Discolored Red Seaweed Pyropia yezoensis with Low Commercial Value Is a Novel Resource for Production of Agar Polysaccharides

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Abstract: The red seaweed *Pyropia yezoensis* has been demonstrated to be a novel resource for the production of high quality agar. *P. yezoensis* is grown for the food industry in large-scale Japanese mariculture operations. However, discolored *P. yezoensis* is typically discarded as an industrial waste. Here, we evaluated the utility of discolored *P. yezoensis* as a resource for agar production. The quality of agar from the discolored seaweed was comparable to that from normal seaweed. In addition, agar yield was higher from discolored seaweeds than from normal types. Moreover, we successfully used agar from discolored *P. yezoensis* for bacterial plate media and DNA electrophoresis gels without agarose purification. Thus, our results demonstrate that discolored *P. yezoensis* is suitable for agar production and use in life science research. Diverting discolored *P. yezoensis* from disposal to agar production provides a solution to the current industrial waste problem in mariculture, as well as a secure source of agar for research purposes.

Keywords Agar・Polysaccharide・Red seaweed・*Pyropia yezoensis*・Discoloration・Industrial waste

Introduction

Agar is an essential material not only for food production but also in the preparation of solid plate media and electrophoretic gels for use in life science research. Red seaweeds that are members of the Floridophyceae, including species from the Gelidales and Gracilariaceae, are currently utilized for agar production (Lee et al. 2017a; Lee et al. 2017b). In Japan, industrial production of agar as a plate medium was developed with *Gelidium* species (McHugh 1991; Bixler et al. 2011), and depends on the import of materials primarily from Morocco. The price of these materials is increasing (Callaway 2015) to the point that substitutes from other red seaweeds are required to support sustainable agar production.

Currently, red seaweed species from the Bangiophyceae, including *Pyropia haitanensis* (Mu et al. 2009; Wang et al. 2001; Rou et al. 1994) and *Porphyra capensis* (Zhang et al. 2005), are being processed for agar. *Pyropia* and *Porphyra* species contain a precursor of agar as a cell wall polysaccharide called porphyran (consisting of D-galactose, 3,6-anhydro-L-galactose (3,6-AG), 6-O-methyl-D-galactose, and L-galactose-6-sulfate (Morrice et al. 1983; Bhatia et al. 2010)), which has properties as an anti-oxidant and an anti-inflammatory, and functions in lipid metabolism (Tsuge et al. 2004; Inoue et al. 2009; Isaka et al. 2015). Although a high concentration of sulfate in porphyran prevents gelling, its removal by alkali treatment (conversion of L-galactose-6-sulfate to 3,6-AG) results in a usable, gelling agar product (Bixler et al. 2011; Bhatia et al. 2010; Cao et al. 2015). Thus, *Pyropia* and *Porphyra* species have a potential to become key resources for agar production.

We have recently demonstrated that the red seaweed *Pyropia yezoensis* is useful as a material for agar production (Sasuga et al. 2017). Indeed, bacterial growth on *P. yezoensis* agar plates is comparable to growth on plates prepared with commercially supplied *Gelidium* agar. Moreover, *P. yezoensis* agar is effective for gel electrophoresis of DNA without agarose purification. *P. yezoensis* is a major cultivated seaweed in the sea surface farming and mariculture industries in Japan, with approximately 300,000 metric tons (wet weight) produced per year (Statistics of Agriculture, Forestry and Fisheries: http://www.maff.go.jp/j/tokei/kouhyou/kaimen_gyosei/index.html). All of this production is dedicated for manufacture of nori sheets, a traditional Japanese food. Thus, the use of maricultured *P. yezoensis* in the production of agar for plate medium may conflict with its importance to the food industry. This problem needs to be resolved if *P. yezoensis* is to be utilized in the production of high quality agar for application

in the life sciences.

P. yezoensis changes from red to green with the depletion of nitrogen sources in seawater (Zhang et al. 2004; Nishikawa et al. 2007; Oyama et al. 2008; Kakinuma et al. 2008; Nishikawa et al. 2010; Kakinuma et al. 2017), and such discoloration reduces the commercial value of nori sheets. Currently, a large amount of discolored *P. yezoensis* arises in mariculture (Zhang et al. 2004; Nishikawa et al. 2007; Oyama et al. 2008; Kakinuma et al. 2008; Nishikawa et al. 2010; Kakinuma et al. 2017) and is discarded as an industrial waste. We reasoned that if this discolored *P. yezoensis* could be utilized for agar production, the mariculture industry may realize a substantial cost savings. In fact, it has been reported that the yield of porphyran from discolored nori was 20.6% whereas it was only 10.6% from normal nori (Isaka et al. 2015), indicating that discoloration does not affect the quality of cell wall polysaccharides and actually increases the efficiency of porphyran extraction.

In the present study, we demonstrate the usefulness of discolored *P. yezoensis* for agar production. The yield, as well as rheological and chemical properties, of agar from discolored *P. yezoensis* was evaluated in comparison with agar from normal seaweeds. In addition, the applicability of discolored agar as a bacterial plate medium and for DNA electrophoresis was also examined. Our results clearly indicate that discolored *P. yezoensis* can be used a novel resource for agar production.

Materials and methods

Algal materials and agar extraction

The marine red seaweed *Pyropia yezoensis* was maricultured in Shichigahama, Miyagi, Japan by the Miyagi Prefecture Fisheries Cooperative Association. Normal and discolored seaweeds were harvested in February 2017 and April 2016, respectively. As materials for agar extraction, commercial nori sheets were provided by members of the Shichigahama branch office of the Miyagi Prefecture Fisheries Cooperative Association. Dry nori was produced by air-drying at room temperature in our laboratory (Fig. S1). Partially dried *Gelidium* sp., harvested between June and September 2013, was purchased after import from Morocco and further dried at room temperature in the laboratory. Agar from normal and dry nori was extracted as described (Sasuga et al. 2017).

Rheological and chemical analyses

Analyses of rheological properties (including rupture stress, rupture strain, melting and gelling temperatures) and chemical properties [weight-average molecular weight (Mw), polydispersivity index (Mw/Mn, where Mn is the number-average molecular weight), and sulfate and 3,6-AG contents] were performed according to established methodologies (Sasuga et al. 2017). These analyses were conducted in triplicate for all three seaweed materials. All results are presented as the mean ± standard deviation (S.D.). All statistical analyses were performed using Statcel for Windows (OMS Ltd., Saitama, Japan). One-way ANOVA was followed by the Tukey–Kramer test for multiple comparisons. Differences were considered significant when the calculated p value was less than 0.05.

Bacterial growth

Growth of *Escherichia coli* (NBRC12713), *Staphylococcus aureus* (NBRC13276), *Pseudomonas aeruginosa* (NBRC13275), and *Bacillus subtilis* (NBRC3134) was compared among plates containing 0.75% agar from *P. yezoensis* or *Gelidium* sp., prepared as described (Sasuga et al. 2017), or agar from discolored *P. yezoensis*. Agar from seaweeds was not pretreated with diatomite filtration prior to use.

Protease activity of *B. subtilis* on all agar medium treatments was examined by comparison of the size of clear zones (halos). For this experiment, *B. subtilis* was cultured on 1 % agar containing 0.5 % skim milk, and incubated at 35 °C for 15 h.

Gel electrophoresis of DNA

DNA size markers [including 100 bp DNA Ladder (Takara Bio, Japan) and 1 kb DNA Ladder (New England Bio Lab, UK)] were separated on 1.0 % w/v gels made using agar from *Gelidium* sp. (rupture stress 2.0 x 10^5 Pa), normal nori sheets (rupture stress 2.0 x 10^5 Pa), discolored nori sheets (rupture stress 2.0 x 10^5 Pa), and commercially prepared agarose (rupture stress 2.0 x 10^5 Pa; Agar Division of SSK Sales Co., Ltd.). Gel electrophoresis and visualization of DNA bands were performed as described in

(Sasuga et al. 2017).

Results

Yield

Air-dried nori and commercially-produced nori sheets of both normal and discolored *Pyropia yezoensis* (see Fig. S1) were used for extraction of agar according to established methods (Sasuga et al. 2017). Agar was not obtained at 0% NaOH, but alkali pre-treatment allowed the extraction of agar from both normal and discolored seaweeds (Fig. 1 and Table 1). After alkali pretreatment, high agar yields were achieved for both normal and discolored *P. yezoensis*, with discolored nori sheets having significantly greater yields than the rest. Agar yields from normal dry nori and nori sheets were 10.9‑12.3% and 10.7‑15.3%, respectively, consistent with our previous report (Sasuga et al. 2017), and from discolored dry *P. yezoensis* nori yielded 10.9–13.8% agar. By contrast 16.8–19.7% agar was produced from discolored nori sheets. Overall, the maximum agar yield was 19.7% at 4% NaOH in nori sheets of discolored *P. yezoensis*.

Physical properties

Rheological properties of agar from normal and discolored *P. yezoensis* were compared in terms of rupture stress and rupture strain, as well as melting and gelling temperatures. Rupture stress and rupture strain are indicators of gel strength and agar elasticity, respectively. Nori sheets of normal and discolored *P. yezoensis* both had relatively high values for both rupture stress and rupture strain at all alkali concentrations (Table 1). As the alkali concentration increased, rupture stress levels also increased, but rupture strain was basically unchanged (Table 1 and Fig. S2). Melting and gelling temperatures were essentially equal between normal and discolored *P. yezoensis* at each alkali concentration, with slight increases as NaOH concentration increased.

Chemical properties

Molecular weight distribution is a chemical parameter of gel strength, reported as weight- average molecular weight (Mw) and in molecular weight distribution curves. The Mw of discolored *P. yezoensis* agar was higher than that from normal agar, and the Mw of all materials increased with greater alkali concentration (Table 2). In addition, the molecular distribution curves displayed a shift toward a higher molecular weight in agars from dry nori and nori sheets of normal *P. yezoensus* (Fig. 2 a, b). For agars from dry nori and nori sheets of discolored *P. yezoensus*, molecular weights were higher than those from normal *P. yezoensis* at 4% and 6% NaOH. However, within the discolored *P. yezoensus*, there was very little shift to high molecular weight as NaOH concentration increased (Fig. 2 c, d).

Rupture stress and rupture strain were positively correlated with Mw (Fig. 3). In addition, the polydispersivity index (Mw/Mn, an indicator of the heterogeneity of the molecular weight of agar) increased with increasing NaOH concentration (Table 2). Agars from nori sheets of normal and discolored *P. yezoensis* had greater heterogeneity compared to agar from dry nori (Table 2). This finding is consistent with the results of the molecular weight distribution curve, which showed an increase in molecular weight of agar from nori sheets at high NaOH concentrations (Fig. 2).

The gelling property of agar is related to its sulfate content. For agars from *Gracilaria* species, alkali treatment using 3–7% NaOH does not influence the rheological properties or the contents of sulfate or 3,6-AG (Freile-Pelegrín et al. 2005), supporting the relationship between gel properties and sulfate and 3,6-AG contents. By contrast, we found that alkali pre-treatment dramatically reduced the amount of sulfate in agar from both normal and discolored *P. yezoensis*, although there were no significant changes in 3,6-AG content (Table 2 and Fig. S3). Compared to *Gracilaria* species, the sulfate concentration at 4% NaOH was relatively high in *P. yezoensis* (Table 2), which might be responsible for the lower values of rupture stress and melting and gelling temperatures in *P. yezoensis* agar prepared with 4% NaOH pre-treatment (see Table 1).

Suitability for life scientific researches

Semi-solid medium was prepared with agar extracted from normal and discolored *P. yezoensis*. Using this medium, we compared the growth of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis.* All four bacteria species grew well on plates from all agar sources (Fig. 4). There were no statistical differences in colony numbers (Table 3), although the sizes of *B. subtilis* colonies were different among the sources of agar (Fig. 4). Moreover, the sizes of the halos produced by *B. subtilis* were the same on each seaweed agar treatment medium prepared with skim milk (Fig. S4).

When agar from normal *P. yezoensis* is used to prepare gels for electrophoresis, DNA molecules are well separated due to the fairly low sulfate content (Sasuga et al. 2017). Since the sulfate content was also low in agar from discolored *P. yezoensis* (Table 2 and Fig. S3), we compared DNA molecule separation by agar from normal and discolored *P. yezoensis*. When 1 kb and 100 bp DNA ladders were separated via electrophoresis using 1% gels prepared with either agar from nori sheets, *Gelidium* sp. agar or commercial agarose, high quality band separation was observed for both normal and discolored agar, as well as for commercial agarose. However, band separation was poor with *Gelidium* sp. agar (Fig. 5).

Discussion

Based on our current findings, we propose the redirection of discolored *Pyropia yezoensis* from a valueless waste product of the food industry to a valuable agar resource for the life sciences. Indeed, the physical and chemical properties of agar from discolored *P. yezoensis* were basically the same as those of agar from normal *P. yezoensis*. Plus, alkali treatment of normal and discolored seaweeds increased the content of high molecular weight agar chains and decreased its sulfate content, which is important for gel strengthening. These essential properties of agar derived from discolored *P. yezoensis* make it an excellent resource for life science research, specifically in the preparation of semi-solid medium plates and gels for DNA electrophoresis. Remarkably high yields of agar were extracted from discolored *P. yezoensis*, especially when nori sheets were used (~17–20%). Such yields proved to be much greater than yields obtained from normal *P. yezoensis* (10–15%), as well as yields achieved through previous work using *P. yezoensis* (4–16%; Ogawa et al. 1994), and experiments with *P. haitanensis* (1.3–9.0%, Mu et al. 2009; 4–16%, Wang et al. 2001; 4.9–9.5%, Rou et al. 1994) and other red seaweeds (14.5–22.1%, Freile-Pelegrín et al. 1997; 13–39%, Freile-Pelegrín et al. 2005; 3.5–6.5%, Yousefi et al. 2013; 9.5–27%, Meena et al.

2007a; Meena et al. 2007b; 15–17%, Arvizu-Higuera et al. 2008; 12–38%, Vergara-Rodarte et al. 2010; 9.0–13.5%, Rodríguez-Montesinos et al. 2013; 9.3–11.5%, Rao et al. 1976; 12%, Ibrahim et al. 2015). In short, nori sheets of discolored *P. yezoensis* are highly suitable for agar production compared to other red algal materials.

It has been reported that the yield of porphyran, a precursor of agar, is higher from discolored *P. yezoensis* (20.6%) than from the normal variety (10.6%) (Isaka et al. 2015). Although the underlying causes require further investigation, it seems likely that the high agar yields from discolored *P. yezoensis* are related to porphyran in some way. There are at least two possible reasons for these substantial differences in yield. One possibility is that the discoloration is based on damage in cell wall structure that enhances the extraction rate. A second explanation is that porphyran might be highly accumulated under the conditions that cause discoloration. In addition, nori sheets of normal *P. yezoensis* also showed higher yields compared with dry nori, consistent with previous findings (Sasuga et al. 2017), suggesting that the manufacturing process for nori sheets might contribute to increase the agar yield. As discussed previously (Sasuga et al. 2017), mechanical chopping of *P. yezoensis* into small pieces during nori sheet production increases the surface area exposed to NaOH, which may promote higher agar yields. If so, it is possible that discolored *P. yezoensis* can be processed more easily than normal seaweeds, resulting in a greater number of smaller pieces. Future research will focus on these hypotheses.

Our results indicate that agar processed from normal and discolored *P. yezoensis* had similar physical and chemical properties, and similar applicability for use in plate media and gel electrophoresis. Thus, the overall quality is basically identical between these two agars. However, there was a difference in molecular weight distribution. As discovered previously (Sasuga et al. 2017), agar from dry nori and nori sheets of normal *P. yezoensis* shifted to higher molecular weights as alkali concentration increased. By contrast, there was only a minor shift in the molecular weight distribution of agar from dry nori and nori sheets of discolored *P. yezoensis*. Moreover, molecular weights of discolored agar at 4% and 6% NaOH were clearly higher than those of normal agar, and corresponded to those of normal agar at 8% and 10% NaOH. We found that pre-treatment of discolored *P. yezoensis* with 4% or 6% NaOH produces high molecular weight agar chains with high gel strength, whereas pre-treatment with 8% and 10% NaOH resulted in production of agar with similar gel strength for both normal and discolored *P. yezoensis*. Extraction of high molecular weight agar chains from discolored *P. yezoensis* at low NaOH

concentrations was similar to the treatment of industrially used *Gelidium* sp. agar (Sasuga et al. 2017). The results indicate that discolored *P. yezoensis* could be employed as an agar resource alongside *Gelidium* sp. or as a substitute for it.

In summary, discolored *P. yezoensis* is an excellent source of high quality agar, with high yield and gel strength, which can be used for life science research. Discoloration of *P. yezoensis* is currently severe in Japanese mariculture, and has led to increases in the amount of industrial waste and consequently to rising costs for disposal. Thus, the development of effective, alternative uses of discolored seaweeds is an urgent issue that affects the long-term sustainability of *P. yezoensis* mariculture. Our results indicate that the development of agar production methods using discolored *P. yezoensis* may provide a compelling solution for the mariculture industry, while not directly competing with the food industry.

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Conflicts of Interest The authors declare no conflict of interest.

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Table 1 Effect of NaOH concentration during pretreatment of dry nori and nori sheets on agar yield and rheological properties. Mean values are indicated with ± SD from triplicate experiments. Alphabetical letters denote statistically significant differences ($p<0.05$) as determined by one-way ANOVA.

	Normal		Discolored	
	Dry nori	Nori sheets	Dry nori	Nori sheets
Agar yield (%)				
0% NaOH	0^{g}	0^{g}	0^{g}	0^{g}
4% NaOH	12.2 ± 0.6^{ef}	15.3 ± 0.3^{cde}	12.7 ± 1.5^{ef}	19.7 ± 1.1^{a}
6% NaOH	10.9 ± 0.6^{f}	13.4 ± 1.3^{ef}	13.8 ± 1.0^{def}	18.4 ± 0.8^{ab}
8% NaOH	12.3 ± 0.7^{ef}	11.6 ± 1.0^{f}	13.3 ± 2.4^{ef}	17.4 ± 1.4^{abc}
10% NaOH	11.5 ± 0.4^{f}	10.7 ± 1.4^{f}	10.9 ± 1.9^{f}	16.8 ± 0.8^{bcd}
Rupture stress ($\times10^{5}$ Pa)				
0% NaOH	—	—	—	—
4% NaOH	0.47 ± 0.07^{h}	0.52 ± 0.11^{h}	0.59 ± 0.03^{h}	0.80 ± 0.08^{h}
6% NaOH	1.50 ± 0.12^{fg}	1.95 ± 0.23^{cde}	1.35 ± 0.16^{fg}	1.77 ± 0.19^{def}
8% NaOH	1.66 ± 0.24^{efg}	2.61 ± 0.05^{ab}	1.52 ± 0.23^{fg}	2.18 ± 0.14^{bc}
10% NaOH	2.14 ± 0.05^{cd}	2.66 ± 0.10^{a}	1.95 ± 0.13^{cde}	2.34 ± 0.04^{abc}
Rupture strain (%)				
0% NaOH	—	—	—	—
4% NaOH	12.4 ± 1.2^{c}	16.5 ± 2.1^{b}	18.7 ± 0.5^{b}	19.1 ± 1.2^{b}
6% NaOH	17.5 ± 0.6^{b}	20.2 ± 1.8^{ab}	16.6 ± 1.7^{b}	20.1 ± 0.6^{ab}
8% NaOH	16.8 ± 1.8^{b}	23.0 ± 0.7^{a}	17.2 ± 2.3^{b}	21.8 ± 1.0^{a}
10% NaOH	18.4 ± 0.3^{b}	24.0 ± 1.1^{a}	20.3 ± 1.5^{ab}	23.8 ± 0.9^{a}
Melting temperature (°C)				
0% NaOH	—	—	—	—
4% NaOH	79.9 ± 0.7^{f}	81.4 ± 1.1^{f}	80.0 ± 0.6^{f}	82.0 ± 1.2^{f}
6% NaOH	88.6 ± 0.5^{d}	91.2 ± 1.1^{bc}	85.7 ± 1.1^{e}	88.8 ± 0.8^{cd}
8% NaOH	89.8 ± 1.2^{cd}	95.3 ± 0.6^{a}	87.2 ± 1.2^{de}	90.9 ± 1.0^{bc}
10% NaOH	90.9 ± 0.1^{bc}	96.3 ± 0.8^{a}	88.9 ± 1.0^{cd}	92.4 ± 0.4^{b}
Gelling temperature (°C)				
0% NaOH	—	—	—	—
4% NaOH	31.8 ± 0.3^{h}	31.8 ± 0.3^{h}	33.2 ± 0.6^{g}	33.8 ± 0.3^{fg}
6% NaOH	34.8 ± 0.3^{ef}	34.8 ± 0.3^{ef}	36.5 ± 0.3^{bcd}	36.8 ± 0.3^{abc}
8% NaOH	35.2 ± 0.3^{e}	35.7 ± 0.3^{de}	37.2 ± 0.3^{ab}	37.1 ± 0.5^{ab}
10% NaOH	35.8 ± 0.6^{cde}	36.7 ± 0.3^{abc}	37.8 ± 0.6^{a}	37.0 ± 0.5^{ab}

Table 2 Effect of NaOH concentration during pretreatment of dry nori and nori sheets on weight-average molecular weight (Mw), polydispersivity index (Mw/Mn), and sulfate and 3,6-AG contents of agar. Mean values are indicated with ± SD from triplicate experiments. Alphabetical letters denote statistically significant differences ($p<0.05$) as determined by one-way ANOVA.

	Normal		Discolored	
	Dry nori	Nori sheets	Dry nori	Nori sheets
Mw ($\times 10^5$)				
0% NaOH	—	—	—	—
4% NaOH	1.71 ± 0.16^{e}	2.33 ± 0.23^{de}	2.41 ± 0.21^{cde}	2.79 ± 0.19^{cd}
6% NaOH	2.13 ± 0.16^{de}	2.90 ± 0.33^{bcd}	2.27 ± 0.40^{de}	2.92 ± 0.25^{bcd}
8% NaOH	2.04 ± 0.26^{de}	3.62 ± 0.28^{abc}	2.24 ± 0.53^{de}	3.26 ± 0.70^{bcd}
10% NaOH	2.28 ± 0.07^{de}	4.44 ± 0.86^{a}	3.06 ± 0.57^{bcd}	3.88 ± 0.59^{ab}
Mw/Mn				
0% NaOH	—	—	—	—
4% NaOH	1.70 ± 0.05^{b}	1.96 ± 0.07^{ab}	1.72 ± 0.06^{b}	1.91 ± 0.09^{ab}
6% NaOH	1.69 ± 0.09^{b}	2.09 ± 0.07^{ab}	1.80 ± 0.07^{b}	2.05 ± 0.27^{ab}
8% NaOH	1.84 ± 0.05^{ab}	2.11 ± 0.02^{ab}	1.92 ± 0.28^{ab}	2.16 ± 0.31^{ab}
10% NaOH	1.86 ± 0.03^{ab}	2.18 ± 0.05^{ab}	2.07 ± 0.02^{ab}	2.36 ± 0.46^{a}
Sulfate content (%)				
0% NaOH	—	—	—	—
4% NaOH	0.66 ± 0.01^{a}	0.69 ± 0.10^{a}	0.71 ± 0.08^{a}	0.55 ± 0.05^{b}
6% NaOH	0.14 ± 0.01^{c}	0.13 ± 0.01^{c}	0.09 ± 0.01^{c}	0.09 ± 0.02^{c}
8% NaOH	0.05 ± 0.02^{c}	0.06 ± 0.01^{c}	0.03 ± 0.01^{c}	0.05 ± 0.02^{c}
10% NaOH	0.03 ± 0.01^{c}	0.05 ± 0.01^{c}	0.04 ± 0.01^{c}	0.04 ± 0.01^{c}
3,6-AG content (%)				
0% NaOH	—	—	—	—
4% NaOH	42.2 ± 1.4^{a}	39.5 ± 2.3^{a}	41.6 ± 4.0^{a}	43.5 ± 1.3^{a}
6% NaOH	44.7 ± 2.7^{a}	41.5 ± 3.3^{a}	43.9 ± 3.5^{a}	44.5 ± 2.9^{a}
8% NaOH	42.5 ± 1.9^{a}	42.1 ± 3.0^{a}	42.9 ± 2.5^{a}	41.7 ± 0.4^{a}
10% NaOH	42.8 ± 0.5^{a}	39.6 ± 4.4^{a}	41.7 ± 1.5^{a}	39.4 ± 0.8^{a}

Table 3 Number of colonies observed from four bacterial species grown on semi-solid plates containing 0.75% agar prepared from *Gelidium* sp., normal dry nori, normal nori sheets, discolored dry nori, and discolored nori sheets. Mean values are indicated with ± SD from three independent experiments. No statistically significant differences of colony numbers were obtained among the five agar treatments within each species (one-way ANOVA, $p<0.05$).

	E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*
Gelidium sp.	42±10.7	43±1.5	62±6.4	21±8.1
Normal dry nori	40±2.9	37±5.0	78±4.2	25±3.2
Normal nori sheets	44±7.1	46±4.7	75±3.0	33±5.1
Discolored dry nori	52±1.2	41±6.2	63±18.8	30±9.0
Discolored nori sheets	40±2.5	40±3.8	70±9.9	35±11.1

Figure captions

Fig. 1 Agar yield from normal dry nori (grey bar), normal nori sheets (brown bar), discolored dry nori (light green bar), and discolored nori sheets (green bar) after treatment with 4%, 6%, 8% and 10% NaOH. Mean values (n=3) ± SD are shown. Alphabetical letters denote statistically significant differences ($p<0.05$) as determined by one-way ANOVA.

Fig. 2 Molecular weight distribution curves from GPC experiments of four types of agar from normal dry nori (a), normal nori sheets (b), discolored dry nori (c), and discolored nori sheets (d).

Fig. 3 Relationships between weight-average molecular weight (M_w) and physical properties of gels made by agar from dry nori and nori sheets.

Fig. 4 Bacterial growth on *P. yezoensis* and *Gelidium* sp. agar. Colony formation and growth of *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. subtilis* were compared on plates containing 0.75% agar prepared from *Gelidium* sp., normal dry nori, normal nori sheets, discolored dry nori, and discolored nori sheets.

Fig. 5 Suitability of *P. yezoensis* agar from normal and discolored nori sheets for gel electrophoresis of DNA. Separation of 100 bp and 1 kb DNA ladder fragments was tested using 1% agar from *Gelidium* sp., *P. yezoensis*, and 1% commercial agarose.

Electronic Supplementary Materials

Fig. S1 Seaweed samples used in the present study. Dry nori and nori sheets were from Shichigahama.

Fig. S2 Rupture stress (a) and rupture strain (b) of agar from dry nori normal (grey bar), normal nori sheets (brown bar), discolored dry nori (light green bar), and discolored nori sheets (green bar) after treatment with 4%, 6%, 8%, and 10% NaOH. Alphabetical letters denote statistically significant differences ($p<0.05$) as determined by one-way ANOVA. Mean values (n=3) ± SD are shown.

Fig. S3 Sulfate content (a) and 3,6-AG content (b) of agar from normal dry nori (grey bar), normal nori sheets (brown bar), discolored dry nori (light green bar), and discolored nori sheets (green bar) after treatment with 4%, 6%, 8%, and 10% NaOH. Alphabetical letters denote statistically significant differences ($p<0.05$) as determined by one-way ANOVA. Mean values (n=3) ± SD are shown.

Fig. S4 Comparison of the size of halos produced by *B. subtilis* on skim milk plates made with agar from *Gelidium* sp. (a), normal dry nori (b), normal nori sheets (c), discolored dry nori (d), and discolored nori sheets (e).

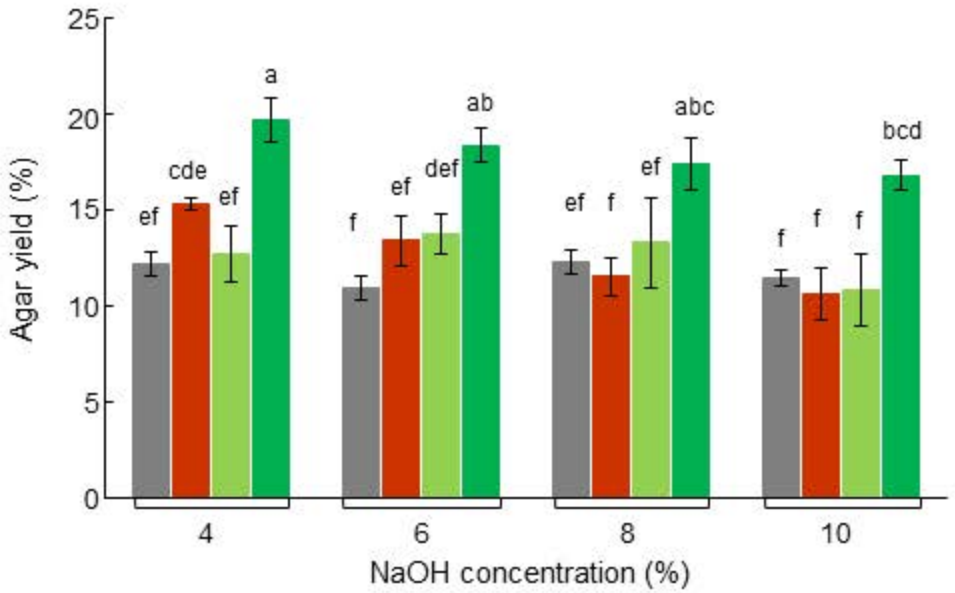

25
20
15
10
5
0
Agar yield (%)
ef
cde
ef
a
f
ef
def
ab
ef
f
ef
abc
f
f
f
bcd
4
6
8
10
NaOH concentration (%)

Fig. 1

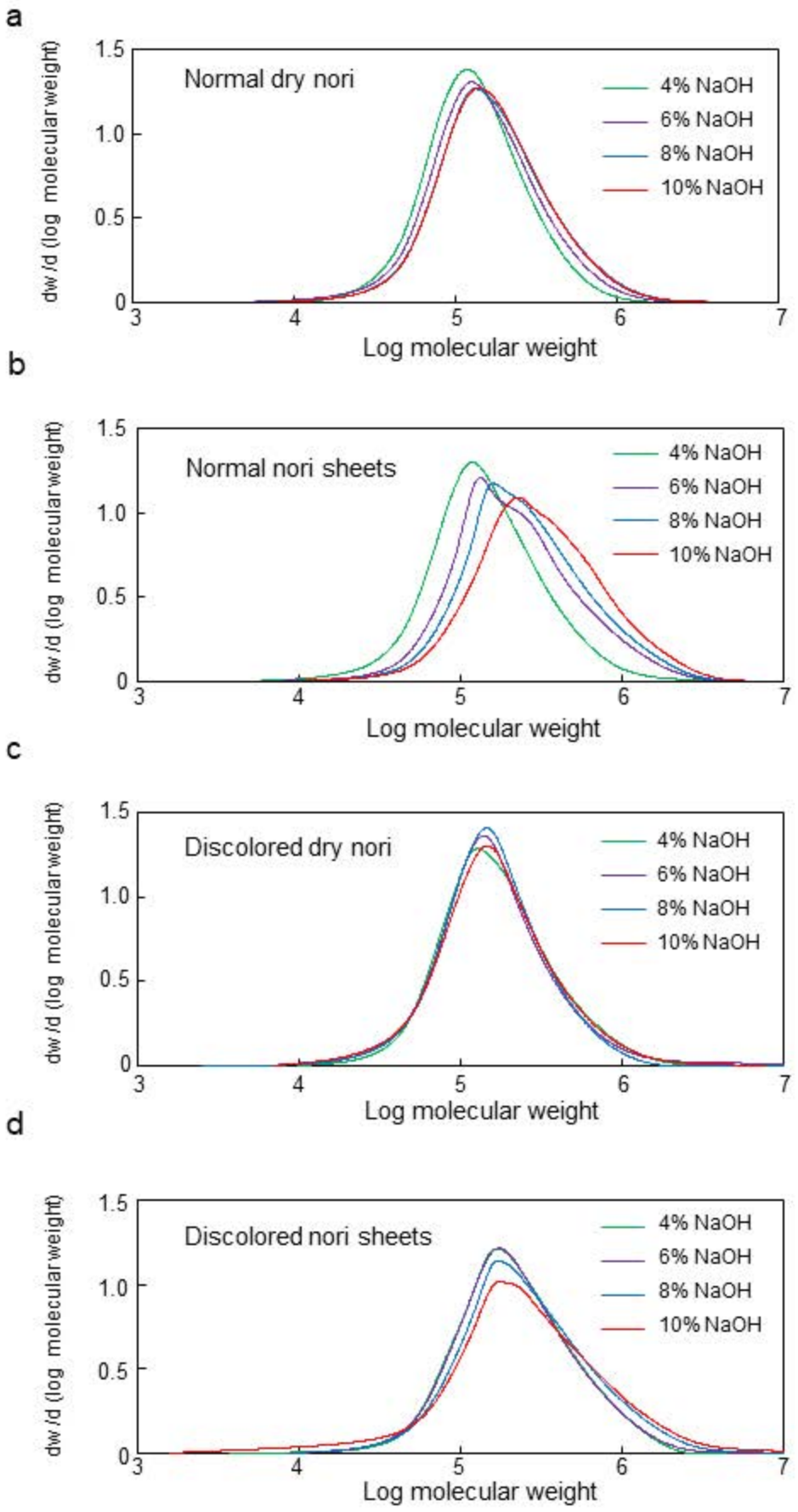
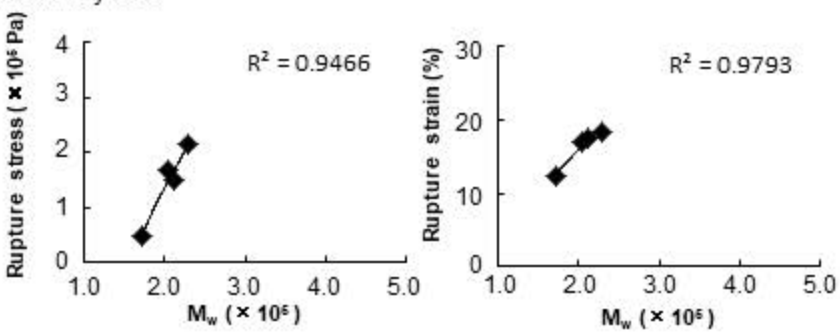
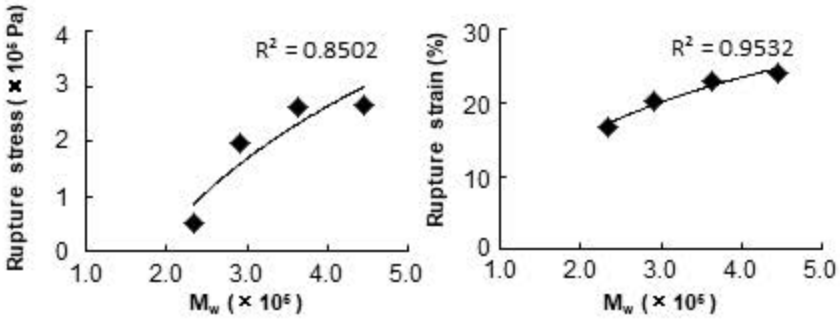

a
Normal dry nori
4% NaOH
6% NaOH
8% NaOH
10% NaOH
dw /d (log molecular weight)
Log molecular weight
b
Normal nori sheets
4% NaOH
6% NaOH
8% NaOH
10% NaOH
dw /d (log molecular weight)
Log molecular weight
c
Discolored dry nori
4% NaOH
6% NaOH
8% NaOH
10% NaOH
dw /d (log molecular weight)
Log molecular weight
d
Discolored nori sheets
4% NaOH
6% NaOH
8% NaOH
10% NaOH
dw /d (log molecular weight)
Log molecular weight

Fig. 2

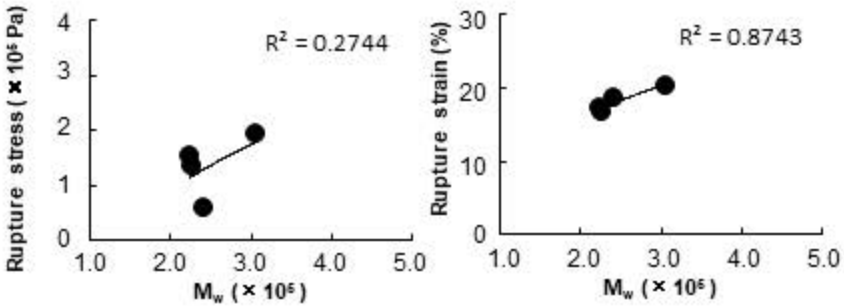
Normal dry nori



Normal nori sheets



Discolored dry nori



Discolored nori sheets

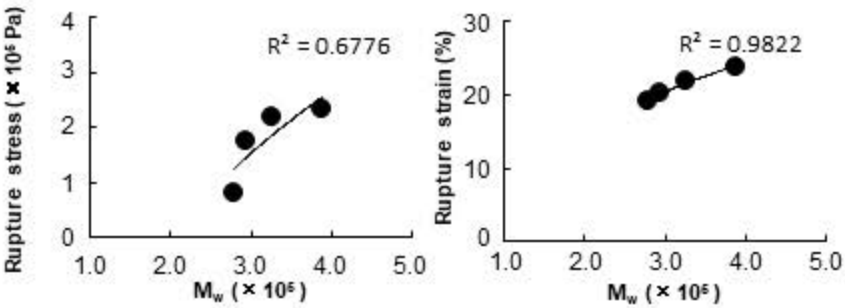


Fig. 3

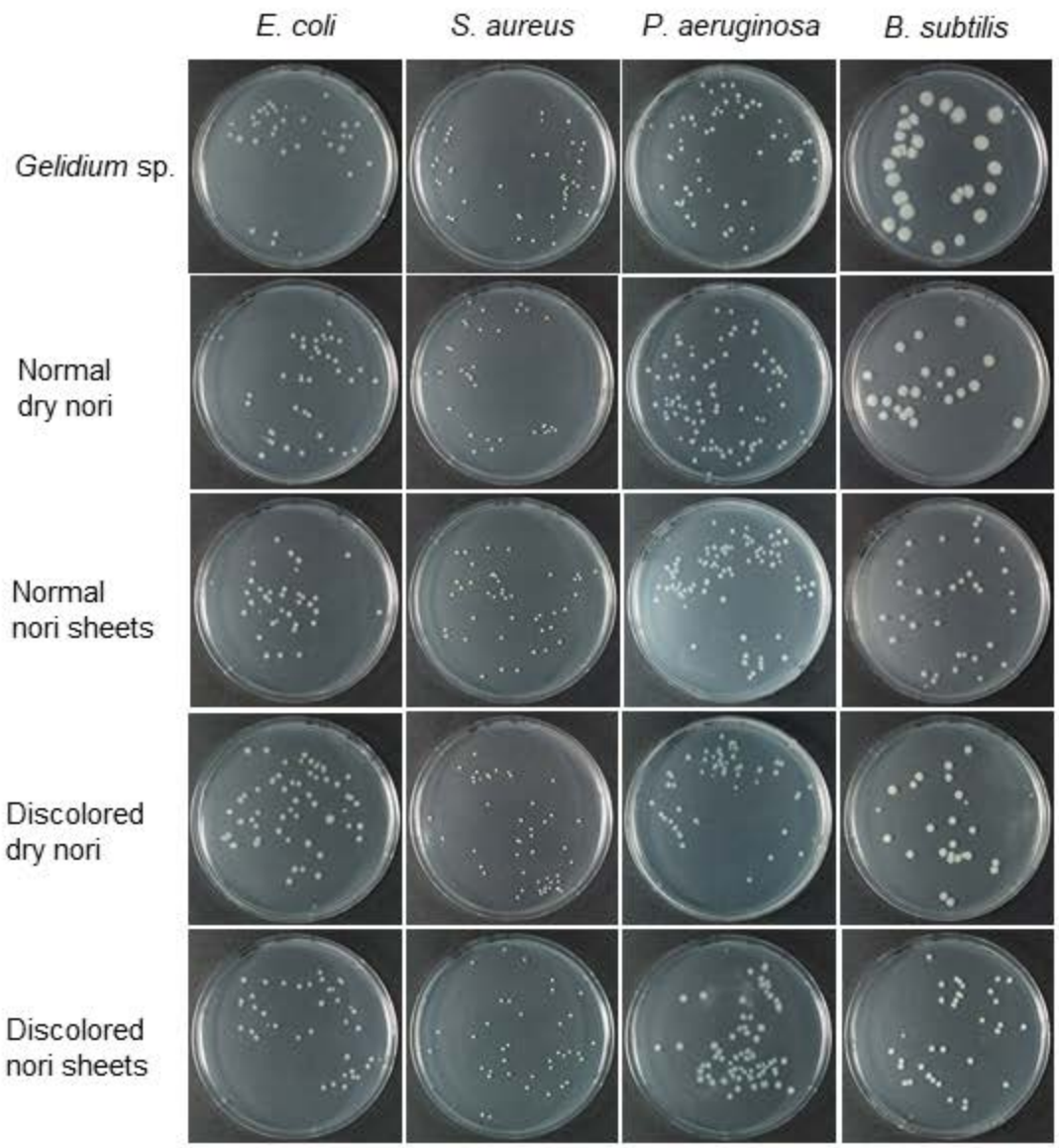

E. coli
S. aureus
P. aeruginosa
B. subtilis
Gelidium sp.
Normal dry nori
Normal nori sheets
Discolored dry nori
Discolored nori sheets

Fig. 4

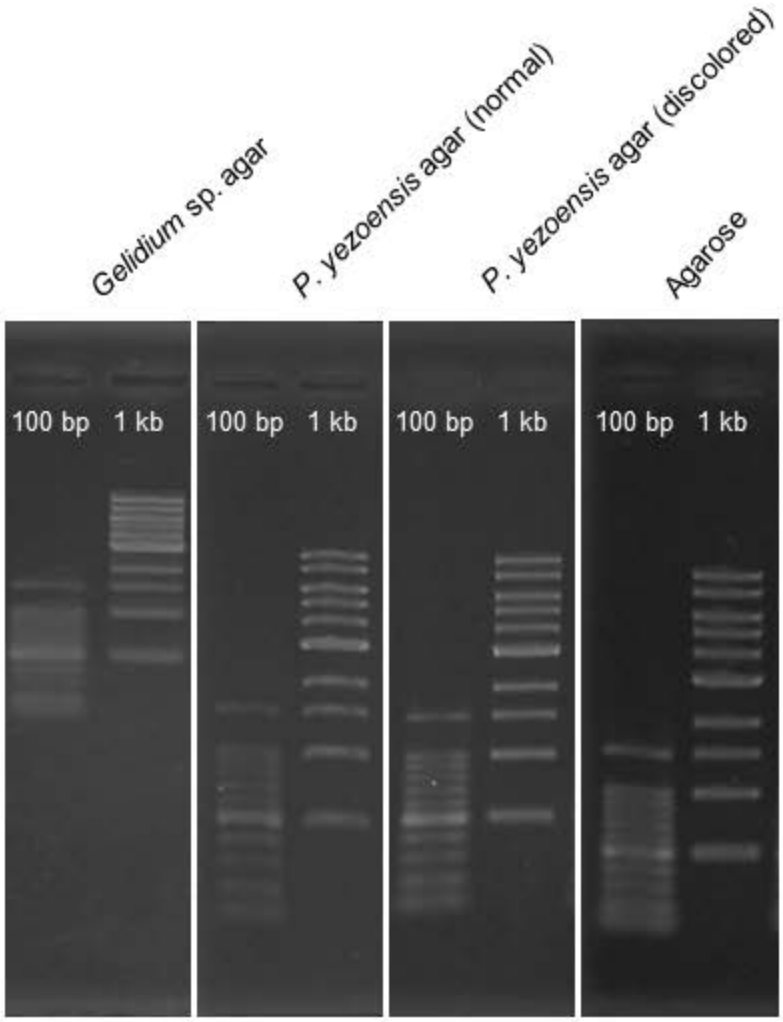
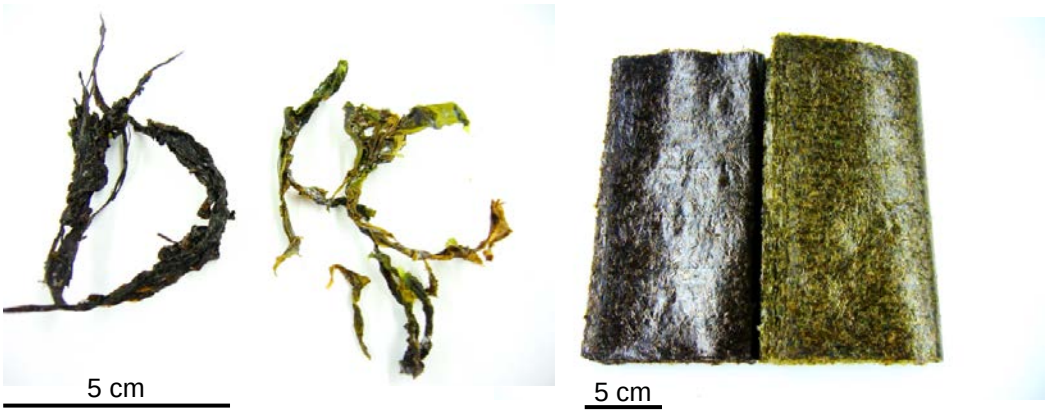

Gelidium sp. agar
P. yezoensis agar (normal)
P. yezoensis agar (discolored)
Agarose
100 bp
1 kb
100 bp
1 kb
100 bp
1 kb
100 bp
1 kb

Fig. 5



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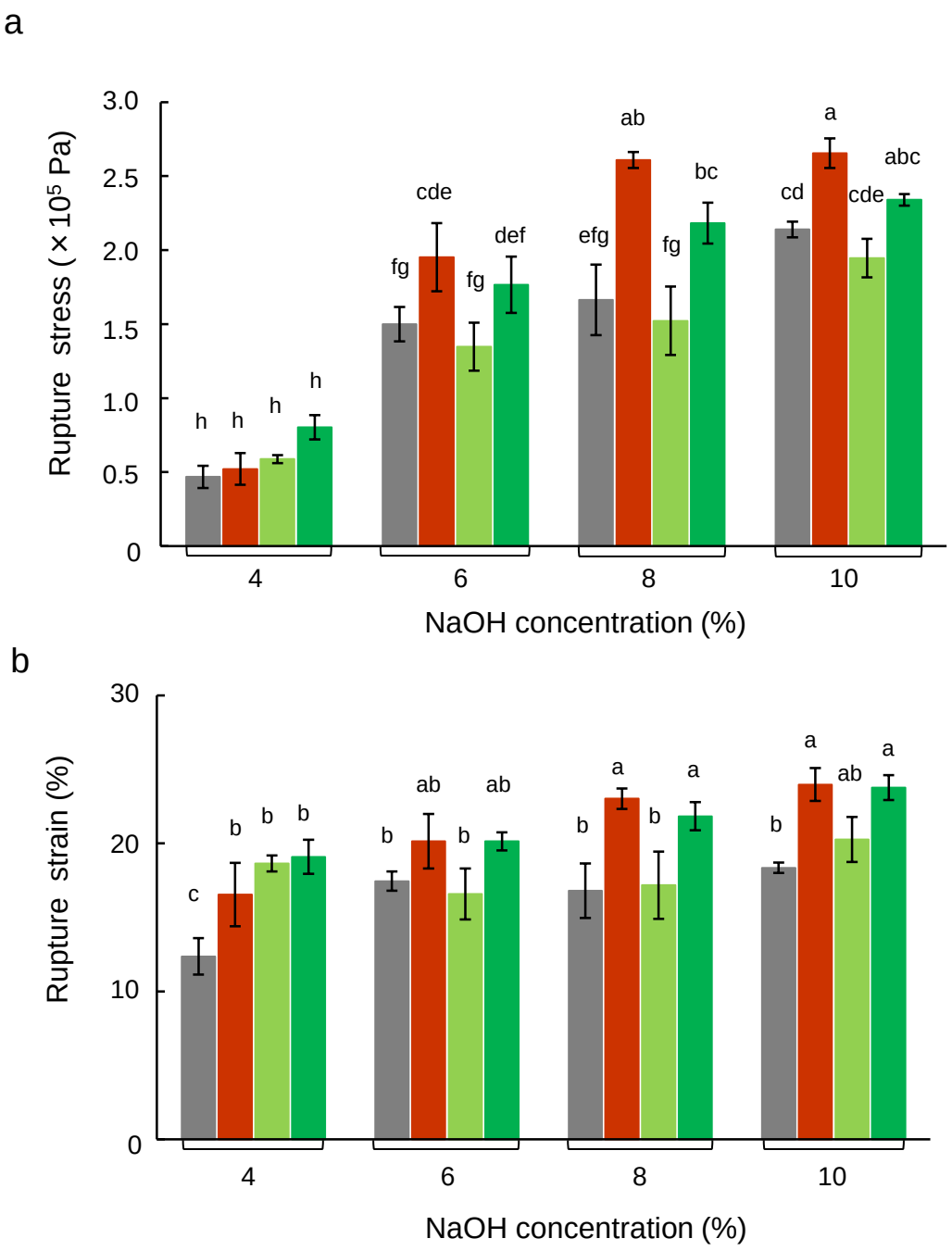

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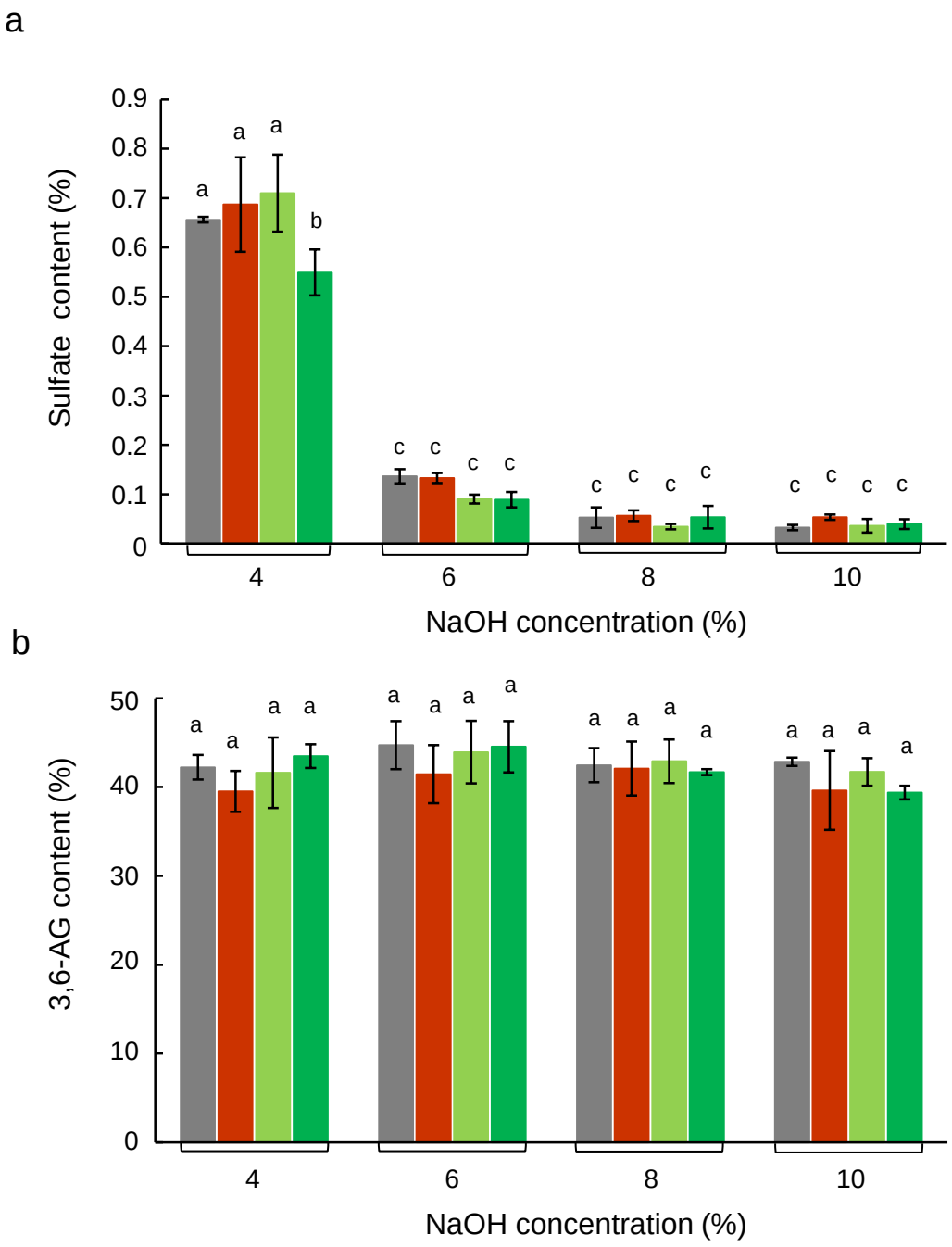
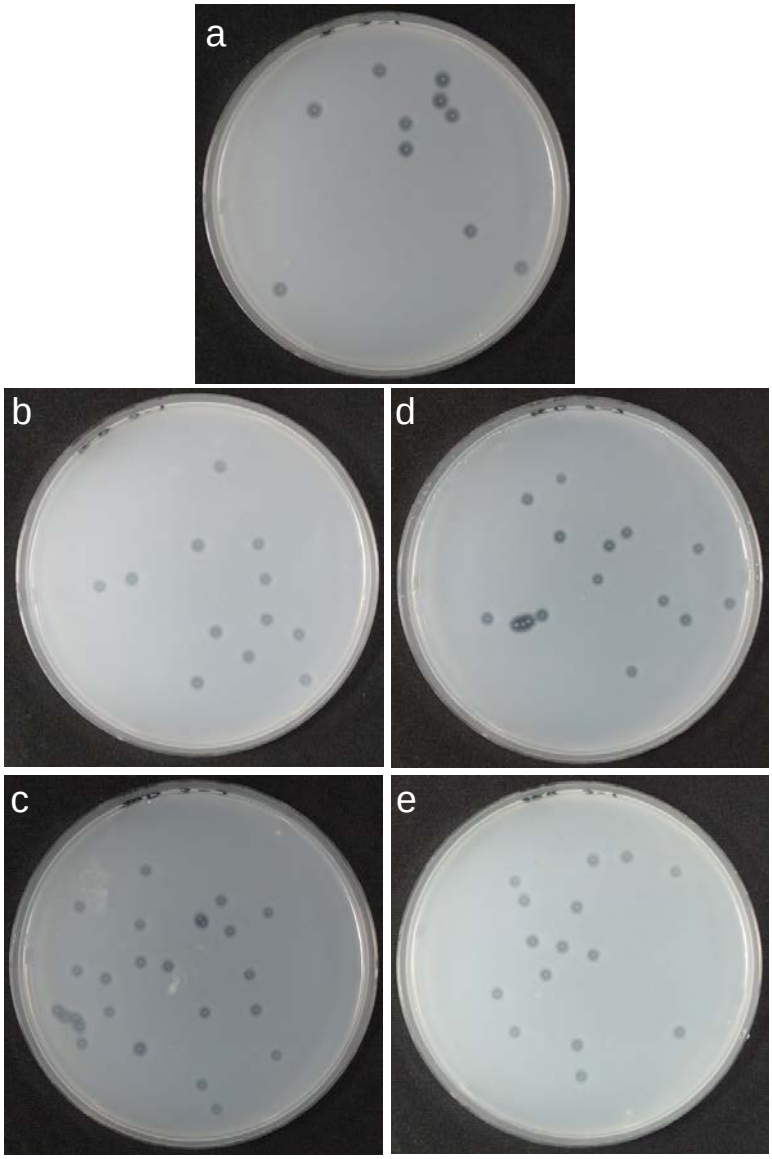


Fig. S3 Sulfate content (a) and 3,6-AG content (b) of agar from normal dry nori (grey bar), normal nori sheets (brown bar), discolored dry nori (light green bar), and discolored nori sheets (green bar) after treatment with 4%, 6%, 8%, and 10% NaOH. Alphabetical letters denote statistically significant differences ($p < 0.05$) as determined by one-way ANOVA. Mean values (n=3) ± SD are shown.



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