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Strictly anaerobic halophiles isolated from canned Swedish fermented herrings (Surströmming)

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Abstract

Strictly anaerobic halophiles were isolated from canned Swedish fermented herrings (Surströmming). All isolates were phenotypically uniform with some exceptions and were identified as the genus *Haloanaerobium* and assigned to either *Haloanaerobium praevalens* or *Haloanaerobium alcaliphilum.* A comparative analysis of 16S rDNA sequences revealed that the representative strain S-8 of the isolates was identical to that of *Haloanaerobium praevalens* DSM 2228^T. Furthermore, this strain exhibited high levels ($>80\%$) of DNA–DNA homology with *Haloanaerobium praevalens* DSM 2228^T. This is a novel report of halophilic anaerobes isolated from a food product. Such anaerobes may contribute to the intense flavor and the swollen can characteristics of Swedish fermented herring.

Keywords: Strictly anaerobic halophile; Canned fermented herrings (Surströmming); *Haloanaerobium*

1. Introduction

Canned fermented herring is a traditional product in the northern region of Sweden (Alm, 1965; Beddows, 1985), and is referred to as Surströmming. A strong and unique flavor and the swollen appearance of the can are the notable features of this food. To prepare Surströmming, salted herrings are fermented for several months in barrels and canned without sterilization. The fermentation continues in the can, and the can swells.

Since food cans are airtight and dissolved oxygen is generally low in highly salted foods such as these fermented herrings, it is likely that strictly anaerobic halophiles are present and must have some role in the fermentation process, though no microbial study indicating the presence of these organisms has been reported to date. Norberg (1977) described a microbiological analysis of Surströmming, but these isolates were not identified and strictly anaerobic halophiles were not described.

Several investigations of the ecological importance and physiological functions of strictly anaerobic halophilic bacteria have been described (Oren, 1986, 1988). For example, *Haloanaerobium praevalens* appears to have been important to the carbon decomposition process in the Great Salt Lake (Oren et al., 1984). *Haloanaerobacter chitinovorans,* which

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exhibits chitinolytic activity was isolated from a saltern in California, where chitin derived from brine shrimp and brine flies may be abundant (Liaw and Mah, 1992). However, to our knowledge, there have been few reports of halophilic anaerobes present in food products and associated with the fermentation processes of their manufacturing process. A halophilic anaerobe, *Bacteroides halosmophilus* was isolated from Mediterranean salted anchovies (Baumgartner, 1937), but this isolate has been lost. Similar anaerobes were also isolated from a spoiled sample of sugar salted herring (Knøchel and Huss, 1984a,b), but these isolates were not identified in detail.

In the present study, we isolated and characterized strictly anaerobic halophiles from fermented herrings and report a unique occurrence of such anaerobes present in a food product.

2. Materials and methods

2.1. Canned fermented herrings

Two cans of the fermented herrings (sample A and sample B) were purchased from a local retail shop in Sweden. Cans were transported to Japan by air. Sample A was manufactured in 1996 and was opened in May 1997. Sample B was manufactured in 1997 and was opened in September 1997. There was no means of knowing the manufacturing process and detailed composition of the products. In general, however, Baltic herring, *Clupea harengus* var. *membras* is freshly caught just prior to spawning (from May 1 until the first week of July) and is first treated in saturated brine for 30–40 h, eviscerated with roe and milt retained, following which the fillets are packed in barrels and stored for fermentation (Alm, 1965).

2.2. Bacterial strains

Fermented fillets were taken out from each can. Viable bacteria were enumerated by ordinary plate counting. To enumerate and purify isolates, anaerobic bacterial culture medium (ABCM) (E-MG19, Eiken, Tokyo, Japan) containing 13 or 20% of NaCl was incubated in a GasPak anaerobic system (BBL, Cockeysville, MD, USA). After 7–10 days incubation, counts of viable bacteria were taken and individual colonies were restreaked. A total of 41 isolates were obtained in this study. Type strains of *Haloanaerobium praevalens* DSM 2228^T and *Haloanaerobium alcaliphilum* DSM 8275^T were obtained from the German Strain Collection (DSM) (Braunschweig, Germany).

2.3. Identification of strictly anaerobic bacteria

All strictly anaerobic bacteria were incubated at 30°C in 10% NaCl, under anaerobic conditions using a GasPak anaerobic system. Gram stain, cell form, and motility were observed by light microscopy. To test for formation of endospores, growth was observed after pasteurization at 70°C for 10 min. Catalase activity was tested with 3% H_2O_2. Hydrogen sulfide production was determined with sulfide indole motility (SIM) medium (E-MA32, Eiken). Nitrate reduction was tested by using Gifu anaerobic medium (GAM) (05460, Nissui, Tokyo, Japan) supplemented with 0.1% KNO_3 and 0.15% agar. Salt-tolerance tests were carried out with ABCM semisolid medium (E-MG20, Eiken) containing NaCl at concentrations ranging from 0 to 25%. The range of growth temperatures and pH values were also determined in ABCM semisolid medium. To test the ability to ferment various carbohydrates, the basic medium containing 40 mM PIPES buffer (pH 6.6) was used according to methods described by Oren et al. (1984). For analysis of fermentation products from the medium, strains were incubated in 15-ml portions of the medium in 30-ml serum bottles under nitrogen gas. For analysis of broth samples, Shimadzu organic acid analysis system (Shimadzu, Kyoto, Japan) using ion-exclusion chromatography was employed. Ethanol in broth samples was analysed by enzymatic kits (176290, Boehringer Mannheim, Mannheim, Germany). Gas phase was analyzed by a Hitachi G-5000A gas chromatograph (Hitachi, Tokyo, Japan) equipped with a thermal-conductive detector. DNA base composition was determined by a HPLC method as previously reported (Katayama-Fujimura et al., 1984).

2.4. Identification of lactic acid bacteria

Gram stain, cell form, and motility were observed by light microscopy. To determine gas formation,

GYP broth medium with a Durham tube was used (Kobayashi et al., 1995). Glucose fermentation was tested by the broth medium described previously (Uchida, 1982). Nitrate reductions were tested by the method of Davis (1955). Salt-tolerance tests were carried out in MRS medium (De Man et al., 1960). To measure growth at different pH values, MRS broth at pH 4.2 and pH 8.5 were also used.

2.5. 16S rDNA sequencing and DNA–DNA homology

Sequencing of 16S rDNA was carried out by using an Applied Biosystems commercial PCR, Dye Terminator Cycle Sequencing Kit (402079, Applied Biosystems, Foster City, CA, USA). For amplification of 16S rDNA, a universal primer set, corresponding to positions 8–27 (forward primer) and 1492–1510 (reverse primer) of the *Escherichia coli* numbering system, was used (Weisburg et al., 1991). The reaction mixture was analyzed with an Applied Biosystems 373A DNA sequencer as described previously (Satomi et al., 1997). The 16S rDNA sequences of the new isolate S-8 and *Haloanaerobium praevalens* DSM 2228^T determined in this study have been deposited in the DDBJ (DNA data bank of Japan) data base under accession nos. AB022035 and AB022034, respectively. DNA–DNA pairing studies were carried out by the microplate hybridization method with photobiotin labeling and fluorometric detection as described previously (Ezaki et al., 1989).

2.6. Maintenance of the culture

All stains were stabbed into ABCM agar medium containing 10% NaCl and 0.5% $CaCO_3$. They were maintained at 5°C, and subcultured every 6 months.

2.7. Quantitative analysis of organic acid in fermented herrings

The organic acid values were analyzed by Shimadzu organic acid analysis system according to methods described by Kawashima and Yamanaka (1996).

Table 1
Characterization of fermented herrings (Surströmming)

Characteristics	Sample A	Sample B
pH	7.5	6.3
NaCl conc. (%)	9.0	9.5
Organic acids (mg/100 g)		
Lactic acid	9	46
Formic acid	3	2
Acetic acid	288	189
Propionic acid	65	52
Butyric acid	140	163
Viable cells counts (cfu/g)		
On 13% NaCl medium	5.6×10^6	2.2×10^6
On 20% NaCl medium	4.8×10^5	2.2×10^6

3. Results

Table 1 shows the pH values, salt concentrations, organic acid concentrations, and anaerobic viable counts in the fermented herrings. Both fermented herring samples (fermented fillets) were found to contain 10% NaCl (w/w). Both samples showed the accumulation of considerable amounts of acetic acid, butyric acid, and propionic acid, in that order. Anaerobic viable cell counts of both were 10^6/g.

As shown in Table 2, all the anaerobically isolated strains could be divided into two groups based on phenotypic characteristics. Twenty-two strains were strictly anaerobic rods, motile, gram-negative, able to grow even in 20% NaCl, and had mol% G + C content of the DNA in the range of 30–32. The other 19 strains were homofermentative lactic acid bacteria which were gram-positive, catalase-negative, tetrad-forming cocci, able to grow at pH 8.5 but not at pH 4.2, and tolerant of 15% NaCl. The former strains were identified as the order *Haloanaerobiales* (Rainey et al., 1995) and the latter strains as *Tetragenococcus halophila* (Sakaguchi, 1958; Collins et al., 1990; Kozaki et al., 1992).

All strictly anaerobic isolates were selected for further studies to determine their taxonomic positions. The morphological, physiological, and biochemical characteristics of the strictly anaerobic isolates and two reference strains are shown in Table 3. All the isolates grew well at 10 and 45°C, but not at 50°C. They could not grow in the presence of 3% NaCl. They grew well at pH 5.5 and pH 8.5. Spores were not observed microscopically, and no growth was obtained after pasteurization. All the isolates

Table 2
Characteristics of isolates from fermented herrings (Surströmming)[a]

Characteristics	Anaerobes	Lactic acid cocci
Shape	R	C
Gram stain	−	+
Motility	+	−
Nitrate reduction	−	−
Production of catalase	−	−
Gas from glucose	+	−
Strict anaerobe	+	−
Facultative anaerobe	−	+
Spore formation	−	−
G + C content (mol%)	30 ~ 32	NT
Growth in NaCl		
0%	−	+
10%	+	+
15%	+	+
20%	+	+
Growth in pH		
4.2	NT	−
8.5	+	+
No. of isolates from 13% NaCl medium		
Sample A	7	6
Sample B	5	3
No. of isolates from 20% NaCl medium		
Sample A	5	5
Sample B	5	5

[a] +, positive; −, negative; D, differs among strains; NT, not tested; R, rod; C, coccus.

produced sulfide and fermented D-glucose and produced hydrogen, carbon dioxide, and acidic end products such as lactate, acetate, propionate, and butyrate but not ethanol. After 6 days incubation, the representative strain S-8 from 22 anaerobes produced 4.9 mg of lactic acid/100 ml, 11.8 mg of acetic acid/100 ml, 0.3 mg of propionic acid/100 ml and 2.6 mg of butyric acid/100 ml in the broth culture. There was variation in the ability to ferment three substrates. Twelve strains (91% of the total number of strains) could ferment N-acetylglucosamine, fifteen strains (68% of the total number of strains) could ferment D-mannose, and twelve strains (54% of the total number of strains) could ferment maltose.

From the profile of phenotypic characterization, isolates best fit categories of either *Haloanaerobium praevalens* or *Haloanaerobium alcaliphilum*. These characteristics of the isolates coincided with that of a type strain of *Haloanaerobium praevalens* DSM 2228^T except for the motility and its variable properties of fermenting D-mannose, maltose and N-acetylglucosamine. The isolates were also similar to *Haloanaerobium alcaliphilum* DSM 8275^T in their profile of phenotypic characteristics except for sucrose and ribose fermentation and the variable properties of fermenting the above three substrates.

To investigate the phylogenetic relationships of the isolates further, 16S rDNA analyses by PCR were subjected to sequence analysis. Almost complete 16S rDNA sequences of representative strain S-8 and *Haloanaerobium praevalens* DSM 2228^T were determined. The *Haloanaerobium praevalens* sequence has been described previously (database accession number M59123), but there were many ambiguous bases in the 16S rDNA sequence of *Haloanaerobium praevalens*. Therefore, we determined the 16S rDNA sequence of *Haloanaerobium praevalens* DSM 2228^T to study the difference between our isolate and *Haloanaerobium praevalens*. A comparison of the 16S rDNA sequence revealed not a single nucleotide difference between representative isolate S-8 and a type strain of *Haloanaerobium praevalens*, while a 25-nucleotide difference (98.01% homology) was observed between the strain S-8 and *Haloanaerobium alcaliphilum* DSM 8275^T available from the data base (database accession number X81850) in 1357 positions of all sequences that could be aligned.

A DNA–DNA hybridization test was done and the results are summarized in Table 4. *Haloanaerobium praevalens* DSM 2228^T exhibited high levels ($>80\%$) of homology with strain S-8. On the other hand, *Haloanaerobium alcaliphilum* DSM 8275^T exhibited low levels ($<60\%$) of homology with strain S-8. This information verifies the taxonomic position of strain S-8 as a member of *Haloanaerobium praevalens*.

4. Discussion

Currently, strictly anaerobic halophilic eubacteria, order *Haloanaerobiales*, include two families (*Halobacteroidaceae* and *Haloanaerobiaceae*). The family *Haloanaerobiaceae* has three genera: *Haloanaerobium*, *Halothermothrix* and *Halocella*. Five genera, *Halobacteroides*, *Haloanaerobacter*, *Acetohalobium*, *Orenia* and *Spoloholobacter* belong to the family *Halobacteroidaceae* (Rainey et al.,

Table 3
Characteristics of strictly anaerobic bacteria from fermented herrings (Surströmming) and two reference strains[a]

Characteristics	Anaerobes (22 strains)	*H. praevalens* DSM 2228^T	*H. alcaliphilum* DSM 8275^T
Shape	R	R	R
Gram stain	−	−	−
Motility	+	−	+
Nitrate reduction	−	−	−
Production of			
Catalase	−	−	−
H_2S	+	+	+
Gas from glucose	+	+	+
Obligate anaerobe	+	+	+
Spore formation	−	−	−
G + C content (mol%)	30.2 ~ 32.1	30.7	30.4
Growth in NaCl			
0%	−	−	−
3%	−	−	−
5%	+	+	+
7%	+	+	+
10%	+	+	+
20%	+	+	+
Growth in pH			
5.5	+	+	+
6.0	+	+	+
7.0	+	+	+
8.5	+	+	+
Growth at			
10°C	+	+	+
15°C	+	+	+
20°C	+	+	+
30°C	+	+	+
40°C	+	+	+
Fermentation of			
L-Arabinose	−	−	−
D-Fructose	+	+	+
L-Rhamnose	−	−	−
D-Xylose	−	−	−
D-Mannose	D	+	+
Lactose	−	−	−
Sucrose	−	−	+
D-Ribose	+	+	−
Galactose	−	−	−
N-Acetylglucosamine	D	+	+
Cellobiose	−	−	−
Maltose	D	+	+
Raffinose	−	−	−
Starch	−	−	−
Sodium pyruvate	−	−	−
Glycerol	−	−	−
Products of glucose fermentation:			
Lactic acid	+	+	+
Acetic acid	+	+	+
Propionic acid	+	+	+
Butyric acid	+	+	+
Ethanol	−	−	−

[a] +, positive; −, negative; D, differs among strains; R, rod.

Table 4
Degree of DNA–DNA relatedness between the representative strain S-8 and two species in the genus *Haloanaerobium*

Source of unlabeled DNA	Relatedness to labeled DNA (%) from		
	Haloanaerobium praevalens DSM 2228^T	*Haloanaerobium alcaliphilum* DSM 8275^T	S-8
Haloanaerobium praevalens DSM 228^T	100	55	80
Haloanaerobium alcaliphilum DSM 8275^T	49	100	41
S-8	83	55	100

1995). Sixteen species belonging to the above eight genera have been isolated from various hypersaline environments: *Haloanaerobium praevalens* (Zeikus et al., 1983) and *Haloanaerobium alcaliphilum* (Tsai et al., 1995) from the Great Salt Lake, *Sporohalobacter lortetii* (Oren, 1983), *Halobacteroides halobius* (Oren et al., 1984) and *Orenia marismortui* (Oren et al., 1987) from the Dead Sea, *Haloanaerobium salsuginis* (Bhupathiraju et al., 1994) from oil well brine in Oklahoma, *Haloanaerobium acetoethylicus* (Rengpipat et al., 1988) from oil injection filters in the Gulf of Mexico, *Haloanaerobium congolense* (Ravot et al., 1997) from oil fields in Congo, *Haloanaerobium saccharolyticum* subsp. *saccharolyticum* (Zhilina et al., 1992b), *Halocella cellulolytica* (Simankova et al., 1993) and *Acetohalobium arabaticum* (Zhilina and Zavarzin, 1990) from Sivash Lake, *Haloanaerobacter lacunaris* (Zhilina et al., 1992a) from Chokrak Lake, *Haloanaerobium saccharolyticum* subsp. *senegalense* (Cayol et al., 1994b), and *Haloanaerobium lacusrosei* (Cayol et al., 1995) from Retba Lake, *Haloanaerobacter chitinovorans* (Liaw and Mah, 1992) from a solar saltern in California and *Halothermothrix orenii* from a Tunisian hypersaline lake (Cayol et al., 1994a). However, to our knowledge, there have been few descriptions concerning the isolation and characterization of the strictly anaerobic halophiles from food products like Mediterranean salted anchovies (Baumgartner, 1937) and sugar salted herring (Knøchel and Huss, 1984a,b). Recently, slight halophilic anaerobe *Haloanaerobium butiricum* (Kobayashi and Ueno, 1997) was isolated from seafish and shellfish, but its phylogenetical position has not been described until now.

In the present study, all the isolates from the fermented herrings were identified as *Haloanaerobium praevalens*. Taxonomically, the present data show several discrepancies with the previous papers. *Haloanaerobium praevalens* and *Haloanaerobium alcaliphilum* are differentiated by motility (Zeikus et al., 1983; Rainey et al., 1995; Tsai et al., 1995), the former being immobile. However, the representative strain (S-8) identified as *Haloanaerobium praevalens* by the 16S rDNA sequence and DNA–DNA homology in this study was motile. Therefore, it seems not to possible to differentiate *Haloanaerobium praevalens* and *Haloanaerobium alcaliphilum* by motility alone. *Haloanaerobium praevalens* could also be differentiated by its G + C content (*Haloanaerobium praevalens*, 27%; *Haloanaerobium alcaliphilum*, 31%) in the literature (Zeikus et al., 1983; Tsai et al., 1995). However, in our study there appeared to be no significant difference in the G + C content between these two species. The G + C content of *Haloanaerobium praevalens* DSM 2228^T determined in this study was 30.7%, whereas *Haloanaerobium alcaliphilum* DSM 8275^T was 30.4%. Such levels are considered insufficiently dissimilar to differentiate two species. Previous reports also described differences in fermentation properties that are useful to distinguish these halophiles (Zeikus et al., 1983; Rainey et al., 1995; Tsai et al., 1995). *Haloanaerobium praevalens* produced acetate, propionate, butyrate, hydrogen and carbon dioxide, whereas *Haloanaerobium alcaliphilum* produced lactate, acetate, butyrate, hydrogen and carbon dioxide. However, in this study all strains, including the type strains of *Haloanaerobium praevalens* and *Haloanaerobium alcaliphilum*, had the same fermentation properties and were able to produce lactate, acetate, propionate, butyrate, hydrogen and carbon dioxide. Other discrepancies were observed in such growth characteristics as growth temperature, pH

value, and NaCl concentration. For example the growth of *Haloanaerobium alcaliphilum* was reported to be inhibited below 20°C (Tsai et al., 1995), whereas the *Haloanaerobium alcaliphilum* DSM 8275^T tested in our laboratory was able to grow even at 10°C. These phenomena could be a consequence of the different compositions of the culture media. The above results indicate that the phenotypically major differences between these two species were in the ability to ferment sucrose and ribose. Genotypical typings such as sequencing of 16S rDNA and DNA–DNA hybridization studies are also accurate methods of differentiation.

A strong and unique flavor characterizes Swedish fermented herrings (Alm, 1965; Beddows, 1985). As shown in Table 1, significant amounts of organic acids were detected in the fermented herrings. Among them, the amount of two of the volatile organic acids, propionic acid (the average of two samples: 58.5 mg/100 g) and butyric acid (the average of two samples: 151.5 mg/100 g), were much higher than those observed in Japanese fermented seafood products such as fish sauce (propionic acid: ND ~ 5.2 mg/100 g, butyric acid: ND ~ 13.2 mg/100 g) (Fujii and Sakai, 1984; Fujii et al., 1992a), sardine fermented with rice bran (butyric acid: 20 ~ 30 mg/100 g) (Chang et al., 1991), and crucian carp fermented with rice (propionic acid: ND ~ 46.0 mg/100 g, butyric acid: 9 ~ 12 mg/100 g) (unpublished data in our laboratory), with an exception of mackerel fermented with rice (propionic acid: 103 mg/100 g) (Fujii et al., 1992b). The existence of these organic acids may be due to the activity of the halophilic anaerobes isolated in this study, and they may contribute the specific aroma of the fermented herrings, because these bacteria demonstrated a strong propensity to produce these volatile acids (Table 3). Also, the can of the fermented herrings was usually swollen in appearance, and this is characteristic of this food product. During the incubation of these anaerobes, a significant amount of the gas was produced in the medium causing cracks in the solid medium. Therefore, it is likely that our isolates cause expansion of the can of fermented herrings.

Further studies should clarify the physiological significance of strictly anaerobic halophiles in fermented herrings. Previously, we reported that the halophilic eubacteria that grew only anaerobically were distributed widely in a traditional Japanese food product, fermented puffer fish ovaries (Kobayashi et al., 1995). The study of detailed characteristics of these isolates is also in progress to elucidate the distribution and physiological roles of these organisms in fermented foods.

In this study we focused on the occurrence of strictly anaerobic halophiles, because these anaerobes have been poorly studied in fermented food products. Besides these bacteria, the lactic acid bacterium *T. halophila* was also isolated from Surströmming. *T. halophila* is used to ferment soy sauce and is known to produce lactic acid and other useful organic acids in high-salt concentrations (O'Toole, 1997). Thus, it is likely that these bacteria also play an important role in the fermentation of Surströmming. These aspects should be studied in the future. Also, marine fish contain TMAO which is known to influence the Eh (the oxidation–reduction potential) and the growth condition of microorganisms (Huss and Larsen, 1979). The Eh values and TMAO contents of Surströmming and the bacterial reaction to these have not been determined in this study. The elucidation of these aspects should be helpful for a better understanding of microbial succession in Surströmming.

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References

Alm, F., 1965. Scandinavian anchovies and herring tidbits. In: Borgstrom, G. (Ed.), Fish as Food, Vol. 3, Academic Press, New York, pp. 195–216.

Beddows, C.G., 1985. Fermented fish and fish products. In: Wood, B.J.B. (Ed.), Microbiology of Fermented Foods, Vol. 2, Elsevier, London, pp. 1–39.

Baumgartner, J.G., 1937. The salt limits and thermal stability of a new species of anaerobic halophile. Food Res. 2, 321–329.

Bhupathiraju, V.K., Oren, A., Sharma, P.K., Tanner, R.S., Woese,

C.R., McInerney, M.J., 1994. *Haloanaerobium salsugo* sp. nov., a moderately halophilic, anaerobic bacterium from a subterranean brine. Int. J. Syst. Bacteriol. 44, 565–572.

Cayol, J.-L., Ollivier, B., Lawson Anani Soh, A., Fardeau, M.-L., Ageron, E., Grimont, P.D.A., Prensier, G., Guezennec, J., Magot, M., Garcia, J.-L., 1994a. *Haloincola saccharolytica* subsp. *senegalensis* subsp. nov isolated from the sediments of a hypersaline lake, and amended description of *Haloincola saccharolytica*. Int. J. Syst. Bacteriol. 44, 805–811.

Cayol, J.-L., Ollivier, B., Patel, B.K.C., Prensier, G., Guezennec, J., Garcia, J.-L., 1994b. Isolation and characterization of *Halothermothrix orenii* gen. nov., sp. nov., a halophilic, thermophilic, fermentative, strictly anaerobic bacterium. Int. J. Syst. Bacteriol. 44, 534–540.

Cayol, J.-L., Ollivier, B., Patel, B.K.C., Ageron, E., Grimont, P.A.D., Prensier, G., Garcia, J.-L., 1995. *Haloanaerobium lacusroseus* sp. nov., an extremely halophilic fermentative bacterium from the sediments of a hypersaline lake. Int. J. Syst. Bacteriol. 45, 790–797.

Chang, C.M., Oshima, T., Koizumi, C., 1991. Lipid, free amino acid, and organic acid compositions of rice bran-fermented sardine (in Japanese, with English abstract). Nippon Suisan Gakkaishi 58, 1579–1585.

Collins, M.D., Williams, A.M., Wallbanks, S., 1990. The phylogeny of *Aerococcus* and *Pediococcus* as determined by 16S rRNA sequence analysis: description of *Tetragenococcus* gen. nov. FEMS Microbiol. Lett. 70, 255–262.

Davis, G.H.G., 1955. The classification of lactobacilli from the human mouth. J. Gen. Microbiol. 13, 481–493.

De Man, J.C., Rogosa, M., Sharpe, M.E., 1960. A medium for the cultivation of lactobacilli. J. Appl. Bact. 23, 130–135.

Ezaki, T., Hashimoto, Y., Yabuuchi, E., 1989. Fluorometric deoxyribonucleic acid–deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int. J. Systm. Bacteriol. 39, 224–229.

Fujii, T., Sakai, H., 1984. Chemical composition and microflora of fish sauce 'Shottsuru' (in Japanese, with English abstract). Nippon Suisan Gakkaishi 50, 1061–1066.

Fujii, T., Nikkuni, S., Iida, H., 1992a. Chemical composition and puterscible potential of commercial 'Shottsuru' Japanese fish sauce (in Japanese, with English abstract). Nippon Shokuhin Kogyo Gakkaishi 39, 702–706.

Fujii, T., Sasaki, T., Okuzumi, M., 1992b. Chemical composition and microbial flora of Saba-narezushi (fermented mackerel with rice) (in Japanese, with English abstract). Nippon Suisan Gakkaishi 58, 891–894.

Huss, H.H., Larsen, A., 1979. The post mortem changes in the oxidation–reduction potential of fish muscle and internal organs. In: Sobolewska-Ceronik, K., Ceronik, E., Zaleski, S. (Eds.), Food as an Ecological Environment for Pathogenic and Index Microorganisms, Vol. 2, Ars Polona, Warszawa, pp. 265–279.

Katayama-Fujimura, Y., Komatsu, Y., Kuraishi, H., Kanako, T., 1984. Estimation of DNA base composition by high performance liquid chromatography of its nuclease P1 hydrolysate. Agric. Biol. Chem. 48, 3169–3172.

Kawashima, K., Yamanaka, H., 1996. Influences of seasonal variations in contents of glycogen and its metabolites on browning of cooked scallop adductor muscle. Fisheries Sci. 62, 639–642.

Knøchel, S., Huss, H.H., 1984a. Ripening and spoilage of sugar salted herring with and without nitrate. I. Microbiological and related chemical changes. J. Food Technol. 19, 203–213.

Knøchel, S., Huss, H.H., 1984b. Ripening and spoilage of sugar salted herring with and without nitrate. II. Effect of nitrate. J. Food Technol. 19, 215–224.

Kobayashi, T., Okuzumi, M., Fujii, T., 1995. Microflora of fermented puffer fish ovaries in rice-bran 'Fugunoko Nukazuke'. Fish. Sci. 61, 291–295.

Kobayashi, T., Ueno, K., 1997. Distribution of halophilic nonsporeforming anaerobic gram negative rods in seafish and shellfish on the market (in Japanese, with English abstract). Jpn. J. Food Microbiol. 14, 155–162.

Kozaki, M., Okada, S., Uchimura, T., 1992. Identification diagram for lactic acid bacteria. In: Laboratory Manual for Lactic Acid Bacteria, Asakura-syoten, Tokyo, pp. 126–135, (in Japanese).

Liaw, H.J., Mah, R.A., 1992. Isolation and characterization of *Haloanaerobacter chitinovorans* gen. nov., sp. nov., a halophilic, anaerobic, chitinolytic bacterium from a solar saltern. Appl. Environ. Microbiol. 58, 260–266.

Norberg, P., 1977. Bacteriological analysis of fermented Baltic herring (in Swedish, with English abstract). Var föda 29, 263–266.

Oren, A., 1983. *Clostridium lortetii* sp. nov., a halophilic obligatory anaerobic bacterium producing endospores with attached gas vacuoles. Arch. Microbiol. 136, 42–48.

Oren, A., Weisburg, W.G., Kessel, M., Woese, C.R., 1984. *Halobacteroides halobius* gen. nov., sp. nov., a moderately halophilic anaerobic bacterium from the bottom sediments of the dead sea. System. Appl. Microbiol. 5, 58–70.

Oren, A., 1986. The ecology and taxonomy of anaerobic halophilic eubacteria. FEMS Microbiol. Rev. 39, 23–29.

Oren, A., Pohla, H., Stackebrandt, E., 1987. Transfer of *Clostridium lortetii* to a new genus *Sporohalobacter* gen. nov. as *Sporohalobacter lortetii* comb. nov., and description of *Sporohalobacter marismortui* sp. nov. System. Appl. Microbiol. 9, 239–246.

Oren, A., 1988. Anaerobic degradation of organic compounds at high salt concentrations. Antonie van Leeuwenhoek 54, 267–277.

O'Toole, D.K., 1997. The role of microorganisms in soy sauce production. In: Meidleman, S.L., Laskin, A.I. (Eds.), Advances in Applied Microbiology, Vol. 45, Academic Press, New York, pp. 87–152.

Rainey, F.A., Zhilina, T.N., Boulygina, E.S., Stackebrandt, E., Tourova, T.P., Zavarzin, G.A., 1995. The taxonomic status of the fermentative halophilic anaerobic bacteria: description of *Haloanaerobiales* ord. nov., *Halobacteroidaceae* fam. nov., *Orenia* gen. nov. and further taxonomic rearrangements at the genus and species level. Anaerobe 1, 185–199.

Ravot, G., Magot, M., Ollivier, B., Patel, B.K.C., Ageron, E., Grimont, P.A.D., Thomas, P., Garcia, J.-L., 1997. *Haloanaerobium congolense* sp. nov., an anaerobic moderately halophilic, thiosulfate- and sulfur-reducing bacterium from an African oil field. FEMS Microbiol. Lett. 147, 81–88.

Rengpipat, S., Langworthy, T.A., Zeikus, J.G., 1988. *Halobac-*

teroides acetoethylicus sp. nov., a new obligately anaerobic halophile isolated from deep subsurface hypersaline environments. System. Appl. Microbiol. 11, 28–35.

Sakaguchi, K., 1958. Studies on the activities of bacteria in soy sauce brewing. Part III. Taxonomic studies on *Pediococcus soyae* nov. sp., the soy sauce lactic acid bacteria. Bull. Agric. Chem. Soc. Japan 22, 353–363.

Satomi, M., Kimura, B., Mizoi, M., Fujii, T., 1997. *Tetragenococcus muriaticus* sp. nov., a new moderately halophilic lactic acid bacterium isolated from fermented squid liver sauce. Int. J. System. Bacteriol. 19, 832–836.

Simankova, M.V., Chernych, N.A., Osipov, G.A., Zavarzin, G.A., 1993. *Halocella cellulolytica* gen. nov., sp. nov., a new obligately anaerobic, halophilic, cellulolytic bacterium. System. Appl. Microbiol. 16, 385–389.

Tsai, C.-R., Garcia, J.-L., Patel, B.K.C., Cayol, J.-L., Baresi, L., Mah, R.A., 1995. *Haloanaerobium alcaliphilum* sp. nov., an anaerobic moderate halophile from the sediments of Great Salt Lake, Utah. Int. J. Syst. Bacteriol. 45, 301–307.

Uchida, K., 1982. Multiplicity in soy pediococci carbohydrate fermentation and its application for analysis of their flora. J. Gen. Appl. Microbiol. 28, 215–223.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173, 697–703.

Zeikus, J.G., Hegge, P.W., Thompson, T.E., Phelps, T.J., Langworthy, T.A., 1983. Isolation and description of *Haloanaerobium praevalens* gen. nov. and sp. nov., an obligately anaerobic halophile common to Great Salt Lake sediments. Curr. Microbiol. 9, 225–234.

Zhilina, T.N., Zavarzin, G.A., 1990. Extremely halophilic, methylotrophic, anaerobic bacteria. FEMS Microbiol. Rev. 87, 315–322.

Zhilina, T.N., Miroshnikova, L.V., Osipov, G.A., Zavarzin, G.A., 1992a. *Halobacteroides lacunaris* sp. nov., new saccharolytic, anaerobic extremely halophilic organism from the lagoon-like hypersaline lake Chokrak. Microbiology 60, 495–503.

Zhilina, T.N., Zavarzin, G.A., Bulygina, E.S., Kevbrin, V.V., Osipov, G.A., Chumakov, K.M., 1992b. Ecology, physiology and taxonomy studies on a new taxon of *Haloanaerobiaceae*, *Haloincola saccharolytica* gen. nov., sp. nov. System. Appl. Microbiol. 15, 275–284.