Fermentation Properties of Low-Quality Red Alga Susabinori Porphyra yezoensis by Intestinal Bacteria

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Susabinori (Porphyra yezoensis), a red alga, is cultured and processed into a sheet-style dried food, nori, in Japan. But significant amounts of cultured susabinori, which has a low protein content is discarded because of its low quality. The protein content of nori has been reported to be correlated inversely with the carbohydrate content. In this study, we examined the relationship between the protein content and the fermentation of nori by means of bifidobacteria. nori with a low protein content (25% on dry base) was strongly fermented by bifidobacteria, whereas nori with a high protein content (41% on dry base) was not. nori with a low protein content contained large amounts of glycerol galactoside (GG, floridoside: 2-O-glycerol-C11-D-galactoside, isofloridoside: 1-O-glycerol-C11-D-galactoside), more than 10% w/w in the dried condition, and GG was the main substrate for fermentation by bifidobacteria. GG was not digested by digestive enzymes, and was not absorbed in the small intestine. These results suggest that GG can be used as a substrate for fermentation by bifidobacteria, and possibility of GG as a prebiotic.

Key words: nori; Porphyra yezoensis; glycerol galactoside; floridoside; fermentation

Susabinori (Porphyra yezoensis), a red alga, is extensively cultured in Japan. The amount of annual production of susabinori reaches about 300,000 metric tons (wet weight).13 Susabinori is processed into sheet-style dried nori. Nori is popularly consumed in East and Southeast Asia, and is an important component of sushi. Generally, the quality of nori is highly correlated with its blackness. The degree of blackness depends on the amount of pigments contained in it, such as chlorophylls, carotenoid, and phycobilins.23 Every year, a significant amount, exceeding 2% in a bad year, of cultivated nori is disposed of because of its low quality, associated with a low degree of blackness. In order to add value to this low quality nori, various studies of the food functionality of the nori and its components have been carried out.1–12

Low quality nori is characterized by its low degree of blackness and lower protein content than that of normal quality products. It has been reported that the protein content of nori is inversely correlated with the carbohydrate content,13 and hence low-quality nori contains rich carbohydrates. Some of the non-digestible carbohydrates are known to act as prebiotics that enhance intestinal bacterial flora.14 Kawadu et al. studied the effect of dietary normal-quality nori on the cecum microflora of rats. They reported that the ratio of bifidobacteria to total counts of cecum microflora increased with administration of normal-quality nori.15 Porphyran, a major carbohydrate in nori, and xylan, mannan, floridean starch, and glycerol galactoside (GG), constitute the carbohydrate fraction of nori.16 However, the carbohydrate composition of low-quality nori has not been sufficiently studied. The influence of dietary low-quality nori on gut microflora also has not been studied.

In this study, we examined fermentation properties of normal and low quality nori by bifidobacteria, which play an important role in human health among intestinal flora. We fractionated and identified the carbohydrates that are utilized in fermentation by bifidobacteria.

Materials and Methods

Nori samples. Ten commercial samples of nori cultivated and processed in Kumamoto, Japan, which had different levels of quality (protein content) were used. The protein content varied between 17 and 41%
Nori samples were ground and filtered through a 0.5 mm mesh filter. For following experiments, a nori sample having 25% protein content was used as a low-protein content sample, and one having 41% protein content was used as a high-protein content sample.

Chemical analyses. Total sugar contents were measured by the phenol-sulfuric acid method\(^{[17]}\) using glucose as a standard. Galactose was measured using F-kit lactose/galactose (Roche, Basel, Switzerland). Glucose was measured using Glucose E-test kit (Wako Pure Chemical Industries, Osaka, Japan). Protein content was measured by the Kjeldahl method.

Intestinal bacteria. Seventeen strains of intestinal bacteria supplied by the Japan Collection of Microorganisms (Riken Bioresource Center, Saitama, Japan) were used in the fermentation experiments. Bacterial strains were precultured in GAM broth (Nissui Pharmaceutical, Tokyo) at 37°C under anaerobic conditions using an anaerobic jar, AnaeroPack (Mitsubishi Chemical, Tokyo).

Fermentation of nori by bifidobacteria. Five strains of *Bifidobacterium* were used in this experiment. Two nori samples (1.5 g, low and a high protein content) were suspended in 30 ml of medium, which was composed of equal volumes of modified GAM semisolid without dextrose and physiological saline,\(^{[18]}\) The same medium without nori was used as a control. After being sterilized at 115°C for 15 min, each medium was inoculated with 0.75 ml of an active culture of bifidobacteria that had been subcultured in the medium, which was composed of equal volumes of modified GAM semisolid without dextrose and physiological saline, for 4 d at 37°C under anaerobic conditions. pHs of samples were measured with a pH meter on the 0 and 4 d. \(\Delta pH\) was calculated from each pH by the following definition for estimation:

\[
\Delta pH = \frac{pH_{control}(4\text{ d}) - pH_{sample}(0\text{ d})}{pH_{sample}(4\text{ d}) - pH_{sample}(0\text{ d})},
\]

where \(pH_{control}\) and \(pH_{sample}\) mean pH of control and sample at 0 and 4 d.

The degree of fermentation was estimated using the following standards.

- : \(\Delta pH \leq 0\)
- : \(0 < \Delta pH \leq 0.5\)
+ : \(0.5 < \Delta pH \leq 1.0\)
+++ : \(1 < \Delta pH \leq 1.5\)
++++ : \(1.5 < \Delta pH\)

Gel filtration chromatography (GFC) of carbohydrate fraction. The water-soluble carbohydrate fraction was prepared from nori samples by the method of Osumi *et al.*\(^{[19]}\) with a modification. Nori samples (50 g each) with protein contents of 25 and 41% were soaked in 750 ml of 7% formaldehyde solution. Twelve h later, 250 ml of distilled water was added to each sample, and the sample solution was incubated for 8 h in a boiling water bath. After filtration through diatomaceous earth, the filtrate was concentrated to 1/4 volume with a rotary evaporator. The concentrate was used as the water-soluble carbohydrate solution.

The water-soluble carbohydrate solution (5 ml) was applied to a gel filtration column (2.6 x 80 cm) embedded with HW-40F (Tosoh, Tokyo) and equilibrated with 0.8 M NaCl. Carbohydrates were eluted with 0.8 M NaCl at a flow rate of 1 ml/min. Eluent was collected at 20 ml of each fraction. Total sugar content of each fraction was determined by the phenol-sulfuric acid method. Salt in each fraction was eliminated by Sephadex G-10 (Amersham Japan, Tokyo) column (2 x 30 cm) chromatography.\(^{20}\) The eluents were condensed to 3 ml with a rotary evaporator.

Fermentation of GFC fractions by bifidobacteria. Modified GAM semisolid without dextrose (3 ml) was added to each GFC fraction of carbohydrate of a nori sample of low protein content. After being sterilized at 115°C for 15 min, the mixture was inoculated with 0.2 ml of an active culture of *Bifidobacterium adolescentis* that had been subcultured in the medium, which was composed of equal volumes of modified GAM semisolid without dextrose and physiological saline, for 4 d at 37°C under anaerobic conditions. Then the mixture was incubated under the same conditions. Fermentation of each mixture was confirmed by measurement of pH using a pH meter. As a control, 3% NaCl solution was used in the same way. \(\Delta pH\) was calculated from each pH level by the following definition:

\[
\Delta pH = pH_{control}(4\text{ d}) - pH_{fraction}(4\text{ d}),
\]

where \(pH_{control}\) and \(pH_{fraction}\) mean pH of control and fraction after 4 d.

Carbohydrate composition was analyzed by HPLC for the GFC fractions in which fermentation was observed. CARBOSep CHO-411 (Transgenomic, San Jose, CA) was used as a column, and the eluent was monitored using a refractive index detector (L-3300, Hitachi, Tokyo). The column temperature was maintained at 75°C. Distilled water was used as a mobile phase at 0.5 ml/min. Injection volume was 10 μl.

Extraction of glycerol galactoside (GG) from nori. GG was extracted from nori basically by the method of Noda *et al.*\(^{21}\) One liter of 75% ethanol was added to 150 g of a nori sample of low protein content. The mixture was boiled for 30 min in a 90°C water bath and filtered through 5B filter (Advantec, Tokyo). The residue was extracted 2 times by the same procedure. Each extract was concentrated to about 250 ml under reduced pressure at 35°C with a rotary evaporator, and defatted 2 times with 250 ml of diethyl ether. The defatted extracts were combined, concentrated under reduced pressure at 60°C, and made up to 100 ml.

A twenty-ml portion of the concentrate was applied to
a column (2 x 30 cm) of Diaion HPK25 (H\(^+\) form: Mitsubishi Chemical, Tokyo), and eluted with distilled water at a flow rate of 1 ml/min. The eluent was passed successively through a column (2 x 30 cm) of Diaion HPK75 (OH\(^-\) form, Mitsubishi Chemical), and collected from 0 to 400 ml. The same procedure was repeated 2 times using the rest of the concentrate. Each eluent was combined and concentrated to about 30 ml under reduced pressure at 60\(^\circ\)C, and crystallized by freeze-drying.

HPLC and nuclear magnetic resonance (NMR) spectrometry were used to confirm that this crystal was GG. \(^1\)H and \(^{13}\)C NMR spectroscopy were recorded with a FT-NMR spectrometer (ECA-500, Jeol, Tokyo) in D\(_2\)O solutions with TSP (3-(trimethylsilyl) propionic-2, 2, 3, 3, -d\(_4\) acid sodium salt) as an internal standard. F, Floridoside: 2-O-glycerol-\(\alpha\)-D-galactoside; D, D-isofloridoside: 1-O-D-glycerol-\(\alpha\)-D-galactoside; L, L-isofloridoside: 1-O-L-glycerol-\(\alpha\)-D-galactoside.

For estimation of the purity of GG in this crystal, 1 N HCl was added to 0.1% w/v aqueous solution of the crystal, and the solution was heated to 121\(^\circ\)C for 20 min. After neutralization of this solution with 1 N NaOH, the amount of galactose generated from GG hydrolyzed by heating was measured as described above. The purity of GG was 93.5% w/w by measurement, as described above.

**Determination of GG content in nori samples.** GG content was determined in 10 samples of nori with different protein contents (17–41\%), as described below. Seventy-five percent ethanol of 100 ml was added to 5 g of the nori samples. The ethanol solution was boiled for 30 min in a 90\(^\circ\)C water bath, and filtered through 5B filter. The residue was extracted 2 times with 50 ml of 75% ethanol by the same procedure. Each ethanol extract was concentrated to about 50 ml under reduced pressure at 35\(^\circ\)C, and defatted 2 times with 100 ml of diethyl ether. The defatted extracts were combined, concentrated under reduced pressure at 60\(^\circ\)C, and made up to 50 ml. After filtration with a 0.4\(\mu\)m filter, sample solution was analyzed by HPLC. HPLC conditions are described above. The amount of GG in each sample was estimated from the peak area using GG extracted as described above as a standard.

**Fermentation of GG by intestinal bacteria.** Fermentation of GG by intestinal bacteria was assayed by the method of Osumi \textit{et al.}, \textsuperscript{8) with a modification. GG was dissolved at a concentration of 0.5% in 4 ml of the...
medium, which was composed of 250 ml of modified GAM semisolid without dextrose and 750 ml of physiological saline. For comparison, 0.5% glucose solution was used. After being sterilized at 115°C for 15 min, each solution was inoculated with 0.3 ml of an active culture of intestinal bacteria that had been subcultured in the same medium for 4 d at 37°C, and was incubated at 37°C for 7 d under anaerobic conditions. pH of each solution was measured with a pH meter after 7 d. For a control, the same medium without a sample was treated in the same way.

\[
\Delta pH = pH_{cont} (7 \text{ d}) - pH_{samp} (7 \text{ d})
\]

\(pH_{cont} (7 \text{ d})\) and \(pH_{samp} (7 \text{ d})\) mean pH of control and sample after 7 d. The degree of fermentation was estimated by the following standards:

- : \(\Delta pH \leq 0\),  ± : \(0 < \Delta pH \leq 0.5\),  
+ : \(0.5 < \Delta pH \leq 1.0\),  ++ : \(1 < \Delta pH \leq 1.5\),  
+++: \(1.5 < \Delta pH\)

The fermentation was also confirmed by measuring turbidity (absorbance) of the medium at 650 nm.

In vitro digestion of GG. Digestibility of GG was assayed with human saliva \(\alpha\)-amylase, artificial gastric juice, porcine pancreatic \(\alpha\)-amylase, and rat intestinal acetone powder. Assay conditions were as follows:

i) \(\alpha\)-Amylase from human saliva: GG (0.2 g) was dissolved in 2 ml of 45 mM bis-tris buffer (pH 6, 0.9 mM CaCl\(_2\)) containing 40 units of \(\alpha\)-amylase from human saliva (Sigma), incubated for 30 min at 37°C, and then deactivated by heating at 85°C.

ii) Artificial gastric juice: GG (0.1 g) was dissolved in 1 ml of 16.7 mM HCl-KCl buffer (pH 2), and incubated for 100 min at 37°C.

iii) Porcine pancreas: GG (0.2 g) was dissolved in 2 ml of 45 mM bis-tris buffer (pH 6.6, 0.9 mM CaCl\(_2\)) containing 80 units of \(\alpha\)-amylase from porcine pancreas (Boeringer-Manheim Yamanouchi, Osaka), incubated for 6 h at 37°C, and then deactivated by heating at 85°C.

iv) Rat intestinal acetone powder: GG (0.2 g) was dissolved in 2 ml of 45 mM bis-tris buffer (pH 6.6, 0.9 mM CaCl\(_2\)) containing 20 units of \(\alpha\)-amylase from porcine pancreas (Sigma), incubated for 3 h at 37°C, and then deactivated by heating at 85°C.

The amount of galactose generated from hydrolyzed GG in the reaction mixtures was measured by HPLC, as described above.

Absorption of GG by everted sacs of rat small intestine. The absorption test was carried out by the method of Gee et al.\(^24\) and Kinter et al.\(^25\), with a modification. Male Wistar rats (5 weeks old) obtained from an animal supplier (Charles River Japan, Kanagawa) were fasted overnight before the experiment. Each rat was killed immediately before removal of the small intestine via an abdominal incision.

![Fig. 2. \(^{13}\)C Nuclear Magnetic Resonance NMR Spectroscopy of Glycerol Galactoside Extracted from Nori. \(^{13}\)C NMR spectroscopy was recorded with a FT-NMR spectrometer (ECA-500, JEOL, Tokyo) in D\(_2\)O solutions with TSP (3-(trimethylsilyl)propionic-2, 3, 3, -d4 acid sodium salt) as an internal standard. F, Floridoside: 2-O-glycerol-\(\alpha\)-D-galactoside; D, D-isofloridoside: 1-O-D-glycerol-\(\alpha\)-D-galactoside; L, L-isofloridoside: 1-O-L-glycerol-\(\alpha\)-D-galactoside.](image-url)
A jejunum section of about 10 cm was cut, inserted in a glass probe, and ligatured at one end. These segments of jejunum were everted over the glass probe and ligatured tightly at the other end. Segments were filled with 1 ml of Krebs-Henseleit buffer (Sigma, pH 7.4, containing 2 g/l of glucose, 2.1 g/l of sodium bicarbonate, and 0.373 g/l calcium chloride dihydrate) with disposable syringes after cutting at about 5 cm from the end of the glass probe side, tied tightly, and withdrawn with syringes at the same time. These everted sacs were incubated in test tubes containing 6 ml of buffer containing 0.3 w/v% GG. The test tubes were incubated for 1 h at 37°C. During incubation, the buffer in a test tube was bubbled with 5% CO₂/95% O₂ gas. The contents of glycerol galactoside and glucose were measured for the buffer solution in everted sacs and test tubes. To measure the contents of GG, 10 μl of 2 N HCl was added to 0.2 ml of the buffer solution, and the buffer solution was heated to 121°C for 20 min. After neutralization of this solution with 1 N NaOH, the amount of galactose, which was generated from glycerol galactoside hydrolyzed by heating, was measured as described above for measurement kit. The content of GG was calculated from measured amount of galactose. The content of glucose was measured as described above.

Results

Fermentation of nori by bifidobacteria

Table 1 shows the results of the fermentation experiment on nori with five strains of Bifidobacterium. Nori with a low protein content (25% w/w) was strongly fermented by all the strains, except for Bifidobacterium bifidum, whereas nori with a high protein content (41% w/w) was not.
Fermentation of GFC fractions by bifidobacteria

Figure 3 shows the pH (the degree of fermentation) of GFC fractions, prepared from nori with a low protein content, due to Bifidobacterium adolescentis. As shown in Fig. 3, a clear peak of pH was observed at around 300 ml of the fraction. This peak of pH coincided with the second peak of total sugar content observed in GFC for nori with a low protein content.

Subsequently, we analyzed the carbohydrate composition contained in the peak of pH by HPLC. Figure 4A shows a HPLC chromatogram of the concentrated (2 ml) sample solution from fractions 260–340 ml in which fermentation was observed, as in Fig. 3. The first peak, at 7.2 min, contained NaCl used for GFC and porphyran, a major water-soluble polysaccharide in nori. The second peak appeared at 16.5 min. From comparison with the HPLC chromatogram for 0.1% w/v aqueous solution of glycerol galactoside (GG) extracted from nori (Fig. 4B), the second peak in Fig. 4A was identified as GG. It was also confirmed that this peak decreased extremely under heating after the addition of 0.1 N HCl, and instead of this peak, equimolar peaks of galactose and glycerol appeared. This strongly suggests that the second peak in Fig. 4A is GG.

Fermentation of GG by intestinal bacteria

We examined whether fermentation of nori by bifidobacteria was due to glycerol galactoside. Table 2

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>0.5% glycerol galactoside</th>
<th>0.5% glucose</th>
<th>ΔpH (glucose) – ΔpH (glycerol galactoside)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides ovatus</td>
<td>+</td>
<td>++</td>
<td>0.19</td>
</tr>
<tr>
<td>Bacteroides distasonis</td>
<td>±</td>
<td>++</td>
<td>0.84</td>
</tr>
<tr>
<td>Bacteroides vulgatus</td>
<td>±</td>
<td>+</td>
<td>0.46</td>
</tr>
<tr>
<td>Bacteroides thetaiotaomicron</td>
<td>++</td>
<td>+++</td>
<td>0.72</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>+</td>
<td>++</td>
<td>0.61</td>
</tr>
<tr>
<td>Clostridium ramosum</td>
<td>++</td>
<td>+++</td>
<td>0.58</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>++</td>
<td>+++</td>
<td>0.71</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>–</td>
<td>+++</td>
<td>2.34</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>–</td>
<td>+++</td>
<td>2.23</td>
</tr>
<tr>
<td>Eubacterium limosum</td>
<td>–</td>
<td>+++</td>
<td>1.55</td>
</tr>
<tr>
<td>Collinsella (Eubacterium) aerosaciens</td>
<td>–</td>
<td>+++</td>
<td>1.6</td>
</tr>
<tr>
<td>Lactobacillus acidophilis</td>
<td>–</td>
<td>+</td>
<td>2.53</td>
</tr>
<tr>
<td>Bifidobacterium breve</td>
<td>+++</td>
<td>+++</td>
<td>–0.04</td>
</tr>
<tr>
<td>Bifidobacterium longum</td>
<td>+++</td>
<td>+++</td>
<td>0.13</td>
</tr>
<tr>
<td>Bifidobacterium infantis</td>
<td>+++</td>
<td>+++</td>
<td>0</td>
</tr>
<tr>
<td>Bifidobacterium bifidum</td>
<td>–</td>
<td>+</td>
<td>0.82</td>
</tr>
<tr>
<td>Bifidobacterium adolescentis</td>
<td>+++</td>
<td>+++</td>
<td>0.29</td>
</tr>
</tbody>
</table>

*Judgement of fermentation by bacteria (estimated as ΔpH calculated from following definition: ΔpH = [pHcont (7 d) – pHsamp (7 d)] / pHcont (7 d) and pHsamp (7 d) mean pH of control and sample at 7 d)

−, ΔpH ≤ 0; ±, 0 < ΔpH ≤ 0.5; +, 0.5 < ΔpH ≤ 1.0; ++, 1 < ΔpH ≤ 1.5; ++++, 1.5 < ΔpH

**In Vitro Effects of Glycerol Galactoside on Fermentation by Various Intestinal Bacteria**

**Fermentation of GFC fractions by bifidobacteria**

Figure 3 shows ΔpH (the degree of fermentation) of GFC fractions, prepared from nori with a low protein content, due to Bifidobacterium adolescentis. As shown in Fig. 3, a clear peak of ΔpH was observed at around 300 ml of the fraction. This peak of ΔpH coincided with the second peak of total sugar content observed in GFC for nori with a low protein content.

Subsequently, we analyzed the carbohydrate composition contained in the peak of ΔpH by HPLC. Figure 4A shows a HPLC chromatogram of the concentrated (2 ml) sample solution from fractions 260–340 ml in which fermentation was observed, as in Fig. 3. The first peak, at 7.2 min, contained NaCl used for GFC and porphyran, a major water-soluble polysaccharide in nori. The second peak appeared at 16.5 min. From comparison with the HPLC chromatogram for 0.1% w/v aqueous solution of glycerol galactoside (GG) extracted from nori (Fig. 4B), the second peak in Fig. 4A was identified as GG. It was also confirmed that this peak decreased extremely under heating after the addition of 0.1 N HCl, and instead of this peak, equimolar peaks of galactose and glycerol appeared. This strongly suggests that the second peak in Fig. 4A is GG.

**Fermentation of GG by intestinal bacteria**

We examined whether fermentation of nori by bifidobacteria was due to glycerol galactoside. Table 2
shows the results of fermentation experiments of GG by 17 strains of intestinal bacteria. The strongest fermentation was observed for all the strains of *Bifidobacterium*, except for *B. bifidum*. Fermentation judged by turbidity of the medium agreed almostly with the results for ΔpH (data not shown).

**Determination of GG content of nori samples**

GG content was determined for 10 samples of nori with different protein contents (17–41%). The protein content was found to correlate inversely with the GG content (Fig. 5).

**In vitro digestion of GG**

From *in vitro* digestion tests of GG, no galactose generated from hydrolyzed GG by *in vitro* digestion was detected by HPLC measurement of any assay (human saliva α-amylase, artificial gastric juice, porcine pancreatic α-amylase, or rat intestinal acetone powder). It was estimated from detection limit of HPLC (<2 × 10⁻³% w/v galactose) that the decomposition rate of GG in this test was below 3%.

**Absorption of GG by everted sacs of rat small intestine**

We examined absorption of GG and glucose by everted sacs of rat small intestine. The concentration of GG in the everted sac was below 1/10 in the test tube, whereas the concentration of glucose in the test tube was about 1/4 in the everted sac (Table 3).

**Discussion**

In the present *in vitro* study, we found that nori with a low protein content was strongly fermented by bifidobacteria, whereas nori with a high protein content was not fermented (Table 1). To determine the reason for this difference, we fractionated the water-soluble carbohydrate solutions prepared from nori samples with low and high protein contents by GFC and identified the carbohydrates that were fermented by bifidobacteria. GFC showed that the second peak of total sugar content, which was not observed in nori with a high protein content, was observed at around 300 ml of the fraction in that with a low protein content (Fig. 3). Fermentation *in vitro* by *Bifidobacterium adolescentis* was observed in the fraction of this second peak (Fig. 3). By GFC and identification by HPLC, the fermentable GFC fraction contained glycerol galactoside (GG, floridoside: 2-O-glycerol-D-galactoside, isofloridoside: 1-O-glycerol-D-galactoside) and poryphyrin as carbohydrates (Figs. 3, 4). Considering that porphyrin was not utilized *in vitro* by *Bifidobacterium adolescentis*,24) we concluded that GG was the fermentable component. We also confirmed that glycerol galactoside was strongly fermented *in vitro* by bifidobacteria, and that nori with a low protein content contained large amounts of GG, more than 10% w/w on a dry-weight basis (Table 2 and Fig. 5). These results explain why nori with a low protein content is strongly fermented *in vitro* by bifidobacteria.
The present results show that the protein content of nori correlated inversely with the amount of GG (Fig. 5). Noda et al. have reported that the total amount of free amino acids in nori correlated inversely with the total amount of sugars, including GG. Their report agrees with our present results, because the protein content of nori correlated with the total amount of free amino acids. A point of difference is that the maximum content of GG was only 1% w/w in their report. The reason is probably that their experiment dealt with nori with a relatively high protein content. We examined nori with a wide range of protein content, and found that nori with 17% w/w protein content contained about 15% w/w of GG, 10 or more times the amount contained in nori with a high protein content.

The present in vitro fermentation experiment showed that four strains of bifidobacteria, except for B. bifidum, fermented GG at the highest degree of 17 strains of intestinal bacteria (Table 2). Bacteroides thetaiotaomicron, Clostridium ramosum, and Escherichia coli showed fermentation at the second-highest degree, and no or very weak fermentation was observed for the other strains. Taking into consideration that all 17 strains of intestinal bacteria used in the in vitro fermentation experiment showed a high enough degree (+ + + + + + +) of fermentation in the medium with glucose (Table 2), we concluded that these 17 strains of intestinal bacteria could grow in this medium, and that the results for the medium with GG reflected the degree of utilization of GG. Comparing ΔpH (glucose) and ΔpH (GG) in Table 2, GG was fermented in vitro to nearly the same degree of glucose by the four strains of bifidobacteria. On the other hand, the other strains fermented GG at lower degrees than glucose. These in vitro results mean that the four strains of bifidobacteria can utilize GG at higher degrees than the other strains of intestinal bacteria. Kawadu et al. reported that porphyran of nori was not fermented by bifidobacteria in vitro, and weak fermentation by Bifidobacterium adolescentis was observed in a mixture of mannan and xylan of nori. Osumi et al. have reported the biological activities of oligosaccharides from porphyran. In their report, fermentation of oligosaccharides by bifidobacteria was not observed in vitro. In the fermentation experiment on non-digestible carbohydrates in brown algae, fucoidan, laminarin, and sodium alginate were not significantly fermented in vitro by bifidobacteria. Based on these reports, it may be an unusual case that GG contained in algae have a strong fermentation nature by bifidobacteria. On the basis of the present results of the fermentation experiment, we must confirm the growth of intestinal bacteria in further in vivo studies.

In a study regarding absorption and digestion of GG, Sugawara reported that GG prepared from monogalactosyl diacylglycerol contained in wheat flour was not absorbed or digested in the intestinal tract in vivo experiments. The structure of this GG is 1-O-glycerol-β-D-galactoside. On the other hand, the structure of GG from nori is 1-O-glycerol-α-D-galactoside or 2-O-glycerol-α-D-galactoside for which the absorption and digestion properties have not been reported. In the present study, we examined small-intestinal absorption of GG by the in vitro everted sac method involving the rat small intestine, which is often performed to investigate the intestinal absorption of nutrition components, the mechanism of intestinal transport, etc. As shown in the present in vitro absorption test using everted sacs of rat small intestine, GG was not significantly absorbed from rat small intestine, and was not degraded in the intestinal tract (Table 3). We also confirmed that GG was not hydrolyzed by in vitro digestion tests. These results suggest that most of the orally ingested GG reaches the large intestine without digestion or absorption in vivo.

Our present results suggest that GG can be used as a substrate for fermentation by bifidobacteria, and possibility of GG as a prebiotic. Further studies, including in vivo and human experiments, are necessary to evaluate prebiotic activity of GG. We think the present data should contribute to development of a new way of using nori.

### References


