

REVIEW

Algal Fermentation—The Seed for a New Fermentation Industry of Foods and Related Products

Motoharu UCHIDA* and Tatsuo MIYOSHI

Coastal Fisheries and Environment Division, National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency (Hatsukaichi, Hiroshima 739-0452, Japan)

Abstract

Many kinds of fermented products are now being consumed as food and dietary items, although those produced from algae have yet to be developed. A recent observation that seaweed could be used as a substrate for lactic acid fermentation opened the possibility of obtaining such products as foods, diets and fertilizers by algal fermentation. This manuscript reviews past studies on the lactic acid fermentation of algae. Both macroalgae (seaweeds) and microalgae can be used as the materials for lactic acid fermentation, as successful fermentation has been observed regarding all the seaweed species tested to date. Saccharification by cellulase treatment is considered a significant element for inducing algal fermentation. The addition of a starter culture of lactic acid bacteria and salt also promotes successful fermentation. A wide range of *Lactobacillus* species can be used for inducing algal fermentation, with *Lactobacillus brevis*, *Lactobacillus casei* and *Lactobacillus plantarum* in particular showing a superior ability to dominate in seaweed fermentation cultures. A starter culture of halophilic lactic acid bacteria that is now being developed will make it possible to prepare algal fermented products containing a high (>10%) salt content and having long-term preservation. As for application, a prototype of ‘seaweed sauce’ containing a high quantity of amino acids was obtained from *Porphyra* sp. (Rhodophyta). Some functional effects are also demonstrated when fish and animals were fed algal fermented products. Studies on the ethanol fermentation of seaweeds are also making progress. All these advances in algal fermentation are expected to lead to the creation of a new genre of algal fermentation industry.

Discipline: Fisheries

Additional key words: algae, ethanol, lactic acid bacteria, macroalgae, microalgae

Introduction

There have been many kinds of fermentation technology and products since ancient times. For example, fermented food items from soybean are common in the East Asian countries of China, Korea and Japan, while those from fish are common in Southeast Asian countries⁵. Despite the long history of fermentation technology, fermented food items produced from algae have yet to be developed (Fig. 1). Many studies were conducted on methane fermentation of seaweeds during the 1970s and 1980s^{1, 2, 6}. However, methane fermentation is a technology for supplying energy, not for foods and food production.

Macroalgae (macrophytes) can be divided into four groups: brown algae (Phaeophyta), red algae (Rhodophyta), green algae (Chlorophyta), and seagrass (Magnoliophyta). Carbohydrates are the major component of

seaweeds and seagrass (ca. 50-70% on a dry basis)^{11, 29}, containing mostly polysaccharides to construct algal tissue. For example, brown algae contain alginate and fucoidan as major components. Red algae contain galactan (e.g. agar, carrageenan) as a major component. Green algae and seagrasses contain cellulose and hemicellulose as major components. These major algal polysaccharides are known to be unfavorable substrates for fermentation. This may be one of the reasons why algal fermentation technology has yet to be developed. However, it was recently reported that seaweed could be used as a substrate for lactic acid and ethanol fermentation, provided that the algal tissue was saccharified with cellulase enzymes. This finding opened the possibility of obtaining foods and related items from algal fermentation^{17, 18, 19}.

This manuscript reviews past studies on the lactic acid fermentation of algae^{17, 22}. It also refers to other kinds of algal fermentation that are now being developed, such

* Corresponding author: e-mail uchida@affrc.go.jp

Received 21 September 2011; accepted: 17 April 2012.

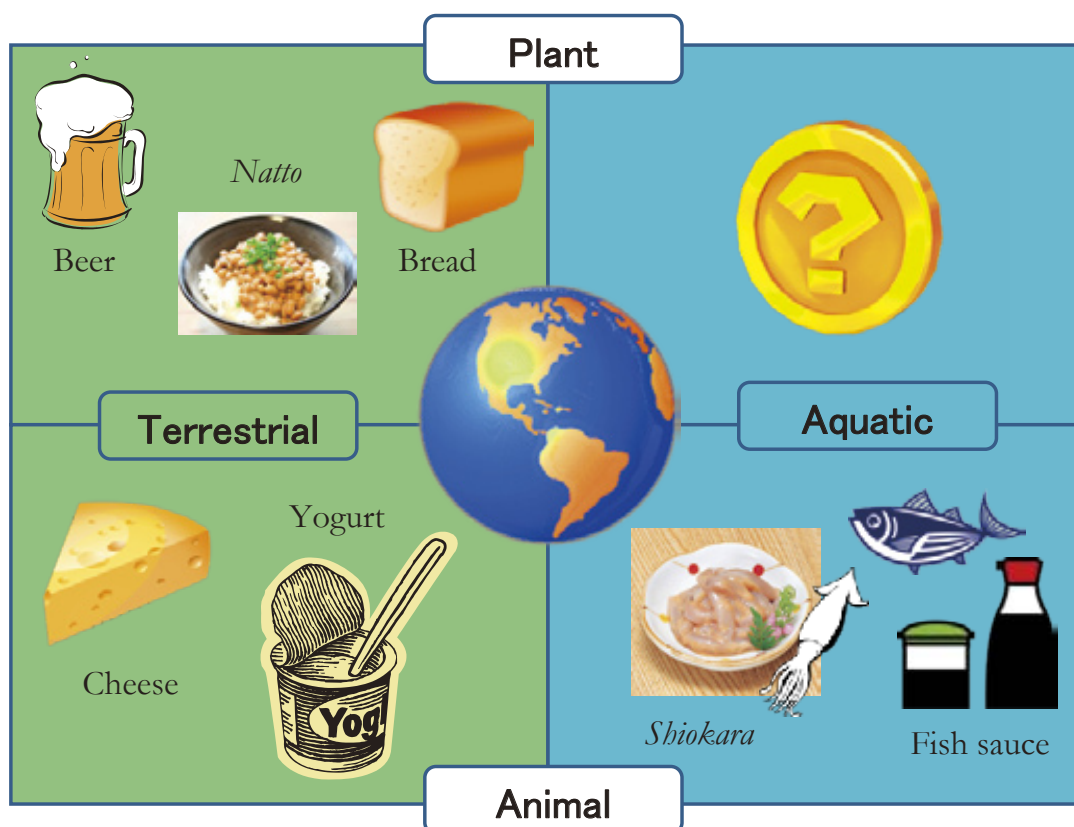


Fig. 1. Category of fermented foods based on raw materials
 Fermented foods prepared from aquatic plants (algae) have yet to be developed.

as ethanol fermentation^{9, 27, 29}. With all these advances in algal fermentation, we suggest that there is a great possibility of creating a new industry based on algal fermentation technology in the near future²⁸.

Methods of fermentation

1. Beginning of the study on lactic acid fermentation of seaweeds

A fermented material of *Ulva* spp. (Chlorophyta) was first obtained on 9 October 1998, after treating the fronds with enzymes containing cellulase activity and leaving the material for 17 months at 2°C¹⁹. The fermented material obtained was then transferred with cellulase to a new *Ulva* culture at an interval of several tens of days, with the induction of fermentation being demonstrated repeatedly. Microbial analysis based on rRNA gene nucleotide sequences allotted the predominant microorganisms in the culture to *Lactobacillus brevis* (lactic acid bacteria, LAB), *Debaryomyces hansenii* var. *hansenii* (typical marine yeast), and *Candida zeylanoides*-related specimens (yeast) (Fig. 2). Therefore, the primarily observed ‘fermentation’ was regarded as a mixture of lactic acid fermentation and

ethanol fermentation. The inoculation of three kinds of separately cultured microorganisms induced fermentation on various kinds of seaweed. This is the first report on an intentional induction of fermentation (except for methane fermentation) of seaweed (Table 1)¹⁹. The addition of salt (5% w/v) besides the starter microorganisms (initial concentration, ca. 10⁶⁻⁷ cfu/mL) and cellulase (0.1-1%) also promoted the predominance of LAB and yeast, and successfully induced fermentation^{17,18,19}.

2. Microorganisms used for the lactic acid fermentation of seaweeds

In order to determine the suitable combinations of LAB and yeast strains for use as a starter culture for the lactic acid fermentation of seaweed, fermentation culture was prepared with different combinations of LAB strains (i.e. *L. brevis* FERM BP-7301, *Lactobacillus acidophilus* IAM10074, *Lactobacillus plantarum* IAM12477¹) and yeast strains (i.e. the two isolates above, plus *Saccharomyces cerevisiae*)²¹. The inoculation of LAB with or without yeast strains yielded the successful induction of fermentation, while inoculation of the yeast strains alone yielded unsatisfactory results, along with some contaminant bac-

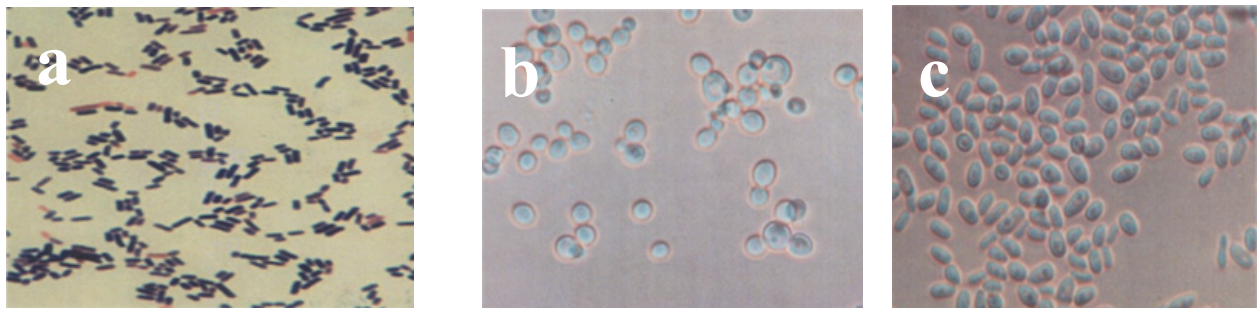


Fig. 2. Microorganisms initially isolated from an algal fermented culture as a starter culture¹⁹

A kind of bacterium (a: *Lactobacillus brevis*) and two kinds of yeast (b: *Debaryomyces hansenii*, and c: *Candida zeylanoides*-related specimen)

Table 1. Fermentation results on various kinds of seaweed with the addition of cellulase and the microbial starter culture

Seaweeds*	Group	pH		Gas	Production (g 100mL/L) of:	
		Initial	After 7Ds		Lactic acid	Ethanol
<i>Chondracanthus teedii</i>	Rhodophyta	5.7	4.5	+	+ (0.16)	+ (0.18)
<i>Chondracanthus tenellus</i>		5.7	3.9	+	+ (0.25)	+ (0.18)
<i>Gelidium linooides</i>		6.5	5.3	+	+ (0.18)	+ (0.12)
<i>Gracilaria incurvata</i>		6.2	4.0	–	+ (0.25)	+ (0.12)
<i>Gracilaria vermiculophylla</i>		6.0	4.0	+	+ (0.31)	+ (0.23)
<i>Hypnea charoides</i>		6.4	6.1	+	+ (0.22)	+ (0.16)
<i>Prionitis angusta</i>		6.3	3.8	+	+ (0.25)	+ (0.17)
<i>Prionitis divaricata</i>		6.4	4.7	+	+ (0.25)	+ (0.41)
<i>Pterocladia capillacea</i>		6.4	5.6	+	+ (0.12)	+ (0.08)
<i>Dilophus okamurae</i>		Phaeophyta	6.7	6.1	–	+ (0.02)
<i>Eisenia bicyclis</i>	5.1		4.2	–	+ (0.02)	+ (0.03)
<i>Hizikia fusiformis</i>	5.4		5.0	+	+ (0.01)	+ (0.24)
<i>Ishige okamurae</i>	4.8		4.9	–	+ (0.01)	+ (0.10)
<i>Laminaria japonica</i>	5.4		3.3	+	+ (0.16)	+ (0.15)
<i>Padina arborescens</i>	6.2		5.8	±	– (<0.01)	+ (0.08)
<i>Sargassum ringgoldianum</i>	5.1		5.0	–	+ (0.01)	+ (0.04)
<i>Undaria pinnatifida</i> (Whole No. 1)	5.7		3.6	+	+ (0.23)	+ (0.38)
<i>Undaria pinnatifida</i> (Whole No. 2)	5.8		3.9	+	+ (0.18)	+ (0.07)
<i>Undaria pinnatifida</i> (Stem)	5.9		3.5	+	+ (0.25)	+ (0.12)
<i>Ulva</i> sp. No. 1	Chlorophyta	5.6	3.3	+	+ (0.76)	+ (0.16)
<i>Ulva</i> spp. No. 2		5.8	4.7	+	+ (0.45)	+ (0.41)
<i>Zostera marina</i>	Vascular plant	6.0	3.3	–	+ (1.14)	+ (0.26)

* 0.5 g of seaweed (dried) and 0.1g of cellulase R-10 were suspended with 9 ml of autoclaved 3.5% NaCl solution. 0.05 ml each of the cultured cell suspensions (OD_{660nm}=1) of the strains *L. casei* B5201, *D. hansenii* Y5201 and *Candida* sp. Y5206 were added as a starter. The culture tubes were incubated for 7 days at 20°C rotating at 5 rpm with the caps closed. Data is the average of the duplicate tests.

teria growth. It therefore follows that the inoculation of yeast is not necessary when intending to induce the lactic acid fermentation of seaweeds.

The species-specific primer sets were developed to identify the LAB strains by using PCR techniques, prior to

the examination of suitable LAB strains for use as a starter culture for seaweed fermentation²¹. Fourteen LAB strains including 11 species were tested under culture conditions prepared with or without salt (Fig. 3)²⁵. A commercial product of *Undaria pinnatifida* (Phaeopyta) powder was

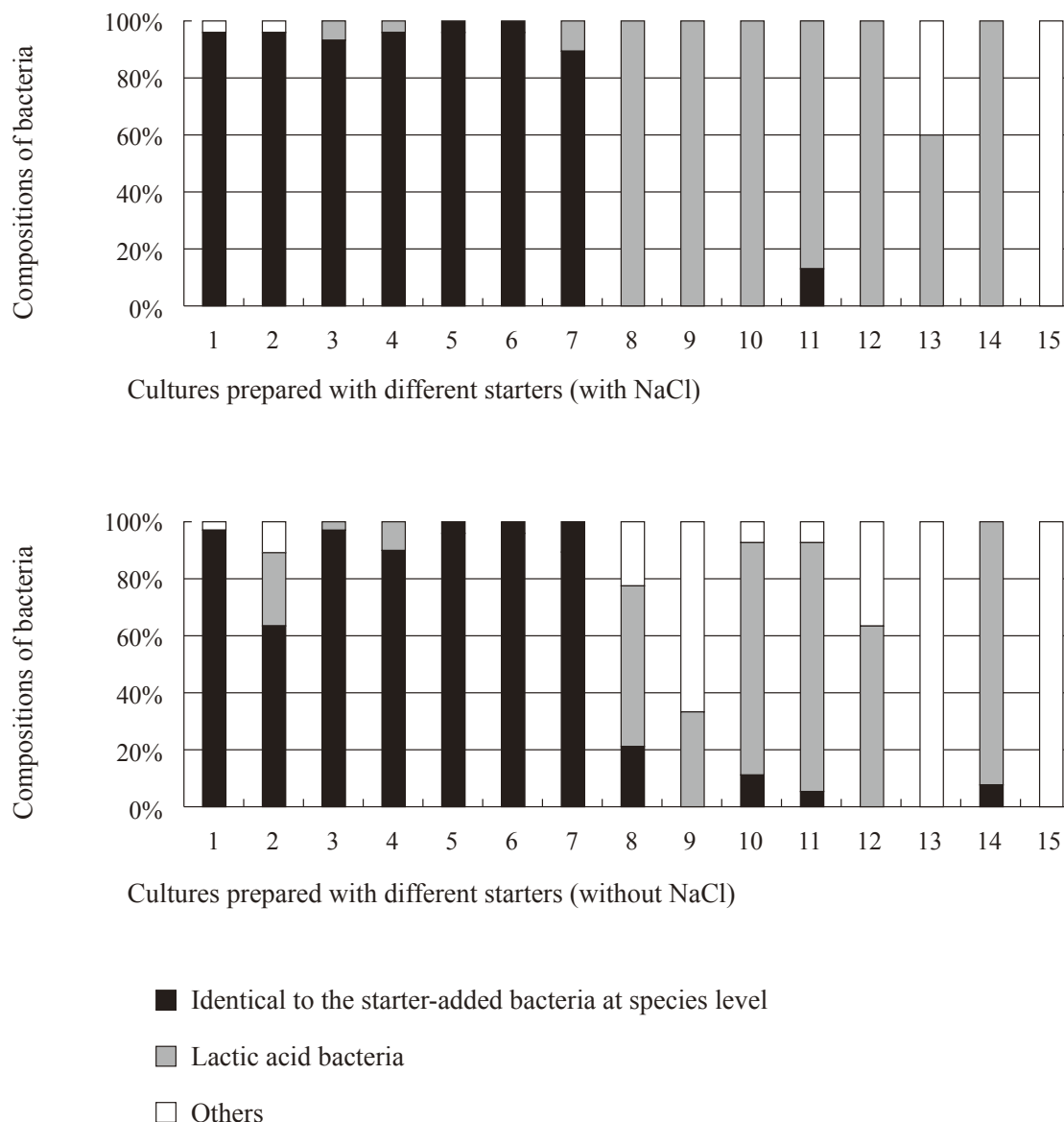


Fig. 3. Results of the test for examining suitable starter culture of lactic acid bacteria for seaweed fermentation

To prepare cultures with NaCl, 2.0 g of the commercial product of *Undaria* powder (Hamamidori, Riken Shokuhin) was mixed with 40 mL of autoclaved 3.5 % (w/v) NaCl solution, 40 mg of cellulase (12S, Yakult Pharmaceutical Ind. Co. Ltd.) and 0.4 mL of bacterial cell suspension. The bacterial cell suspension was prepared for the 14 LAB strains: No. 1; *Lact. brevis* FRA 000033, 2; *Lact. brevis* IAM 12005, 3; *Lact. plantarum* ATCC 14917T, 4; *Lact. plantarum* IAM 12477T, 5; *Lact. casei* IFO 15883T, 6; *Lact. casei* FRA 000035, 7; *Lact. rhamnosus* IAM 1118T, 8; *Lact. zae* IAM 12473T, 9; *Lact. acidophilus* IFO 13951T, 10; *Lact. kefir* NRIC 1693T, 11; *Lact. fermentum* ATCC 14931T, 12; *Lact. delbrueckii* subsp. *bulgaricus* ATCC 11842T, 13; *Streptococcus thermophilus* NCFB 2392, and 14; *Leuconostoc mesenteroides* IAM 13004T. These strains were pre-cultured with MRS medium (Merck Co.), collected by centrifuge (8,000 g × 20 min.), washed with autoclaved 0.85% NaCl solution, re-suspended to make a concentration of O.D.660 nm = 1.0 (containing $7.3 \times 10^7 - 1.1 \times 10^9$ CFU/mL), and then used. To prepare culture without NaCl, autoclaved distilled water was used instead of 3.5% NaCl solution. Cultures without the inoculation of lactic acid bacteria were prepared as being without starter culture controls (No. 15). After incubating for 11 days at 20°C, the microbial composition was investigated. Ten colonies, each formed on the SMA plates prepared for viable counting, were chosen at random from the triplicated trials (Total n = 30), and then transferred to the BCP plates with the % proportion of yellow-colored colonies shown as average ± SE of lactic acid bacteria. *L. brevis*, (Trial Nos.1, 2), *L. plantarum* (2, 4), *L. casei*, (5, 6), and *L. rhamnosus* (7) showed marked ability to be dominant in the *Undaria* cultures²⁵.

used as a substrate for fermentation without sterilizing it. The starter suitability of the LAB strains was assessed from their predominance after 11 days of culture at 20°C. The predominance was assessed by PCR using the developed species-specific primer sets²¹. Among the tested strains, *L. brevis*, *L. plantarum*, *Lactobacillus casei*, and *Lactobacillus rhamnosus* showed high (>90%) predominance in their cultures, while the control cultures prepared without the inoculation of LAB showed no detectable LAB growth and then spoiled²⁵. In those spoiled cultures, *Bacillus* strains such as *Bacillus cereus*-related and *B. fusiformis*-related species were observed to dominate²⁵. The *Undaria* powder prior to fermentation contained culturable microorganisms at $1.4\text{--}3.1 \times 10^2$ CFU/g, but the *Bacillus cereus*-related

strain was not a major component, suggesting a concern about the selective growth of the *Bacillus cereus*-related strain during spoiled fermentation. This study did not test whether the *Bacillus cereus*-related strains isolated from seaweed possess a toxic gene.

3. Optimum conditions for the fermentation and production of algal single cell products

It was observed that cellulase activity can easily fragment algal frond tissue, and that large numbers of single cell detritus (SCD: algal detrital products originating from one cell unit) were produced in the case of *U. pinnatifida* (Fig. 4)²⁰. The optimum conditions for fermentation were studied from two perspectives: the production efficiency of

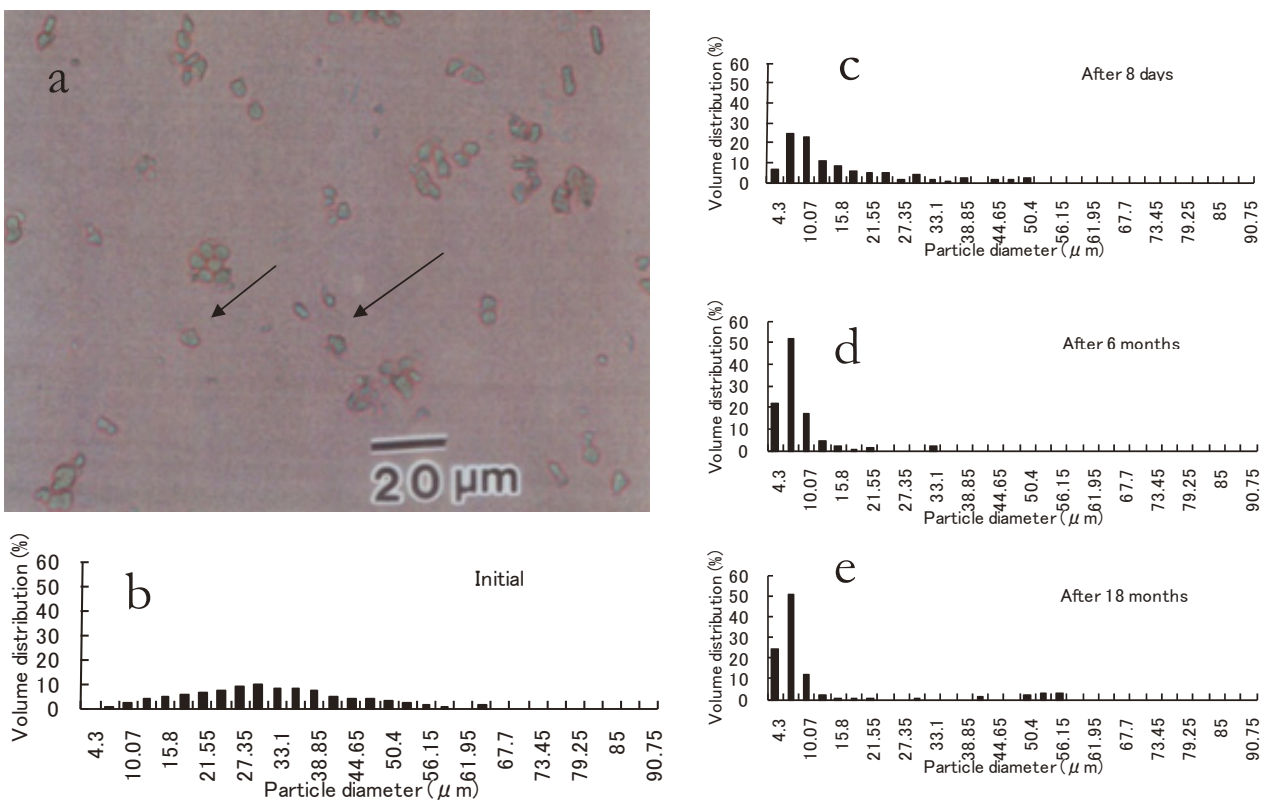


Fig. 4. Microscopic observation of single cell detritus (SCD) prepared from *Undaria pinnatifida* (a, arrows, photo after 8 days of incubation) and time course changes of volume-size distribution of *U. pinnatifida* particles in the fermented culture (b-e)²⁰

One kg of commercially available dried particles of *U. pinnatifida* (Wakamidori, particle size < 74 mm; Riken Co.) was dispensed into bottles containing 9 L of autoclaved 3.3% w/v NaCl solution and 10 g of cellulase (R-10; Yakult Honsha Co. Ltd.), and then mixed well. The culture was inoculated with a microbial mixture composed of *Lactobacillus brevis* strain B5201 (FERM BP-7301), *Debaryomyces hansenii* var. *hansenii* strain Y5201 (FERM BP-7302), and *Candida* sp. strain Y5206 (FERM BP-7303). The bacterium and yeast cells were pre-incubated for 8 days in MRS broth (Merck) and YM broth (Difco/Beckton Dickinson Co.), respectively, collected by centrifuge, washed twice, and then suspended in sterile 0.85% NaCl solution at a concentration of OD_{660 nm} = 1.0. A suspension mixture containing 100 mL of *L. brevis* (5.0×10^{10} cells), 100 mL of *D. hansenii* (5.5×10^7 cells), and 10 mL of *Candida* sp. (1.2×10^8 cells) was used to inoculate the microbial mixture. The culture bottle was tightly capped, incubated at 20°C, and then mixed several times a day to improve dispersion for the first 8 days. The culture bottle was then preserved for 18 months at 20°C.

SCD, and the dominance of lactic acid bacteria (LAB)¹⁸.

As for salt concentration, contaminant bacteria will often grow and spoil cultures that are prepared without salt. However, as *Lactobacillus* group bacteria are not halotolerant, their growth is significantly restricted under a salt concentration exceeding 5%. The suitable salt concentration in the culture water used for SCD preparation was in the range of 2.5 to 3.5% (w/v). The culture will have high viscosity and easily forms an aggregation of algal particles. It is therefore difficult to prepare a homogeneous algal suspension at a salt concentration of less than 2.5%. Conversely, the total volume of the solid part of *Undaria pinnatifida* frond suspension cultures decreased to 100%, 98%, 90%, 71%, 60%, and 39% after being left for six days when the salt concentrations of the cultures were 0%, 1%, 2.5%, 3.5%, 5%, and 10%, respectively¹⁸. As for incubation temperature, a temperature range of 5–50°C can be used for SCD production. The maximum production rate of SCD is achieved at 20°C, and remains almost constant in the range of 20–50°C¹⁸. The final number of SCD products is almost the same in the range of 5–50°C, provided that incubation lasts more than two weeks.

As for incubation time, a period of six to 14 days is sufficient to produce the maximum number of SCD products.¹⁸ LAB will achieve growth above the 10⁸ cfu/mL level within five days, and lactic acid production and pH value will become almost constant after six to 14 days.

As for cellulase concentration, the addition of more than 1% (w/v) was necessary to avoid rotting and obtain a fermented culture¹⁸. Commercial products of cellulase are expensive and saving the use thereof is important in terms of cost. The use of cellulase at 0.1% concentration was observed as being effective enough for the fermentation and production of SCD from *U. pinnatifida* fronds in a 10-L-scale culture²⁰. Under the optimum conditions of a culture containing 5% *U. pinnatifida* (on a dry basis), 3.5% NaCl, and 0.5% cellulase with additional LAB starter, SCD was produced at a rate of 5.8 × 10⁷ cells/mL after six days of incubation at 20°C (Fig. 5)¹⁸.

4. Lactic acid fermentation of microalgae

Lactic acid fermentation was demonstrated on microalgae (i.e. *Chrolorella* sp., *Tetracelmis* sp., *Pavrova lutheri*, *Chaetoceros* sp., *Nannochloropsis* sp.) as well as macroalgae (i.e. seaweeds)²⁹. The pretreatment of microalgae (as well as seaweeds) with cellulase was effective in inducing fermentation. Lactic acid was produced in the range of 1.5–5.4 g/L in the case of microalgae, while 3.6 g/L was produced in the case of *U. pinnatifida*. For example, the use of macerozyme and lactase in addition to cellulase increased the production of lactic acid from 5.4 g/L to 9.6 g/L (*Chrolorella* sp.), and from 1.5 g/L to 4.3 g/L (*Tetra-*

celmis sp.). However, microscopic observation showed quite limited cell wall decomposition in all cases. Suitable enzyme products must therefore be developed to obtain products with the cell wall eliminated or otherwise well-dissolved products.

5. Housing units for conducting algal fermentation

For conducting algal fermentation, plastic tanks or buckets with lids can be used for culture. Plastic bags such as polypropylene bags are cheaper and more convenient for dispersing the aggregate of seaweed and preparing a homogeneous frond suspension. Two 200-L-scale algal fermentation tanks named the ‘marine silos’ were manufactured and set up along the waterside of Lake Hamana (Shirasu-cho, Hamamatsu, Shizuoka, Japan) in 2003²⁸. These marine silos are equipped with a temperature control and mixing facility, and 200-L-scale fermentation was

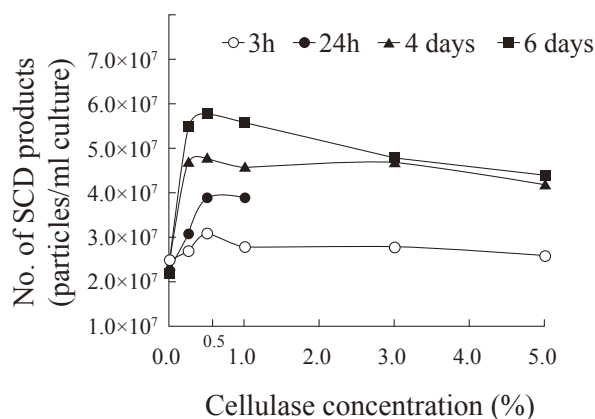


Fig. 5. Relation between SCD production and cellulase concentration in *Undaria* fermentation culture¹⁸

Ten grams of dried particles of *Undaria pinnatifida* (Wakamidori, <74µm, Riken Co.) were dispensed into each 500-mL bottle containing 180 mL of autoclaved distilled water with different concentrations of cellulase (R-10, Yakult Honsha Co.), and then mixed well. The culture bottles were tightly capped and incubated at 20°C, with shaking done several times a day to improve dispersion. The number of detrital particles of *Undaria* was counted using a Coulter Multisizer (Coulter Electronics Ltd.) with a 140-µm orifice. The detrital particles in fractions of 5.8–11.5 µm in diameter were tentatively regarded as SCD products. The weight distribution was calculated from the distribution of detrital particles, based on the hypothesis that the detrital particles were of a spherical form with a specific gravity of 1.0. All cultures were prepared in duplicate and the average values of data are shown.

demonstrated in treatment using *Ulva* sp. fronds collected from Lake Hamana.

Application of fermented products for foods and food-related industry

1. Foods

Many kinds of fermented foods have been developed to date. Most fermented foods are made from such terrestrial (agricultural and stockbreeding) materials as rice, soybean, barley, vegetables, and milk. Some products are produced from aquatic biomaterials, but no products have yet to be produced from aquatic plant materials (i.e. algae) (Fig. 1). The development of the lactic acid fermentation methods described above opened the possibility of producing fermented foods from algae. Seaweed sauce is one possible product to be developed. A major difficulty in developing seaweed sauce having commercial value is the shortage of amino acid compounds contained in the supernatant of fermented products prepared from seaweed.

Typical *Koikuchi* soy sauce contains more than 12 g N/100 mL of amino acid compounds (including peptides and proteins). In contrast, the supernatant of fermented products prepared from seaweed, such as *U. pinnatifida* and *Ulva* sp. (an unpublished observation), only contains 1-3 g N/100 mL of amino acid compounds, which can be explained by the low protein content of seaweed. Fresh seaweed is rich in moisture (contain ca. 87% on average)^{11,29}. And on average, seaweeds contain ca. 15% of protein (on a dry basis), while soy bean contains less moisture and ca. 40% of protein on a dry basis¹¹. However, *Susabinori* seaweed (*Porphyra* sp., Rhodophyta) contains an exceptionally high quantity of protein exceeding 50%¹¹. Amino acid-rich supernatant containing 16.5 g N/100 mL is obtained from the fermented products of *Susabinori* seaweed (an unpublished observation).

2. Marine silage as fisheries diet

Marine silage (MS) is a new dietary item prepared by decomposing seaweed to a cellular unit^{14,15,16} and per-

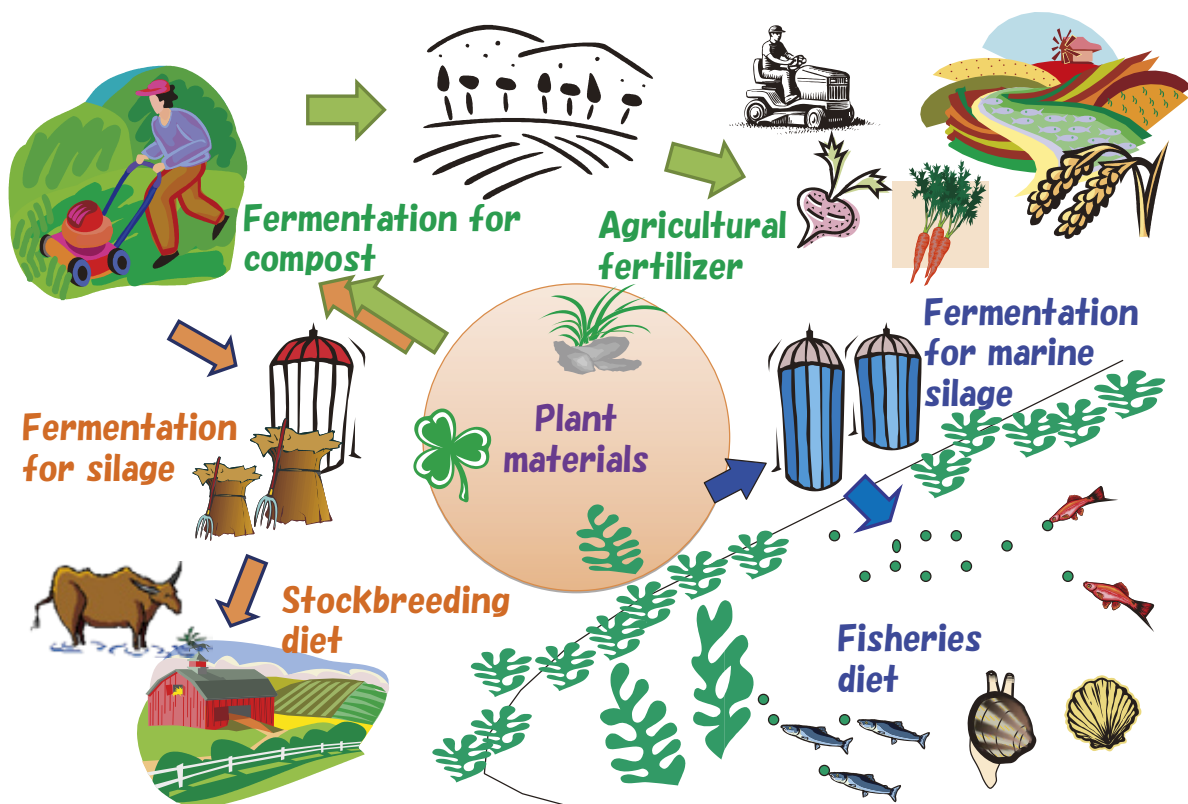


Fig. 6. Use of fermentation skills on plant materials for foods and diet production

Fermentation is rarely or never used in the fisheries industry, and Uchida proposed a conceptual diet of marine silage.²²

forming lactic acid fermentation (Fig. 6)^{24,26}. A 10-L-scale preparation and the long-term preservation (18 months) of MS was demonstrated using *U. pinnatifida* as a substrate (Fig.4)²⁰. The mass preparation of MS is very easy and the prepared MS was shown to be preservative for a long time (more than 18 months). In addition, two separate feeding trials were conducted to demonstrate the dietary value of MS for the Japanese pearl oyster (*Pinctada fucata martensii*)²⁰. In the first trial, pearl oysters grew significantly when fed MS at a rate of 3×10^4 cells/mL per day. The shell growth and survival rates (69 ± 11 $\mu\text{m}/\text{day}$; mean \pm SE and 100%, respectively, $n = 30$) of the pearl oysters were higher than those of the unfed control oysters (-26 ± 8 $\mu\text{m}/\text{day}$ and 100%, $n = 30$), while oysters fed *Chaetoceros calcitrans* (known as one of the most preferable microalgal diets for bivalves and used as a positive control diet) grew 205 ± 12 $\mu\text{m}/\text{day}$ ($n = 29$) and 96.7% survived. Proximate analysis of MS suggested a shortage of such nutritional elements as lipid content. The second rearing trial demonstrated remarkable shell growth when the pearl oysters were fed MS supplemented with a small quantity of *C. calcitrans*, as shown in Table 2. The dietary effect of MS prepared from *U. pinnatifida* was also demonstrated on *Rotifer* (a common hatchery diet for fish rearing)¹³. However, MS prepared from *Ulva* sp. showed a negative effect on the growth of short necked clam (*Ruditapes philippinarum*, (an unpublished observation). Small particles of *Ulva* prepared without fermentation also showed a negative effect on short necked clam growth, suggesting that *Ulva* fronds contain growth inhibitory compounds, and not a compound artificially produced through fermentation treatment.

The diet supplemented with MS prepared from *Eck-*

lonia sp. (Phaeophyta) at 10% w/w was fed to red sea bream challenged by an iridovirus. The survival rate of the fish fed a diet containing MS was found to be higher than that of the control group (Fig. 7)¹³.

3. Diet for stockbreeding

Seaweeds have been utilized as a stockbreeding diet¹⁰. One reason to use seaweed is to supply breeding animals with such valuable minerals such as iodine¹⁰. Usually, seaweed is added to the diet in the form of grain or

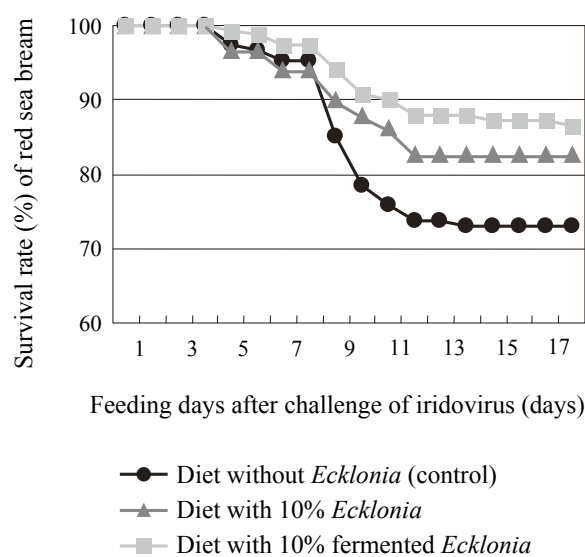


Fig. 7. Results of the feeding test for red sea bream

The fish were challenged by iridovirus and then fed a control diet, a diet containing *Ecklonia* sp. at 10% w/w, and a diet containing fermented *Ecklonia* sp. at 10% w/w.

Table 2. Results of feeding trials of MS and MSNF with young pearl oyster, *Pinctada fucata martensii*

Treatments	Feeding conditions(/ml/day)	Growth rate ($\mu\text{m day}^{-1}$)	Survival(%)
Unfed	No feed	-10 ± 14 ^{dc}	53.3
<i>Chaetoceros</i> (C)	<i>C. calcitrans</i> 3×10^4 cells	168 ± 33 ^a	66.7
1/10C	<i>C. calcitrans</i> 3×10^3 cells	-7 ± 10 ^{cdc}	66.7
MS	SCD prepared from <i>U. pinnatifida</i> (= MS) 2×10^4 particles	23 ± 13 ^{bd}	53.3
MS + 1/10C	MS 2×10^4 particles + <i>C. calcitrans</i> 3×10^3 cells	125 ± 13 ^a	80.0
$\times 2.5$ MS	MS 5×10^4 particles	47 ± 17 ^{bc}	66.7
MSNF	SCD prepared from <i>U. pinnatifida</i> without fermentation (MSNF) 2×10^4 particles	18 ± 16 ^{bc}	66.7
MSNF + 1/10C	MSNF 2×10^4 particles + <i>C. calcitrans</i> 3×10^3 cells	71 ± 14 ^b	93.3
$\times 2.5$ MSNF	MSNF 5×10^4 particles	23 ± 18 ^{bd}	53.7

Results are based on single trial and shown as the average \pm SEM. Treatments with common superscripts are not significantly different (Duncan's multiple arrangement test, $P < 0.05$)

powder, and no fermented product of seaweed has yet to be used. Fermented products prepared from *Porphyra* sp. and *U. pinnatifida* were supplemented in diets for both broiler and egg-laying chickens, and the dietary effects were examined. The addition of *Porphyra* sp. did not show any significant difference (an unpublished observation). In contrast, the addition of *U. pinnatifida* showed some significant effects, such as promoting growth, the moisture-retention ability of meat, and lipid metabolizing activity. However, all of these preferable effects were not observed every time, and more repeated testing is needed. Although the usefulness of seaweed as a stockbreeding diet is well accepted, whether the treatment by fermentation on seaweed is superior remains open for future study.

4. Fertilizer

Many kinds of seaweed have been utilized as fertilizer, such as *Enteromorpha* spp., *Ulva* spp. (Chlorophyta), *Gracilaria chilensis* (Rhodophyta), *Ascophyllum nodosum*, *Ecklonia maxima*, *Laminaria shinzii*, *Durvillaea potato-rum*, and *Sargassum* spp. (Phaeophyta)³⁰. Most algal fertilizer products are produced from seaweeds through a simple drying or extraction treatment¹⁰. Products called compost are produced by a kind of fermentation treatment. For example, compost was prepared from *Ulva rigida* collected in the Venice Lagoon after three weeks of fermentation³. Such treatment using fermentation is expected to promote the uptake of nutrients through the degradation of algal components, and prove useful in avoiding excess heat emission in soils and subsequent plant damage. We have conducted some preliminary studies on the fertilizing effects of seaweed products prepared by lactic acid fermentation. For example, the supernatant of fermented products prepared from *Ulva* sp. was given to a Japanese green tea farm. The farmer commented that the spines of the green tea leaves became sharper and more developed. However, this observation was not based on statistical analysis. Although the usefulness of seaweed as a fertilizer is well accepted, whether the treatment by fermentation on seaweed is superior remains open for future study.

Future prospects of algal fermentation

As for algal fermentation, the methane fermentation of seaweeds was initially studied during the 1970s and 1980s^{1, 2, 6}, for the purpose of energy supply^{9, 27}. Later studies on the lactic acid fermentation of seaweeds began in 1998, with a scope of developing foods and food production-related items^{17, 18, 19}. The development of halophilic starter cultures of lactic acid bacteria is left for future study. Technology for the ethanol fermentation of seaweeds began development in the 2000s^{9, 27}. Yeast and marine bac-

terium are used for ethanol fermentation^{4, 7, 8, 12}. Development of the ethanol fermentation of seaweeds will thus raise expectations for the acetic fermentation of seaweeds, because acetic acid is easily produced from ethanol by acetic acid bacteria⁴. Many kinds of fermentation require the saccharification process as a primary step. Seaweeds contain unique polysaccharides such as alginate and galactan, which are not contained in terrestrial plants^{9, 29}. Therefore, the development of enzyme products used for saccharification is important for the further development of algal fermentation technology. Development of the *koji* fermentation system (i.e. culture mold utilizing the mold's enzymes for saccharification) and the parallel double-fermentation system (for conducting fermentation in parallel with the saccharification process) could prove to be key technologies for the further development of algal fermentation technology^{4, 23}. Fermentation technology for microalgae also remains open for future study. Microalgae are richer in proteins and lipids than seaweeds¹¹, and have a high potential for obtaining economically valuable products in terms of foods, diets, fertilizers, bio-plastics, and the energy industry.

Conclusion

Fermentation can be conducted without supplying electric power and therefore can be considered an eco-friendly processing system. In particular, many Asian countries have traditional food cultures based on fermentation using agricultural and aquatic (fish) products⁴. We believe that there is a significant possibility of an algal fermentation industry being expanded in the future²⁸.

Acknowledgements

This study was partially funded by the Pioneer *tokubetsu kenkyu* program of the Agriculture, Forestry, and Fisheries Research Council, and by the *Suisan biomass no sigen-ka gijutsu kaihatsu jigyo* program (Development of conversion technology for utilization of fisheries biomass) of the Fisheries Agency. Part of the study on lactic acid bacteria was conducted in cooperation with Yakult Honsha Co., Ltd. And part of the study on fisheries diet was conducted in cooperation with Nippon Suisan Kaisha Ltd. The marine silos were developed in cooperation with Fuyo Ocean Development & Engineering Co., Ltd., EBARA JITSUGYO Co., Ltd., and Mr. Yoshimura (Hamanao Aosa Riyo Kyogikai). The study on the dietary effects for broiler chicken was conducted in cooperation with the Okayama Prefectural Research Institute for Daily and Stockbreeding.

References

1. Aquaculture Associates (1982) Energy from marine biomass. Program review, GRI Contract No. 5081-310-0458.
2. Chynoweth, D. P. (2002) Review of biomethane from marine biomass. <http://www.agen.ufl.edu/~chyn/index.htm>.
3. Cuomo, V. et al. (1995) Utilization of *Ulva rigida* biomass in the Venice Lagoon (Italy): biotransformation in compost. *J. Appl. Phycol.*, **7**, 479-485.
4. Ichishima, E. (1997) *Hakko Shokuhin e no shotai*. Shokabo, Tokyo, Japan, 156 [In Japanese].
5. Ishige, N. (1993) Cultural aspects of fermented fish products in Asia. In *Fish fermentation technology*, eds. Lee, Cherl-Ho et al., United Nations University Press, Tokyo, Japan, 13-32.
6. Japan Ocean Industries Association (JOIA) (1984) Investigation into fuel production from marine biomass, the second report: "total system" (FY Showa 58 nendo, *Kaiyo biomass niyoru nenryo-yu seisan ni kansuru chosa, seika houkokusho, dai-2-bu*; "total system") [In Japanese].
7. Kim, N. J. et al. (2011) Ethanol production from marine algal hydrolysates using *Escherichia coli* KO11. *Bioresour. Technol.*, **102**, 7466-7469.
8. Lee, S. et al. (2011) Converting carbohydrates extracted from marine algae into ethanol using various ethanolic *Escherichia coli* strains. *Appl. Biochem. Biotechnol.*, **164**, 878-888.
9. Notoya, M. (2011) *Seaweed bio fuel*. CMC Publishing, Tokyo, Japan, pp.204 [In Japanese].
10. Ohno, M. (2004) *Yuyo-kaiso-shi*. Uchidarokakuho, Toyko, Japan, pp.575 [In Japanese].
11. Science and Technology Agency (2002) *Standard tables of food composition in Japan 2002*. Kagawa Education Institute of Nutrition, Tokyo, Japan, pp.464 [In Japanese].
12. Takeda, H. et al. (2011) Bioethanol production from marine biomass alginate by metabolically engineered bacteria. *Energy Environ. Sci.*, **4**, 2575-2581.
13. Technical research association for new food creation (New food creation *gijutsu kenkyu kumiai*) (2005) Development of a new next-generation fermentation technology (*Jisedai-gata hakko gijutsu no kaihatsu*). In the annual report of a research project funded by the Ministry of Agriculture, Forestry and Fisheries (FY Heisei 16 nendo *kenkyu seika hokokusho*). Ministry of Agriculture, Forestry and Fisheries, Tokyo, Japan, 81-91 [In Japanese].
14. Uchida, M. (1997) Microbial conversion of macroalgae into a detrital hatchery diet. *JARQ*, **33**, 295-301.
15. Uchida, M. et al. (1997) Introduction of detrital food webs into an aquaculture system by supplying single cell algal detritus produced from *Laminaria japonica* as a hatchery diet for *Artemia* nauplii. *Aquaculture*, **154**, 125-137.
16. Uchida, M. et al. (1997) Conversion of *Ulva* fronds to a hatchery diet for *Artemia* nauplii utilizing the degrading and attaching abilities of *Pseudoalteromonas espejiana*. *J. Appl. Phycol.*, **9**, 541-549.
17. Uchida, M. (2002) Fermentation of seaweeds. *J. Jap. Lactic Acid Bac.*, **13**, 92-113 [In Japanese with English summary].
18. Uchida, M. & Murata, M. (2002) Fermentative preparation of single cell detritus from seaweed, *Undaria pinnatifida*, suitable as a replacement hatchery diet for unicellular algae. *Aquaculture*, **207**, 345-357.
19. Uchida, M. & Murata M. (2004) Isolation of a lactic acid bacterium and yeast consortium from a fermented material of *Ulva* spp. (Chlorophyta). *Journal of Applied Microbiology*, **97**, 1297-1310.
20. Uchida, M. et al. (2004 a) Mass preparation of marine silage from *Undaria pinnatifida* and its dietary effect for young pearl oyster. *Fish. Sci.*, **70**, 456-462.
21. Uchida, M. et al. (2004 b) Combinations of lactic acid bacteria and yeast suitable for preparation of marine silage. *Fish. Sci.*, **70**, 507-517.
22. Uchida, M. (2005) Studies on lactic acid fermentation of seaweed. *Bull. Fish. Res. Agen.*, **14**, 21-85 [Doctoral thesis, In Japanese with English abstract].
23. Uchida, M. et al. (2005) Effects of soy sauce *koji* and lactic acid bacteria on the fermentation of fish sauce from freshwater silver carp *Hypophthalmichthys molitrix*. *Fish. Sci.*, **71**, 422-430.
24. Uchida, M. (2007) Preparation of marine silage and its potential for industrial use. *Proceedings of the Thirty-fourth U.S.-Japan Aquaculture Panel Symposium*, 51-56.
25. Uchida, M. et al. (2007) Lactic acid bacteria effective for regulating the growth of contaminant bacteria during the fermentation of *Undaria pinnatifida* (Phaeophyta). *Fish. Sci.*, **73**, 694-704.
26. Uchida, M. & Miyoshi, T. (2008) Development of a new dietary material from unutilized algal resources using fermentation skills. *Bull. Fish. Res. Agen.*, **31**, 25-29.
27. Uchida, M. (2009) Recent topics in studies on biofuel production from aquatic biomass in Japan. *Nippon Suisan Gakkaishi (J. Jpn. Soc. Fish. Sci.)*, **75**, 1106-1108 [In Japanese].
28. Uchida, M. (2010) *Nihon hatsu kaiso-hakko-san'gyo no sohshutsu*. In *Suisan no 21 seiki*, eds. Tanaka, M. et al., Kyoto University Press, Kyoto, Japan, 442-456

- [In Japanese].
29. Uchida, M. (2011) Analysis and collection of data on algal fiber and sugar contents for fermentative utilization of seaweed biomass. *SEN'I GAKKAISHI*, **67**, 181-186 [In Japanese].
30. Zemke-White, L. W. & Ohno, M. (1999) World seaweed utilization: An-end-of-century summary. *J. Appl. Phycology*, **11**, 369-376.