Development of “marine silage” with a scope for constructing aquaculture for bioremediation, not bioremediation for aquaculture

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SUMMARY: “Marine silage” is a detrital hatchery diet, which is prepared by decomposing seaweed to a cellular level and fermenting it. For developing this new dietary item, firstly, we developed a microbial consortium (MC) composed of a lactic acid bacterium and two species of yeast available as a starter for performing lactic acid fermentation on seaweed. Quantitative preparation of the “marine silage” was conducted on a substrate of *Undaria pinnatifida* and its dietary value was demonstrated by rearing experiments for the pearl oyster, *Pinctada fucata martensii*. We suggest an extensive form of aquaculture, feeding fisheries animals on the “marine silage” prepared from blooming seaweed such as *Ulva* spp. with a scope to remediate excessively eutrophicated coastal waters.

KEY WORDS: hatchery diet, fermentation, seaweed, silage

INTRODUCTION

Fermentation has a long history and has been playing important roles in agriculture and livestock farming for preparing compost and silage diet, respectively. In contrast, fermentation skills are rarely utilized in aquaculture industry. One of this reason is attributable to the fact that fermentation on algal materials has never been developed to date. Furthermore, microorganisms available for performing fermentation are rarely isolated from aquatic environments. The present paper

reports the first isolation of a microbial consortium (MC) that is available as a starter for performing lactic acid fermentation on seaweed. Furthermore, quantitative preparation of single cell detritus (SCD) from seaweed was demonstrated under fermentative conditions with the use of the MC. The potential use of the SCD as a replacement hatchery diet for microalgae will be discussed with possible another application as “marine silage” in an extensive fish farming system.

Isolation of a microbial consortium for

fermenting seaweed

The microbial flora contained in the four times transferred cultures of *Ulva*-fronds were thoroughly investigated. The numbers of microorganisms estimated by the agar plate counting coincided with that by the direct counting, suggesting the predominant microorganisms related with the *Ulva*-fermentation could be isolated from the agar plates used for microbial counting. Lactic acid bacteria and yeast were observed to be predominant for the case of the fermented cultures of *Ulva* while marine bacteria such as *Vibrio*- and *Alteromonas*-groups were predominant for the case of the rotted culture. The lactic acid bacteria predominant in the

fermented cultures were characterized as *Lactobacillus brevis* while predominant yeast were grouped into two types and tentatively characterized as *Debariomyces hansenii* var. *hansenii* and *Candida zeylanoides*, respectively, based on the results from morphological and physiological tests and the determination of nucleotide sequences of 16S (for bacteria) and 18S (for yeast) rRNA gene.

The addition of the cell suspension mixture which were prepared by culturing the representative strains of the dominant three species of *L. brevis*, *D. hansenii*, and *Candida* sp. (i. e., strains B5201, Y5201, and Y5206, respectively), individually, were observed to act as a starter for performing lactic acid fermentation on various kinds of seaweed.

Table 1 Microbial flora in the *Ulva*-fermented and *Ulva*-rotted cultures

Cultures	Supplement of:			D. C. (cells mL ⁻¹)				V. C. (CFU mL ⁻¹)		
	NaCl	Cellulase	Seed	Total microbes	Heterotrophic microbes ^a	Lactic acid bacteria ^b	Yeast and mold ^c	Marine microbes ^d		
No. 5A (Fermented)	+	+	+	6.6±1.5×10 ⁷	6.0±0.1×10 ⁷	7.1±4.1×10 ⁷	2.8±1.5×10 ⁷ (Yeast >98%)	8.1±2.2×10 ⁶ (Yeast >99%)		
No. 5B (Rotted)	-	-	-	2.0±0.8×10 ⁹	7.2±0.4×10 ⁸	4.0±6.9×10 ⁶	<10 ⁶	8.2±0.4×10 ⁸		
No. 5C (Fermented)	+	+	-	1.2±0.8×10 ⁸	1.2±0.2×10 ⁸ (Yeast >99%)	<10 ⁶	7.9±2.2×10 ⁷ (Yeast >99%)	1.2±0.1×10 ⁸ (Yeast >99%)		

^aCounted with Standard Method Agar.

^bCounted with Plate Count Agar with BCP.

^cCounted with Sabouraud Agar.

^dCounted with Marine Agar 2216.

Average ± SD.

Table 2 Characterization and typing of bacteria isolated from *Ulva*-fermented cultures.

Cultures	Strains No.	Characteristics							Groups	Clusters based on partial 16S rDNA seq.
		Gram	Cell shape	Motility	Pigment	O/F	Oxidase	Others		
No. 4 ^a (Seed of No. 5)	B4101-B4120	+	Rod	-	-	F	-	Catalase -	<i>Lactobacillus</i>	<i>Lactobacillus</i> 1
No. 5A ^a	B5201-B5220	+	Rod	-	-	F	-	Catalase -	<i>Lactobacillus</i>	<i>Lactobacillus</i> 1
No. 5B ^b	B5121, B5122, B5124, B5127, B5129, B5130, B5131, B5133, B5135, B5138, B5139, B5117	-	Short rod	+	-	F	+	Gas +	<i>Vibrio</i>	NT ^c
	B5125, B5126, B5129, B5133	-	Rod	+	-	O	+	No rosetta, Gelatin degradation	<i>Vibrio</i>	NT ^c
	B5125, B5126, B5129, B5133	-	Rod	+	-	O	+	No rosetta, Gelatin degradation	<i>Alteromonas</i>	NT ^c
	B5123, B5136, B5140	-	Rod	+	-	-	±	No rosetta, Gelatin degradation	<i>Alcaligenes-Pseudomonas</i>	NT ^c
	B5134	+	Coccoid	+	-	F	+	Catalase -	<i>Streptococcus-Leuconostoc</i>	NT ^c

^a20 colonies were picked up at random from Plate Count Agar with BCP plates.

^b20 colonies were picked up at random from Standard Method Agar plates.

^cNT: Not tested.

Fermentative preparation of SCD from *Undaria pinnatifida*

Optimum culture conditions were investigated for fermentative preparation of single cell detritus (SCD) from an *Undaria pinnatifida* material (a dry material, 200-mesh through) with the use of the MC. The favorable culture compositions were determined for 5-10% (w/v) *Undaria*, 0.1-1% (w/v) cellulase (R-10), and 2.5-3.5% (w/v) NaCl against distilled water with the addition of 1% volume each of cultured cell suspensions (OD₆₆₀=1) of the

strains B5201, Y5201, and Y5206. The incubation of the culture can be performed without shaking under a semi-aerobic condition at 5-50°C. The maximum number of the SCD products could be obtained within 2 weeks. Growth of putrefactive microorganisms were not observed even after leaving the culture for 18 months at 20°C. The SCD products contained in the culture water were ca. 10⁸ cells/ml or 300 mg/ml in a wet basis before preservation, which is a much higher level compared to the usual cases of microalgae cultures.

Fig. 1 Correlation between the cellulase concentrations and the numbers of the SCD products in the *Undaria* cultures prepared without the microbial consortium.

Table 3 Characteristics of the *Undaria*-culture waters prepared without or with the addition of the microbial consortium.

	Cellulase% (w/v) of culture solution				
	0	0.1	0.25	0.5	1.0
Without the microbial consortium	Rotted	Rotted	Rotted	Rotted / Fermented ^a	Fermented
With the microbial consortium	Rotted	Fermented	Fermented	Fermented	Fermented

Judged by the microbial flora investigated by the agar plate method and the sensory order of the duplicated cultures.

^aOne culture was rotted and the other was fermented.

Fig. 2 Concept of trophic enhancement using "marine silage".

Dietary values of the SCD or "marine silage"

Dietary values of the SCD were demonstrated by a feeding test for young pearl oyster, *Pinctada fucata martensii*. Feeding of the SCD diet at 3×10^4 cells/ml/day promoted the growth of the bivalve at $69 \mu\text{m/day}$ increase of shell length and 100% survival when feeding of *Chaetoceros calcitrans* marked $205 \mu\text{m/day}$ increase of shell length and 96.6% survival. Furthermore, combinative feeding of the SCD at 2×10^4 cells/ml/day and the *C. calcitrans* at 3×10^3 cells/ml/day showed synergistic dietary effects at $169 \mu\text{m/day}$ shell increase and 80.0% survival when the feeding of the SCD and the *C. calcitrans* alone marked $30 \mu\text{m/day}$ shell increase and 53.5% survival and $11 \mu\text{m/day}$ shell increase and 66.7% survival, respectively. This observation suggested that supplementing of some nutritional compounds such as, for example, lipid elements might be effective for the use of the SCD diet.

Future prospects

The development of the "marine silage" suggests a new style of aquaculture. Recently, nuisance blooms of *Ulva* spp. are observed in many places of Japanese coastal area. Blooms of *Ulva* are attributed to eutrophication caused by human activities. Therefore, if these *Ulva* fronds are converted to a hatchery diet by the presently developed "marine silage" system and contribute to the coastal production, it will result in the remediation of coastal waters. In this case, aquaculture will be conducted for the purpose of bioremediation, which is quite different from the conventional idea that bioremediation will be considered for aquaculture.

REFERENCES

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