Original article

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

Yoshihiro Takahashi 1, Md. Shaheed Reza 1,2, Nanami Mizusawa 1, Ko Yasumoto 1, Misturu Jimbo 1, Nobuyoshi Nanba 1, Hiroki Koyama 1,3, Kazutoshi Yoshitake 1,4, Takanori Kobayashi 5, Yoichi Sato 6 and Shugo Watabe 1,*

1 School of Marine Biosciences, Kitasato University, Sagamihara, Kanagawa 252-0373, Japan
2 Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
3 Graduate School of Biosphere Science, Hiroshima University, Higashi-hiroshima, Hiroshima 739-8528, Japan
4 Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan
5 Japan Fisheries Research and Education Agency, National Research and Development Agency, Yokohama, Kanagawa 220-6115, Japan
6 Riken Food Co. Ltd., Tagajo, Miyagi 985-0844, Japan
*Correspondence: swatabe@kitasato-u.ac.jp; Tel.: +81-42-778-9094

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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

Preparation 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, Kanagawa Prefecture, as a reference. These were incubated at 20°C under the conditions of 1500 µmol m⁻² 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 Assembly 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     | 4,751×10⁹       | 5,658×10⁹       | 5,689×10⁹       | 1,605×10⁹       |
| Estimated genome size | 99.0×10⁸ | 162.6×10⁸ | 1,896×10⁸ | 89.2 ×10⁸ |

http://www.jsfs.jp/
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

<table>
<thead>
<tr>
<th></th>
<th>Taro male</th>
<th>Taro female</th>
<th>Hayama male</th>
<th>Hayama female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total assembled length</td>
<td>3.64 x 10^9</td>
<td>3.89 x 10^9</td>
<td>4.57 x 10^9</td>
<td>2.49 x 10^9</td>
</tr>
<tr>
<td>Number of contigs</td>
<td>4.92 x 10^3</td>
<td>4.92 x 10^3</td>
<td>4.79 x 10^3</td>
<td>3.78 x 10^3</td>
</tr>
<tr>
<td>Average contig length</td>
<td>739</td>
<td>789</td>
<td>955</td>
<td>657</td>
</tr>
<tr>
<td>N50</td>
<td>971</td>
<td>1,065</td>
<td>1,546</td>
<td>721</td>
</tr>
<tr>
<td>G+C content</td>
<td>50.6 %</td>
<td>50.9 %</td>
<td>51.0 %</td>
<td>52.2 %</td>
</tr>
</tbody>
</table>

Bacterial flora colocalizing with wakame 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

<table>
<thead>
<tr>
<th></th>
<th>Taro male</th>
<th>Taro female</th>
<th>Hayama male</th>
<th>Hayama female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total reads</td>
<td>4.08 x 10^7</td>
<td>4.35 x 10^7</td>
<td>5.28 x 10^7</td>
<td>6.01 x 10^7</td>
</tr>
<tr>
<td>Average length</td>
<td>479</td>
<td>471</td>
<td>479</td>
<td>475</td>
</tr>
</tbody>
</table>

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

![Fig. 1](image)

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

![Fig. 2](image)

Acknowledgements

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References