The JSFS 85th Anniversary-Commemorative International Symposium
"Fisheries Science for Future Generations"
Symposium Proceedings, No. 08007

Original article

The genomic analysis of marine brown algae, wakame (*Undaria pinnatifida*), and its colocalizing bacteria

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Keywords: Bacteria; Gametophytes; Seaweed; 16S rRNA gene.

Received: 21 August 2017 / Accepted: 6 September 2017

Introduction

Wakame (*Undaria pinnatifida*), one of the typical marine brown algae consumed in Japan, is mostly produced by aquaculture in the coastal region of Japan, China and Korea. The Great East Japan Earthquake occurred in March 2011, which led to enormously huge tsunami over the Pacific coastal region, damaging seriously wakame aquaculture field. Since then it has been important for people engaged in wakame aquaculture in these areas to enhance the brand image of wakame products in order to reconstitute their supply chain for recovery from such disaster. This background aroused us to study on wakame produced from these areas whether or not they have any possible genetic differences from other areas.

We report here an attempt to analyze total genome of wakame and its colocalizing bacteria.

Materials and methods

Preparetion of wakame gametophytes
Wakame male and female gametophytes were prepared from live specimens of matured sporophylls produced in Taro, Iwate Prefecture, and from those produced in Hayama, Kanagwa Prefecture, as a reference. These were incubated at 20°C under the conditions of 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 12:12 h light:dark cycle as previously reported [1] with some modification.

DNA extraction and bioinformatic analysis
Wakame gametophytes prepared as above and weighing 50-100 mg were subjected to DNA extraction and purification using only ISOPLANT (Nippon Gene, Tokyo, Japan) or together with the treatments with alginate lyase (Nippon Gene) and GM quicker 2 (Nippon Gene) according to the manufacturer's manuals. Shotgun libraries were constructed using Nextera XT DNA preparation kit (Illumina, San Diego, CA, USA) with the manufacturer's recommendation. The obtained shotgun libraries were subjected to sequencing with an Illumine MiSeq (Illumina) and subsequent data processing using CLC Aseembly Cell™ version 8.0 (QIAGEN, Hilden, Germany). The genome size and K-mer were determined by Jellyfish ver 2.2.4 [2]. For analyzing bacteria colocalizing with wakame, the 16S rRNA gene was amplified by PCR with a primer set specific to bacteria, targeting a V1-V2-V3 region. The amplified products were then sequenced with an Illumina MiSeq and the obtained datasets were analyzed using SILVAngs pipelines [3].

Results and discussion

The outline of wakame genome data obtained
Total reads from Taro male, Taro female, Hayama male and Hayama female gametophytes were 4.8, 5.7, 5.8 and 1.6 Gb, respectively, whereas the genome sizes estimated by Jellyfish were 99, 162, about 1900 and 89 Mb, respectively (Table 1). Since we also determined

Table 1. Total reads of wakame gametophytes and estimated genome sizes by Jellyfish

	Taro male	Taro female	Hayama male	Hayama female
Total reads	$4{,}751\times10^6$	$5{,}658\times10^6$	$5{,}689\times10^6$	$1{,}605\times10^6$
Estimated genome size	99.0×10^6	162.6×10^6	$1{,}896\times10^6$	89.2×10^6

the genome size of wakame as about 600 Mb by flow cytometry with the rice genome as a reference in our preliminary experiments (data not shown), this discrepancy in the genome size between the two analytical methods is probably due to insufficient sizes of the datasets we obtained by Illumina MiSeq sequencing. Thus, further sequence data are necessary for detailed analysis.

The total assembled lengths for Taro male, Taro female, Hayama male and Hayama female gametophytes were 0.36, 0.39, 0.46 and 0.25 Gb, respectively (Table 2). While the average contig lengths were 739, 789, 955 and 657, respectively, in the above order, the numbers of contigs were 492, 492, 479, 378 x 10^3, respectively, and N50s were 971, 1,065, 1,546 and 721 bp, respectively, indicating that the genome sequences are still fragmented. Our present datasets revealed 50.6–53.3% GC content, irrespective of different gametophytes.

Table 2. Summary of the assembly of genome sequences for wakame gametophytes

	Taro male	Taro female	Hayama male	Hayama female
Total assembled length	364×10^6	389×10^6	457×10^6	249×10^6
Number of contigs	492×10^3	492×10^3	479×10^3	378×10^3
Average contig length	739	789	955	657
N50	971	1,065	1,546	721
G+C content	50.6 %	50.9 %	51.0 %	52.2 %

Bacterial flora colocalizing with wakama gametophytes

Total reads of the 16S rRNA gene were 0.4, 0.4, 0.5 and 0.6 Mb for Taro male, Taro female, Hayama male and Hayama female gametophytes, respectively, whereas the average length was about 470, irrespective of different gametophytes (Table 3).

Table 3. Summary of the 16S rRNA gene sequencing for wakame gametophytes

	Taro male	Taro female	Hayama male	Hayama female
Total reads	408×10^3	435×10^3	528×10^3	601×10^3
Average length	479	471	479	475

Then, SILVAngs analyses were performed using default parameters. The contigs contained the sequences of mitochondria and chloroplasts. After removing such sequences of the 16S rRNA gene amplicons, the operational taxonomic units (OTUs) were calculated only for bacteria containing > 1 % or more relative to the total. As a result, different bacterial communities were found between the four gametophytes at the class level (Fig. 1). However, the major bacteria belonged to Alphaproteobacteria and Gammaproteobacteria, irrespective of different gametophytes. Although the major bacteria belonged to *Marinobacter* and *Sphingorhabdus* at the genus level for all gametophytes, the composition of minor bacteria was different among them (Fig. 2).

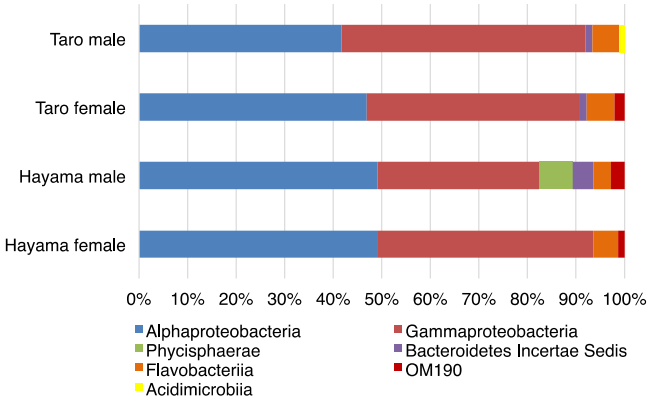

Fig. 1. Taxnomical classification of bacteria colocalizing with wakame genometophytes at the class level. Bacteria contained > 1% are shown.

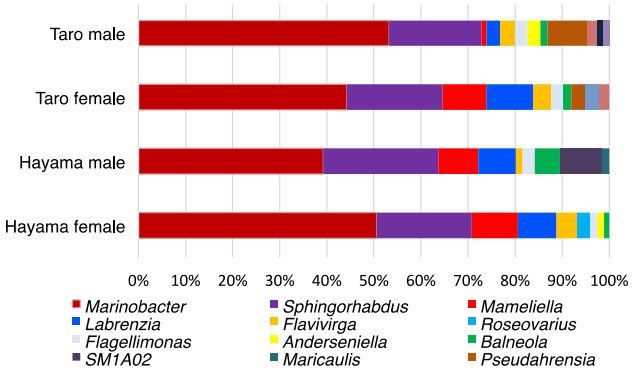

Fig. 2. Taxnomical classification of bacteria colocalizing with wakame genometophytes at the genus level. Bacteria contained > 1% are shown.

Acknowledgements

We would like to express our sincere thanks to Dr. Takahiro Saido, Iwate Fisheries Technology Center, and Dr. Katsunori Yamaki, Hayama Marine Science Laboratory, Kajima Technical Research Institute, for providing us with matured wakame sporophylls. Thanks are also due to Dr. Tomoko Abe, Dr. Hiroyuki Ichida and Mr. Katsunori Ichinose, Accelerator-Based Science Ion Beam Breeding Team, RIKEN Nishina Center, for cytometric analysis of the genome size.

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