Development of a new dietary material from unutilized algal resources using fermentation skills

水研センター研報，第31号，25－29，平成22年
Bull. Fish. Res. Agen. No. 31, 25-29, 2010

Development of a New Dietary Material from Unutilized Algal Resources Using Fermentation Skills

Motoharu UCHIDA * and Tatsuo MIYOSHI *

Abstract：Decreasing fishmeal supply is a major concern for the future of the aquaculture industry. Efforts to use protein from plants to replace the fishmeal diet have been made. However, algal biomass is another candidate to replace dietary fishmeal. Marine silage (MS) is fish dietary material prepared from algae by a fermentation process. During the 34th U.S.-Japan Aquaculture Panel Symposium, we reported on a method to prepare MS from seaweed by lactic acid fermentation. The objective of this work is to report recent progress.

The fermentation of seaweeds can be performed by enzymatic saccharification by cellulase, followed by the fermentation process with the use of lactic acid bacteria. MS was first prepared with *Undaria pinnatifida* (a brown alga). In this study, we prepared MS with *Ulva* spp. (green algae) which was causing nuisance blooms in Japanese coastal waters. We also tested preparing MS from microalgae.

Developing MS is interesting from three viewpoints. First, it makes possible the conversion of algal biomass resources into dietary materials for aquaculture. Secondly, the fermentation product is acidic and long-lasting at room temperature without storage energy costs. Therefore, surplus cultured microalgae can be preserved and utilized depending on a demand. Finally, fermented materials are expected to have some useful functions that contribute to fish health. We believe that MS is a new dietary material to compensate for the lack of fishmeal and can contributes the production of high quality cultured fish.

Key words : fish diet, lactic acid fermentation, marine silage, seaweed

Recently, seafood consumption has remarkably increased in many countries. However, natural fish resources are limited in the world. Therefore, the aquaculture industry is expected to produce more and more fish. The present aquaculture system needs large quantities of fishmeal for fish feeds. However, the fishmeal supply is limited. This is a major concern for the future of the aquaculture industry. To overcome this problem, studies to use plant proteins such as soybean meal and wheat flour have been made to replace the fishmeal in fish feeds. Algal biomass is another candidate to replace fishmeal. Marine silage (MS) is fish dietary material prepared from algae by a fermentation process. During the 34th U.S.-Japan Aquaculture Panel Symposium, we reported on the method to prepare MS from seaweed by lactic acid fermentation (Uchida, 2007). The objective of this work is to report on our recent progress.

The fermentation of seaweeds can be performed by the enzymatic saccharification by cellulase, followed by the fermentation process with the use of lactic acid bacteria. MS was first prepared with *Undaria pinnatifida* (Uchida and Murata, 2002; Uchida and Murata, 2004). In the case of MS prepared from *U. pinnatifida,* algal frond tissues are decomposed during the saccharification process and the fermentation products have a size of 5 ～ 10 μ

2010年 6 月21日受理（Received. June 21. 2010）
* National Research Institute of Fisheries and Environment of Inland Sea, FRA
2-17-5 Maruishi, Hatsukaichi, Hiroshima, Japan, 739-0452
Email: uchida@affrc.go.jp

m in diameter. This size is suitable as a bivalve diet and *Unadaria*-MS was demonstrated to contribute to the growth of a bivalve, *Pinctada martensii* in a rearing experiment (Uchida *et al*., 2004). MS prepared from *Ecklonia maxima* was supplemented in a formulated diet and given to red sea bream challenged with iridovirus (Uchida 2007). That study demonstrated that the MS had a function in promoting fish survival against the pathogen.

In this study, we demonstrated how to prepare MS with *Ulva* spp., which is causing nuisance blooms in Japanese coastal waters. We also examined how to prepare MS from microalgae.

Materials and Methods

Preparation of marine silage from *Ulva*

A sterile 20 L-polycarbonate tank with a screw cap (Nalgene) was used for the preparation of MS. Dried *Ulva* spp. fronds of 1.3 kg (particle size < 2mm) were dispensed into a tank containing 19.5 L of autoclaved distilled water, 26 g cellulase 12S (Yakult Pharmaceutical Ind. Co., Tokyo), 26 g macerozyme 2A (Yakult Pharmaceutical Ind. Co.), 2 g lactic acid bacteria (containing 2×10^{10} CFU of *Lactobacillus casei,* TOA Pharmaceuticals Co., Tokyo), and mixed well. The culture tank was capped, incubated at 20℃ with 70 rpm agitation (Tietech Co., Ltd, Tokyo) for 35 days, and then preserved without agitation. Frond decomposition was evaluated by measuring pecent weight proportion of algal products that passed through 100 μm nylon mesh. The number of algal particles in the culture was determined using a Coulter Multisizer (Beckman Coulter, Inc., Fullerton, CA) with a 140 μm orifice. The algal particles in the fractions of 5.8 ～ 11.5 μm in diameter were tentatively regarded as one cell product of *Ulva*-MS. Lactic acid was measured by an enzymatic method (F-kit D-/L-lactic acid, Roche Diagnostics, Basel). Lactic acid bacteria were counted on Plate Count Agar with BCP (BCP, Nissui Pharmaceutical Co., Ltd, Tokyo).

Preparation of marine silage from microalgae

Sterile 50 mL-polypropylene centrifuge tubes with a screw cap (Iwaki, Tokyo) were used for preparation of MS from microalgae. Five species of dietary microalgae: *Chrolella* sp., *Tetracelmis* sp., *Pavlova lutheri, Chaetoceros* sp., and *Nannochroropsis* sp. were tested. A dried product of a macroalga, *Undaria pinnatifida*, was also tested for comparison. In the cases of *Chrolella* sp. (Nissin Marinetech Co., Ltd) and *U. pinnatifida* (a gift from Riken Vitamin, Wakamidori, particle diameter <74 μm), 2g of the dried products were used for fermentation. In the cases of *Tetracelmis* sp., *P. lutheri*, and *Chaetoceros* sp., fresh cells were collected by centrifuge (3,000 rpm, 10 min.) from 200 mL cultures grown in our laboratory. Inthe case of *Nannochroropsis* sp., fresh cells were collected by centrifuge from 550 mL cultures grown in our laboratory. These algal cell samples were suspended in 40 mL 3.5% (w/v) NaCl solutions containing enzyme mixtures. Tube numbers and the enzyme compositions were: No.1 and No. 2, *U. pinnatifida* with 0.2 g cellulase; No.3, *Chrolella* sp. with 0.2 g cellulase, No.4, *Chrolella* sp. with 0.2 g cellulase and 0.2 g macerozyme; No.5, *Chrolella* sp. with 0.2 g cellulase, 0.2 g macerozyme and 0.2 g lactase Y-AO (Yakult Pharmaceutical Ind. Co.); No.6, *Tetracelmis* sp. with 0.2 g cellulose; No.7, *Tetracelmis* sp. with 0.2 g cellulase and 0.2 g macerozyme; No.8, *Tetracelmis* sp. with 0.2 g cellulase, 0.2 g macerozyme, and 0.2 g lactase; No.9, *P. lutheri* with 0.2 g cellulase; No.10, *Pavlova lutheri* with 0.2 g cellulase and 0.2 g macerozyme; No.11, *Chaetoceros* sp. with 0.2 g cellulose; No.12, *Chaetoceros* sp.with 0.2 g cellulase and 0.2 g macerozyme; No.13, *Nannochroropsis* sp. with 0.2 g cellulose; and No.14, *Nannochroropsis* sp.with 0.2 g cellulase and 0.2 g macerozyme. Four mg of a lactic acid bacteria product (containing 4×10^7 CFU of *Lactobacillus casei*) were added and incubated for nine days at 35℃ with 70 rpm agitation. Fermentation results were evaluated by measuring the pH and lactic acid content. Microscopic observation of the samples was also conducted.

Results and Discussion

Fermentation results of *Ulva* -MS

Time course change of the *Ulva* culture during fermentation is shown on Table 1. The pH value decreased from 6.87 at initial to 3.78 after 35 days of incubation, and remained constant at 3.80 during

preservation for one year. Frond decomposition proceeded from 7.6% initially to 90.5% after 35 days of incubation. One cell products were produced in the culture during incubation (Fig. 1) and the particle diameter of the products was 6.9 μm (mean value) at Day 35 (Fig. 2). The size of the one cell products became slightly smaller at 6.1 μm after one year. Lactic acid was produced at 1.99 g/L at Day 35, and further increased to 6.48 g/L after one year of preservation. Lactic acid bacteria made growth from 2.4×10^6 CFU/mL initially to 5.1×10^8 CFU/mL at Day 35. All of the colonies formed on the BCP plates were yellow colored and no contaminant bacteria were observed. The lactic acid bacteria counts gradually decreased to 1.0×10^3 CFU/mL after one year.

The present study demonstrated that lactic acid fermentation of *Ulva* spp. is possible as well as for *U. pinnatifida* as reported in a previous study. A suitable fermentation period for *Ulva* spp. was regarded as 35 days at 20℃ based on the observations that pH value, frond decomposition score, and one cell product number become constant, and lactic acid bacteria was maximized. The use of cellulase alone is effective enough to obtain the one cell products from *U. pinnatifida* (Uchida and Murata, 2002). In the case of *Ulva* spp, cellulase use alone was not effective in a laboratory study, but the use of macerozyme with cellulase was effective enough to obtain one cell products. The *Ulva*-MS could be preserved at least longer than one year at 20℃ without substantial change of characteristics.

Fermentation results of microalgae

Fermentation results for microalgae are shown in Table 2 with respect to *U. pinnatifida*. Lactic acid

Table 1. Fermentation results of *Ulva* sp.

	Incubation days (with agitation)			Preservation (without agitation)
	Day 0	Day 11	Day 35	After one year
pH	6.87	5.89	3.78	3.80
Frond decomposition (%)	7.6	60.2	90.5	100
No. of one cell products (/mL)	NT	NT	9.5×10^7	6.8×10^7
Particle diameter (mean, μm)	NT	NT	6.9	6.1
Lactic acid content (g/L)	<0.1	NT	1.99	6.48
No. of lactic acid bacteria (CFU/mL)	2.4×10^6	5.0×10^7	5.1×10^8	1.0×10^3

Frond decomposition: Per cent weight proportion of algal products that pass through a 100 μm nylone mesh aginst the total weight. NT: Not tested.

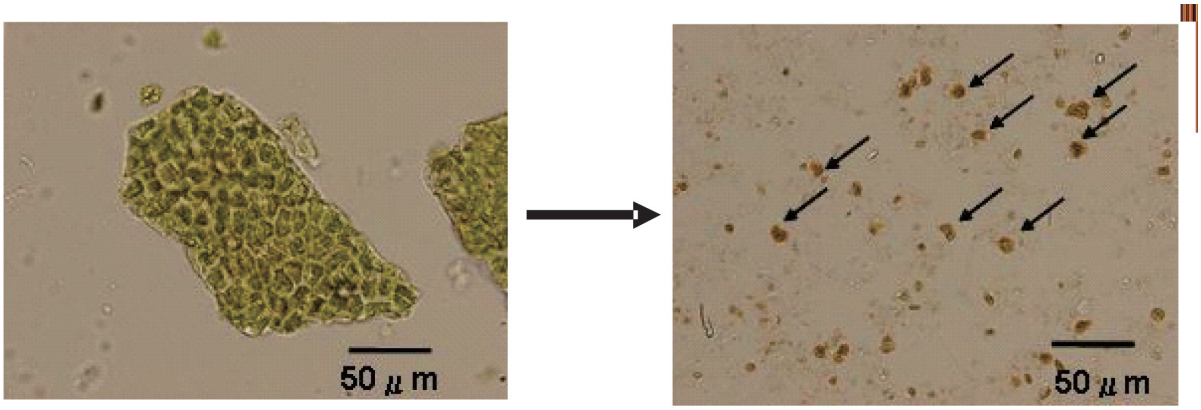

Fig. 1. Formation of one cell product from *Ulva* spp. Microscopic observation before (left) and after (right) 35 days fermentation.

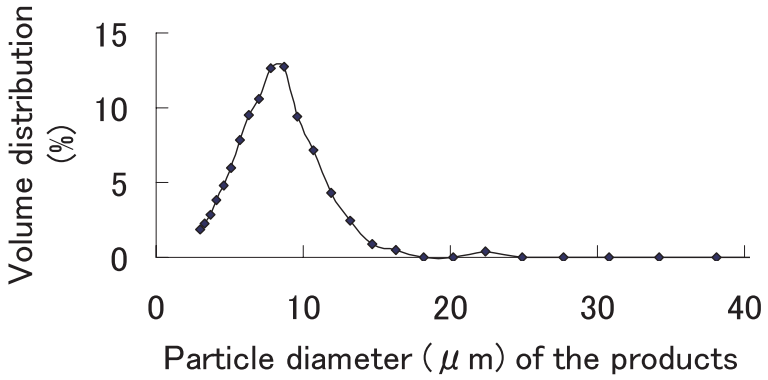

Fig. 2. Particle size distribution of *Ulva*-marine silage after 35 days fermentation.

fermentation could be performed for all the cases of microalgae as well as *U. pinnatifida* with only the use of cellulase. When comparing the results among the cases treated with cellulase alone, pH values were lower in microalgae (pH 3.32–3.80) than in the case of. *U. pinnatifida* (pH 3.87–3.90). Lactic acid contents of the cultures were 3.5 (mean), 5.4, 1.5, 1.3, and 1.3 g/L, for the cases of *U. pinnatifida, Chrolella* sp., *Tetracelmis* sp., *P. lutheri*, and *Chaetoceros* sp., respectively. Combinational use of macerozyme and lactase with cellulase resulted in an increase of lactic acid content in the cultures. Percent proportion of L-lactic acid against the total (L- and D-lactic acid) lactic acid was high (>76.2%) for most cases except that of *Chrolella* sp. In the case of *Chrolella* sp., percent proportion of L-lactic acid increased from 45.1% (with cellulase use) to 83.2% (with cellulose, macerozyme, and lactase use). Based on microscopic observations, microalgae cells looked somewhat changed in shape and were apparently partially damaged after enzymatic treatment, but the decomposition was limited and cell wall tissue remained in all cases. The color of the microalgae cells was also changed to brown, suggesting decomposition of chlorophyll content under the acidic culture conditions. Suitable enzyme product will need to be developed to obtain a cell wall-eliminated product (i.e., protoplast type marine silage).

Table 2. Fermentation results of microalgae

Tube No.	Algae samples	Enzymes			pH		Lactic acid	
		Cellulase	Maceroz.	Lactase	Initial	After ferment.	(Total, g/L)	(L−/Total, %)
1	*Undaria pinnatifida*	+			5.99	3.90	3.6	98.3
2		+			5.99	3.87	3.4	98.4
3	*Chrolella* sp.	+			6.26	3.74	5.4	45.1
4		+	+		6.32	3.64	7.9	59.2
5		+	+	+	6.35	3.29	9.6	83.2
6	*Tetracelmis* sp.	+			6.69	3.32	1.5	91.5
7		+	+		6.81	3.09	3.8	86.5
8		+	+	+	6.78	3.09	4.3	94.1
9	*Pavrova lutheri*	+			6.70	3.35	1.3	97.8
10		+	+		6.82	3.11	3.8	96.5
11	*Chaetoceros* sp.	+			8.63	3.80	1.3	76.2
12		+	+		7.83	3.31	4.0	97.0
13	*Nannochloropsis* sp	+			6.70	NT	NT	NT
14		+	+		6.64	3.15	NT	NT

NT: Not tested.

References

Uchida M. and 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.

Uchida M. and Murata M., 2004: Isolation of a lactic acid bacterium and yeast consortium from a fermented material of *Ulva* spp. (Chlorophyta). *J. Appl. Microbiol.*, **97**, 1297–1310.

Uchida M., Numaguchi K., and Murata M., 2004: Mass preparation of marine silage from *Undaria pinnatifida* and its dietary effect for young pearl oyster. *Fish. Sci.*, **70**, 456–462.

Uchida M., 2007: Preparation of marine silage and its potential for industrial use. Proceedings of the Thity-fourth U.S.-Japan Aquaculture Panel Symposium, 51–56.