwide.

Halophilic Archaea in Korean Foods

It is the predominant view that an excessive sodium intake is having a negative influence on the health of the Korean population, since a Korean's daily salt consumption is approximately 13 g [20], which is more than twice the recommended dietary intake of WHO. In addition, while one of the major causes of death in Korea is cancer, some evidence suggests that the higher rate of gastric cancer, as opposed to other cancers, is related to high sodium consumption [2, 22]. Among Korean traditional foods, there are many high salt-fermented foods that include soy-fermented products, such as soy sauce and soybean paste; and fermented seafoods, such as *jeotgal* (fermented small fish or shellfish) and *sikhae* (fermented fish with salt and starchy grains). Specifically, *jeotgal*, Korea's very own condiment, contains the highest concentration of salt (more than 20% (w/v) NaCl) among high salt-fermented foods, and 164 types of *jeotgals* are being made nationwide [18].

Halophilic archaea isolated from Korean foods using culture-dependent methods include *Haladaptatus cibarius*

Table 1. Halophilic archaea isolated from fermented foods and salts.

Strain name	Source food	Country	NaCl range	Optimal NaCl conc.	Optimal temperature (°C)	Optimal pH	Reference
Haladaptatus cibarius	Fermented shellfish	Korea	10-30%	15%	37	7.0	[43]
Halalkalicoccus jeotgali	Shrimp *jeotgal*	Korea	10-30%	15%	37-45	7.0	[47]
Halarchaeum acidiphilum	Commercial salt	Japan	18-31%	21-24%	37	4.4-4.5	[27]
Haloacular tradensis	Thai fish sauce	Thailand	At least 15%	20~30%	37	7.0	[32]
Haloarcula salaria	Thai fish sauce	Thailand	At least 15%	20~25%	37	7.0	[32]
Halobacterium salinarum (piscisalsi)	Fermented fish (*pla-ra*)	Thailand	At least 2.6 M	3.4~4.3 M	37~40	7.0~7.5	[28, 64]
Halobaculum magnesiiphilum	Commercial salt	Japan	6-30%	9-12%[a]	45	6.5	[55]
Halococcus thailandensis	Thai fish sauce	Thailand	At least 15%	20~30%	37	6-8	[33]
Halogranum salarium	Sea salt	Korea	1.3 - 5.1 M	2.6-3.4 M	37	7.0	[23]
Halorubrum cibi	Shrimp *jeotgal*	Korea	15-30%	23~25%	30~37	7.5	[42]
Halostagnicola alkaliphila	Commercial rock salt	Japan	20-30%	25%	37	9.0	[30]
Haloterrigena jeotgali	Shrimp *jeotgal*	Korea	10-30%	15~20%	37~45	7.0~7.5	[45]
Natrinema gari	Fish sauce (*nam-pla*)	Thailand	At least 1.7 M	2.6~3.4 M	37~40	6.0~6.5	[59]
Natronoarchaeum mannanilyticum	Commercial salt	Japan	At least 2 M	4.0-4.5 M	37	8.0-9.0	[54]
Natronococcus jeotgali	Shrimp *jeotgal*	Korea	7.5-30 %	23~25%	37~45	7.5	[46]
Salarchaeum japonicum	Commercial salt	Japan	1.5-5.3 M	2.5-3.0 M	40	5.2-6.3	[53]

[a]The strain is magnesium-dependent, and the optimal NaCl concentration is in the presence of 2% (w/v) $MgCl_2 \cdot 6H_2O$.

[44], *Halalkalicoccus jeotgali* [47], *Halorubrum cibi* [42], *Haloterrigena jeotgali* [45], and *Natronococcus jeotgali* [46] from various types of *jeotgals*, and also *Halogranum salarium* [23] from bay salt. Their characteristics are shown in Table 1. Whereas most halophilic archaea are extreme halophiles that grow in salt concentrations of at least 10% (w/v) NaCl, with optimal salt concentration for growth being at least 20% (w/v; approx. 3.4 M), *Haladaptatus cibarius* and *Halalkalicoccus jeotgali* showed optimal growth with 15% (w/v) NaCl concentrations, which is relatively low to be referred to as extreme halophiles. In particular, archaea that belong to the genus *Haladaptatus* have been thought to adapt to low salt concentrations. In the case of *Haladaptatus paucihalophilus*, which was isolated for the first time as the genus *Haladaptatus*, it can even grow in very low NaCl concentrations (higher than 0.8 M) [50]. Owing to these reasons, it is expected that the possibility of the existence of archaea belonging to the genus *Haladaptatus* in fermented foods with relatively moderate salt concentrations is high.

Halophilic Archaea in Foods Outside Korea

Halophilic archaea that have been identified in foods outside Korea so far are mostly from Japan and Southeast Asia, especially from Thailand. Almost 80~90% of the total population in Southeast Asia are known to consume fish sauce [4], unlike the Korean and Japanese, who normally use soy sauce for fermented condiments. In particular, Thai fish sauce is well known worldwide as a seasoning for Thai cuisine. Fish sauce, similar to liquid *jeotgal* in Korea (*ackjeot*), refers to the filtered liquid obtained by adding table salt of 20~30% (w/v) to fish or shellfish and by allowing it to ferment for a long time. Most representative Thai fish sauces include nam-pla, which is similar to Korea's fermented anchovy *ackjeot*, and pla-ra, which is fermented by adding starchy grains to fish and salt. Tapinkae *et al*. [60] have identified 156 species of halophilic archaea from various fish sauce products, and new species of halophilic archaea are continuously being reported (Table 1). New strains of halophilic archaea that have been isolated from Thai fish sauce so far include *Halobacterium salinarum* (*H. piscisalsi*) [28, 64], *Natrinema gari* [59], *Haloarcula salaria* [32], *Haloacular tradensis* [32], and *Halococcus thailandensis* [33]. Most of these strains show similar characteristics since a minimum salt concentration of 15% (w/v) is needed for growth, and the optimal salt concentration for growth is at least 20% (w/v).

There are various fermented foods with high sodium concentration in Japan and the likelihood of the existence of haloarchaea has been reported [24]. Japanese researchers have recently reported that halophilic archaea have been isolated from bay salts that are sold commercially in Japan. The fact that halophilic archaea are still alive in salts after being heated by the sun in salterns and dried for at least 10 days suggests that the chances for halophilic archaea to come in contact with humans are much higher than expected. Halophilic archaea that have been isolated and identified from Japanese commercial salts include *Salarchaeum japonicum* [53], *Halobaculum magnesiiphilum* [55], *Natronoarchaeum mannanilyticum* [54], *Halarchaeum acidiphilum* [26], and *Halostagnicola alkaliphila* [30], and these halophilic archaea show a much wider variety of growth conditions compared with those isolated from high salt-fermented foods. Whereas most halophilic archaea are mesophilic, *Halobaculum magnesiiphilum* [55] can grow in temperatures up to 55°C (optimal growth temperature is 45°C), and it also grows in 30% (w/v) $MgCl_2$ concentration since it is magnesium-dependent.

In addition, there are some acidophilic haloarchaea like *Salarchaeum japonicum* [53] and *Halarchaeum acidiphilum* [53], and alkalophilic haloarchaea like *Natronoarchaeum mannanilyticum* [53], showing diverse pH-dependent characteristics. Therefore, it is expected that screening with culture media of various pHs when examining haloarchaea in food products would allow us to study many more varieties of halophilic archaea.

The number of halophilic archaea isolated and identified from foods in and outside Korea by culture-dependent methods is not large. This may be due to conventional screening methods of halophilic archaea that focused on environments around salt lakes or salterns, and the lack of attempts to design new screening media, because of the assumption that the possibility of their existence in foods was low. However, based on the fact that the halophilic archaeal strains isolated until now are very diverse, it needs to be considered that the possibility of having a wide variety of halophilic archaea in food products is higher than would have been expected in the past.

Halophilic Archaea in Foods and Human Intestines Identified by Culture-Independent Techniques

Although culture-dependent methods have been used to analyze microflora within foods or environments for the past few years, there are limitations on the number of microorganisms that researchers can culture with conventional methods. Therefore, culture-independent techniques such as PCR and denaturing gradient gel electrophoresis (PCR-DGGE), library cloning, restriction fragment length polymorphism (RFLP), or next-generation sequencing (NGS) are rapidly being developed for analyzing microflora or microbiomes [62].

Identification of Halophilic Archaea in Foods by Using Culture-Independent Techniques

Studies that have been conducted to explore halophilic archaea in foods by using PCR-DGGE only include research on *jeotgal* [43] and *kimchi* [12] of Korea and pickled olives [1] from the Mediterranean regions, and strains identified through such methods that match the closest to the genetic sequence of the 16S rRNA gene of halophilic archaea are shown in Table 2. Interestingly, although *Halalkalicoccus jeotgali, Natrialba aegyptiaca,* and *Natronococcus jeotgali* isolated by culture-dependent methods were also found in results of PCR-DGGE of *jeotgal* and *kimchi* samples, DNAs of other strains were not found. However, DNAs of *Halorhabdus utahensis, Halorubrum lipolyticum, Halorubrum luteum, Halorubrum* sp. YYJ21, *Halovivax ruber,* and other strains were identified from seven types of *jeotgals* [43]. In the case of *kimchi*, there were changes of PCR-DGGE profile as fermentation progressed, and there was a relatively large number of DNAs for strains such as *Halococcus* spp., *Natronococcus* spp., *Natrialba* spp., and *Haloterrigena* spp. [12].

Aside from the PCR-DGGE method, barcoded pyrosequencing, which is one of the NGS methods, was used in Rho *et al*.'s [43] studies on microbial diversity in *jeotgal*. Their studies showed that 68.6~98.4% of strains of the phylum *Euryarchaeota* in *jeotgal* belonged to the family *Halobacteriaceae*, among which most were found to be *Halorubrum* and *Halalkalicoccus* genera. Furthermore, Park *et al*. [37] used qPCR (quantitative real-time PCR) to enumerate the changes of archaea during the fermentation process of *kimchi*, and it was found to be maintained from the early stage to day 17 of fermentation.

Olive farming takes a great part in Mediterranean agriculture, and not only the well-known olive oil but also green table olives, a type of pickled product of olives submerged in salt water of 6~8% (w/v) for at least 6 months, are popular foods in that area. Abriouel *et al*. [1] analyzed the diversity of microbial populations by PCR-DGGE after obtaining samples by differing the period and method of fermentation from green table olive fermenting companies in the Malaga region of Spain. This study showed that *Halosarcina pallida* and *Halorubrum orientalis* were extensively present in the products. In summary, archaea belonging to the *Halorubrum* genus that has been seldom isolated by culture-dependent methods may be present in various food products.

Table 2. Culture-independent identification of halophilic archaeal 16S rRNA gene sequences of DGGE bands from fermented foods.

Closest strain match	Source	Highest sequence identity (%)	Reference
Halalkalicoccus jeotgali	Various *jeotgal*	100	[43]
Haloarchaeon strain CSW2.24.4	*Kimchi*	92	[12]
Haloarchaeon strain PW.5.4	*Kimchi*	98	[12]
Halobiforma nitratireducens	*Kimchi*	98	[12]
Halococcus dombrowskii	*Kimchi*	100	[12]
Halococcus morrhuae	*Kimchi*	97	[12]
Halorhabdus utahensis	Tiny shrimp *jeotgal*	90.8	[43]
Halorubrum lipolyticum	Various *jeotgal*	99	[43]
Halorubrum luteum	Various *jeotgal*	98.3	[43]
Halorubrum orientalis	Alorena green table olive	100	[1]
Halorubrum sp. YYJ21	Crab *jeotgal*	98.9	[43]
Halorubrum traponicum	*Kimchi*	98	[12]
Halosarcina pallida	Alorena green table olive	100	[1]
Halosimplex carlsbadense	*Kimchi*	99	[12]
Haloterrigena thermotolerans	*Kimchi*	90	[12]
Halovivax ruber	Pollack tripe *jeotgal*	93.5	[43]
Natrialba aegyptiaca	*Kimchi*	100	[12]
Natronococcus jeotgali	*Kimchi*	99	[12]
Natronococcus zabuyensis	*Kimchi*	99	[12]

Halophilic Archaea Inside Human Intestines

The number of microbiomes that live inside the human body is extremely larger, reaching to approximately 10^{13} ~ 10^{14} CFU, and the size of their genome is close to 100 times bigger than that of the human genome [15]. In particular, according to recent studies, microorganisms that live inside human intestines are known to be one of the factors for controlling fat storage and also to be related to obesity [6, 25]. Although more than 90% of these enteric microorganisms are bacteria that belong to divisions of Bacteroidetes and Firmicutes, it is reported that if Firmicutes strains are greater in number than Bacteroidetes strains, the person is more likely to become obese since substances that are hard to digest are digested.

Unlike bacteria, the number of archaea among the enteric microbiomes is very small, most of which are presumed to be methanogenic archaea [49]. Among the methanogenic archaea, *Methanobrevibacter smithii* belongs to the phylum *Euryarchaeota* that takes up nearly 10% of anaerobic microorganisms present in the colon of an healthy adult, which is known to play a central role in removing hydrogen inside human intestines [49]. On the other hand, in the case of halophilic archaea, it had been thought in the past that the low salt concentrations inside human intestines is inadequate for halophilic archaea to survive, but there have been reports [40] that there are halophilic archaea that can survive in salt concentrations close to sea water (approx. 2.5% (w/v)). In addition, Oxley *et al*. [36] have succeeded in identifying DNAs of various halophilic archaea from colonic mucosal biopsies and fecal samples of patients, commercially sold salts, *etc*. Interesting to note is that diversity is quite low for methanogenic archaea inside intestines since most of them belong to the three species *Methanobrevibacter smithii*, *Methanosphaera stadtmanae*, and *Methanobrevibacter arboriphilus*, whereas on the other hand, enteric halophilic archaea have a relatively greater variety, mostly belonging to the genus *Halorubrum*.

These results above also correspond to the results of Nam *et al*.'s [31] study that conducted PCR-DGGE in order to explore the diversity of enteric archaea from fecal samples of Koreans. In this study, DNAs obtained from feces included not only those of methanogenic archaea but also 16S rDNA of strains similar to *Halorubrum koreense*, *Halorubrum alimentarium*, *Halorubrum saccharovorum*, and *Halococcus morrhuae*. In our research laboratory, investigation of halophilic archaea from human fecal samples by using a culture-dependent method resulted in identification of DNAs that all belonged to the genus *Halorubrum* (data unpublished). The genus *Halorubrum* contains very diverse haloarchaea, but it may have special characteristics to be detected. It seems that future studies are needed on why *Halorubrum* is frequently detected as enteric halophilic archaea.

Industrial Usage of Halophilic Archaea

Although there have continually been attempts for using genetic resources and proteins of halophilic archaea for industrial usages, the current status remains mostly in their potential. In particular, there have been studies conducted in the food industry for using haloarchaea in fermentation and the ripening process of foods such as fermented anchovy *ackjeot* or fish sauce that require over 1 year of ripening [5]. It is because various enzymes, including proteases, produced by halophilic archaea promote the ripening of fish sauce, through which desired flavor is achieved in the product. In particular, proteases of halophilic archaea are receiving the most attention, and

Table 3. Proteases from halophilic archaea.

Strain	Protease type	Optimum pH	Molecular mass	Reference
Halobacterium halobium ATCC 43214	Esterase activity	N.D.[a]	66 kDa	[48]
Halobacterium halobium P-353	Serine protease	7.2	41 kDa	[19]
Halobacterium sp. SP1(1)	Serine protease	7.2	42 kDa	[3]
Halobacterium sp. strain TuA4	Serine protease	N.D.	56 kDa	[51]
Haloferax mediterranei strain 1538	Serine protease	8-8.5	41 kDa	[56]
Halogeometricum borinquense strain TSS101	Serine protease	10	86 kDa	[61]
Natrialba asiatica 172P1	Halolysin (alkaline serine protease)	N.D.	42 kDa	[21]
Natrialba magadii	Chymotrypsin-type serine protease	8-10	45 kDa	[16]
Natrinema sp. J7	Serine protease	8.0	62 kDa	[52]
Natronococcus occultus	Chymotrypsin-like extracellular serine protease	N.D.	130 kDa	[57]

[a]N.D. means "not determined".

their potential for use is being widened by purification and cloning from several strains (Table 3). Most of the proteases reported from halophilic archaea are extracellular serine proteases that have characteristics of maintaining their activity even in high salt concentrations.

Akolkar *et al*. [4] have reported that the fermentation period of fish sauce was shortened, in addition to having an improved amino acid profile that is responsible for flavor when *Halobacterium* sp. SP1(1) that produces protease was used as a starter for fish sauce. Moreover, Aponte *et al*. [5] studied the effects of halophilic archaea on the quality and safety of salted anchovies (*Engraulis encrasicolus*), which are traditional foods in the Mediterranean region. The results of this study, comparing the addition of *Halobacterium salinarium* CER6a that produces protease versus *Haloarcula marismortui* 1R strain that has no protease activity, showed that according to a preference survey conducted through sensory test, people preferred salted anchovies with addition of haloarchaea more than the control group despite having no significant differences in the hydrolytic rate of muscle sarcoplasmic protein. The most interesting fact is that in the case of salted anchovies with addition of haloarchaea, production of histamine, which is an anti-nutritional factor, was inhibited during the initial ripening process.

Histamine, which is a representative bionic amine, found much in fish such as mackerels, sauries, and sardines, is an important factor that must be inhibited in fermented foods. Intake of histamine causes scombroid poisoning leading to various toxicities such as rash, hives, nausea, vomiting, diarrhea, flushing, among others [8], and so the US FDA regulates the concentration of histamine to be below 500 ppm, over which would lead to toxicity in humans. Development of methods for reducing histamine is in much need because such histamine is not only produced by fermenting bacteria but is also very stable and hardly broken down except through gamma radiation. Study results of Tapingkae *et al*. [60], in which strains with histamine breakdown activity were examined among the 156 strains of halophilic archaea isolated from anchovy fish sauce, showed that the histamine breakdown activity was present in 60 strains and that the strain (HDS1-1) with the strongest activity was *Natrinema gari*. Accordingly, it is considered that using halophilic archaea as a starter for fermented foods would not only shorten the fermenting period and improve on flavor but also increase the safety of the food product.

Among the proteinous substances produced by halophilic archaea, halocin has the highest potential of uses. Halocin is an antibiotic substance in the form of a small-molecule protein that is produced by most of the rod-type haloarchaea, and until now, not many of its physical and chemical properties characterized from purification have been elucidated; the characteristics of only three halocins have been cloned and studied [13, 39, 58]. However, as NGS technology is being developed and the genomic sequence of halophilic archaea is continuously being reported, orthologs with a similar amino acid sequence to halocin are receiving attention as the new halocin. Although the antibiotic spectrum of halocin has been not studied in detail, the potential for its application can be said to be great since there are no antibiotic substances that can selectively kill archaea for now.

Aside from the above, studies on halophilic archaea of the genus *Haloferax* are being conducted because these archaea produce either extracellular polysaccharides [34, 38] or biopolymers like polyhydroxyalkanoates [17, 41]. It

Table 4. Halocin orthologs from halophilic archaea.

Strain	GenBank number or ORF number([a])	Annotation	Length (A.ac)
Haloarchaeon S8a	AAG10010.1	HalS8 precursor	311
Halobacterium salinarum R1	OE2232F[a]	Hypothetical protein	254
Halobacterium sp. AS7092	AAQ82548.1	Halocin C8 precursor	283
Halobacterium sp. NRC-1	AAG20958.1	Hypothetical protein	253
Haloferax mediterranei	HFX_5264[a]	Halocin H4 precursor	359
Haloferax volcanii	HVO_A0311[a]	Halocin C8 (TBD)	305
Halomicrobium mukohataei	Hmuk_0967[a]	Hypothetical protein	254
Haloterrigena turkmenica	Htur_2012[a]	Hypothetical protein	264
Natrialba magadii	Nmag_1674[a]	Hypothetical protein	312
Natrinema sp. J7-2	NJ7G_4349[a]	Halocin C8 precursor	133

[a]ORF number from genome sequencing data.

is also expected that halophilic archaea could be used for biodegradation of food wastes that contain high salt concentrations and also for treatment of saline wastewater.

Future Outlook

Until now, halophilic archaea have mostly been studied in the environmental and ecological fields, but recently, it is discovered that they have close relationships with daily human life. There have not been many reports of halophilic archaea found in salted foods or animal intestines. This is likely due to the lack of researchers' interest on isolation of strains. Accordingly, there will be continued trials for isolation of strains that have not yet been reported.

Although halophilic archaea had been known to be unable to grow in salt concentrations below 10% (w/v) because cell lysis occurred by osmotic pressure, there are recent reports of halophilic archaea capable of surviving in low salt concentrations. Therefore, the possibility of continued findings of halophilic archaea strains adapting in foods or inside animal guts is high. Yet, the most important issue is how to develop the techniques to grow halophilic archaea by culture-dependent methods instead of DNA discovery through culture-independent methods. The success of culture of halophilic archaea adapting to environments with low salt concentrations would provide a good evidence for understanding adaptation mechanisms of microorganisms.

Moreover, in order to use haloarchaea and their useful enzymes and substances in the food industry, there must be accompanying studies on the safety of haloarchaea itself as well. Interestingly, there is growing interest in the relationship between archaea and infection, as methanogenic archaea *Methanobrevibacter oralis* have been found to be related to periodontitis [26], but until now, there is lack of studies being conducted on the relationship between consumption of halophilic archaea and human health. In particular, there will be needs for future studies on the effects of halophilic archaea surviving in human intestines and studies on the special metabolism of halophilic archaea, because as the human microbiome is considered as another organ [9], humans, bacteria, and archaea are known to have mutualism through metabolisms different from one another [7].

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