Dietary Modulation of Bacterial Fermentative Capacity by Edible Seaweeds in Rats

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The effect of edible seaweeds [nori (*Porphyra tenera*) and wakame (*Undaria pinnatifida*)] on the modulation of colonic microbiota was studied in adult male Wistar rats. Each alga was fed to rats as the only source of dietary fiber and compared with cellulose. After 12 days, animals were sacrificed and cecal contents used as inoculum to ferment lactulose, citrus pectin, cellulose, nori, and wakame in vitro. Dietary treatment did not affect food intake or food efficiency, yet alga caused a significant increase in cecal weight. Nori and wakame were poorly fermented by the cellulose inoculum, with intermediate substrate degradation (76 and 57% for nori and wakame, respectively) and low metabolism to short-chain fatty acids (SCFA) (30% fermentability compared with lactulose). Cecal contents from rats fed nori and wakame showed a reduced ability to ferment all of the studied substrates compared with the cellulose inoculum, causing a reduction in SCFA production and dry matter disappearance. Only nori induced a bacterial adaptation that brought about a higher fermentation of this substrate. The different behaviors of the two algae could be due to their distinct chemical compositions. In conclusion, nondigestible components of edible seaweeds modified the metabolic activity of intestinal microflora, leading to a reduction of its fermentative capacity.

Keywords: *Edible seaweeds; colonic fermentation; microbial modulation*

INTRODUCTION

The human intestinal microflora is a rich ecosystem composed of a wide range of metabolically active microorganisms that play an important role influencing the health of the host (*1*). Bacterial metabolism of substrates present in the large intestine renders a wide range of products that may have different effects at both intestinal and systemic levels. Products originated from the fermentative process depend on the bacterial strains colonizing the large intestine as well as on the type of substrate available (*2*).

Many factors affect the composition of the microbiota in the human large intestine, including host-related factors (e.g., age, intestinal transit time, and immune system), the composition of the diet, interactions between flora components, etc. (*3*). Of these, probably the amount and type of substrate available for the microbial metabolism play the most influential role.

Indigestible dietary residues and endogenous materials make up the pool of metabolizable substrates in the large intestine (*4*, *5*). Thus, diet is likely to influence the intestinal microflora activity and, therefore, the compounds originated from bacterial metabolism and the physiological consequences of these compounds. Additionally, diet can indirectly influence bacteria by modulating intestinal structure and its associated functions. The resulting physicochemical changes in the intestinal environment can be expected to alter the densities, relative proportions, and metabolic characteristics of the resident bacteria.

It is known from animal studies that extreme dietary changes can have significant effects on the composition of the intestinal microflora, because the flora can gradually adapt to changes in substrate supply (*6*).

Dietary fiber (DF) significantly contributes to the nondigestible compounds reaching the large intestine. Among the new potential sources of DF in Western countries, edible seaweeds have gained importance over the past years. Some of the chemical and physicochemical characteristics of these new foods have been described (*7*−*10*), but their physiological properties and nutritional implications are largely unknown.

Nori and wakame are red and brown seaweeds, respectively. Both algae have a high total dietary fiber content (close to 35% of the dry matter), being particularly rich in soluble fraction (14.6 and 17.3%, respectively) (*10*). Predominant polysaccharides depend on the marine macroalgae class. Sulfated galactans (agar and carrageenans), xylans, mannans, and cellulose constitute the main cell wall polysaccharides of Rhodophyceae (red seaweeds), whereas alginates, laminarans, fucans, and cellulose are the main polysaccharides in Phaeophyceae (brown seaweeds) (*11*). The amount of soluble polysaccharides is the most important determinant for the fermentability of fiber-rich substrates (*12*). In land vegetables, this soluble fraction is easily and completely fermented (*5*). Seaweeds constitute a source of DF that differs chemically and physicochemically from those of lands plants and thus may induce different fermentative partners in human.

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Table 1. Composition of the Experimental Diets (Grams per Kilogram of Diet)

ingredient	control	test
casein	140	140
corn starch	465.5	415.5
corn starch dextrinized	155.2	155.2
sucrose	100	100
cellulose	50	
salt mix AIN-93M[34]	35	35
vitamin mix AIN-VX[34]	10	10
L-cystine	1.8	1.8
choline bitartrate	2.5	2.5
tert-butylhydroquinone (TBHQ)	0.01	0.01
soybean oil	40	40
nori or wakame		50

The objective of this work was to study the effect of edible seaweeds on the modulation of colonic microbiota. To this aim, rats were fed diets containing cellulose, nori, or wakame as the only sources of DF, and their cecal contents were used to ferment different substrates in vitro.

EXPERIMENTAL PROCEDURES

Materials. Nori (*Porphyra tenera*) and wakame (*Undaria pinnatifida*) were purchased in local health stores. The algae were dried for 16 h at 60 °C and milled by using a Cyclotec 1093 (Tecator, Sweden) to pass through a 0.5 mm screen. Pulverized algae were extracted (10% w/v) with 90% ethanol, dried, and milled again to a particle size of <0.5 mm.

Citrus pectin (P-9135), lactulose (L-7877), and 4-methylvaleric acid (part 27,782-7) were supplied by Sigma-Aldrich Química S.A. (Madrid, Spain). Cellulose microcrystalline (C-401855) was supplied by Dyets Inc. (Bethlehem, PA). All chemicals were of analytical purity.

Animals and Diets. Thirty male Wistar rats of the same age with an average body weight of 195 g (190−200 g) were supplied by the breeding center at the Faculty of Pharmacy (Universidad Complutense, Madrid, Spain). After weaning, all animals had been fed with a standard maintenance diet (A04, Panlab, Barcelona, Spain) that contained 50 g kg^{-1} cellulose as the source of dietary fiber. Animals were housed in individual metabolic cages in a room maintained at 22 (±1) °C, with 12 h light/dark cycles throughout the 12 day experimental period. Rats were divided into three groups and fed with diets containing cellulose, nori, or wakame as the source of dietary fiber (50 g kg^{-1}). The compositions of the diets, adjusted to allowances for these animals (AIN-93M purified rodent diet, Dyets Inc. (*13*), are shown in Table 1. Food and water were available ad libitum. The study was approved by the Department of Nutrition of the Universidad Complutense. After a 12 day experimental period, animals were killed and cecal contents used for the in vitro fermentation experiments as described below.

In Vitro Fermentation. Fermentations were performed using an in vitro batch culture system under strict anaerobic conditions. Fresh rat cecal contents were used as inoculum, and fermentations were prolonged during 24 h using oxygen-free carbon dioxide to maintain anaerobiosis. The in vitro fermentation method was adapted from a European interlaboratory study (*14*).

Inoculum. Fasted rats were killed in a carbon dioxide chamber. Ceca were removed through abdominal midline incisions and cecal contents collected to prepare the inoculum for each of three in vitro fermentation experiments. In each fermentation, cecal contents from the 10 rats of each experimental group (cellulose, nori, and wakame) were scraped, weighed, pooled, and introduced into a flask containing sterilized anaerobic medium to give a final 100 g L^{-1} (10% w/v) inoculum. The inoculum was mixed for 10 min in a Stomacher 80 Lab Blender (Seward Medical, London, U.K.) and filtered (1 mm mesh) before use. These steps were carried out in an oxygen-free carbon dioxide saturated atmosphere in an Atmosbag (Aldrich 836-2, Milwaukee, WI).

Procedure. One hundred milligrams of substrate (citrus pectin, lactulose, cellulose, nori, or wakame) was weighed into 50 mL serum vials and 8 mL of fermentation medium added into each vial. The medium, adapted from that of Goering and Van Soest (*15*), contained trypticase, micromineral, macromineral solutions and resazurin as anaerobic redox indicator in proportions described elsewhere (*2*). Vials were sealed with rubber caps (no. 407-0-13, Ormacisa, Madrid, Spain), and substrates were left overnight at 4 °C to hydrate. Two milliliters of inoculum was added into each vial and the headspace rinsed with carbon dioxide for 1 min. Vials were placed in a shaking water bath at 37 °C for 24 h. Substrates and blanks (inoculum without substrate) were fermented in triplicate.

After 24 h, the pH was measured in each vial. Fermentations were then stopped by adding an excess of 1 M sodium hydroxide (2.5 mL). Samples were centrifuged at 2500*g* for 10 min and supernatants (3 mL) separated in duplicate for short-chain fatty acid (SCFA) determination. Residues were stored at −20 °C until use.

Determination of Dry Matter Disappearance (Nonfermented Residue). The method followed to quantify the nonfermented residue was adapted from that of Guillon et al. (*16*). Fermentation residues were suspended in 50 mL of saline solution, stirred in a Stomacher 80 for 3 min, and filtered through Dacron cloth (mesh size = 150 μm). Residues were resuspended in saline solution (50 mL) and washed again twice. Finally, they were washed with solvent exchange (95% ethanol and acetone) and dried at 60 °C overnight. Dry matter disappearance (DMD), determined gravimetrically, was calculated as a percentage of the initial substrate.

SCFA Analysis. SCFA production after 24 h of fermentation was determined following the method of Spiller et al. (*17*) slightly modified. Aliquots (400 μL) of supernatants from the fermentation samples were mixed with 100 μL of internal standard (50 μmol mL^{-1} 4-methylvaleric acid) and 50 μL of 850 g L^{-1} phosphoric acid 85% (Sigma Aldrich Química S.A., part 21,510-4) and made up to 1 mL with Milli-Q water. Samples were centrifuged (4 °C, 7300*g*, 15 min) and supernatants filtered through 0.45 μm cellulose filters (Lida 9501-06). Two microliters of sample was injected into a 5890 Hewlett-Packard chromatograph equipped with a flame ionization detector (FID) and connected to an HP ChemStation with software version A.06.03 [509] from Hewlett-Packard (Walbronn, Germany). SCFA were separated in an HP-FFAP column (10 m, 0.53 mm i.d., 1 μm film thickness; HP 19095F-121), using oxygen-free nitrogen as carrier gas and a column temperature of 100−120 °C (initial temperature, 100 °C, 2 min; gradient, 10 °C min^{-1}; final temperature, 120 °C, 2 min). Injector and detector temperatures were 200 and 220 °C, respectively. SCFA were identified and quantified by comparison with known standards. Total SCFA production, in micromoles per milligram of dry substrate, was the sum of lineal (acetic, propionic, butyric, and valeric) and branched (isobutyric and isovaleric) fatty acids corrected for the SCFA supplied by the inoculum by subtracting the SCFA content of a blank sample (with inoculum but no substrate) at time 0 h.

Statistical Analysis. Results are expressed as mean values and standard deviations (SD). One-way analysis of variance (ANOVA) was used to determine the significance of mean differences between groups, by using the StatGraphics computer program (SAS/STAT version 6, SAS Institute, Cary, NC). Significance level was $P < 0.05$.

RESULTS AND DISCUSSION

Although marine algae are totally uncommon in the rat diet, their intake was well tolerated by the experimental animals. No statistically significant differences in food intake, weight gain, and food efficiency were observed in the nori- and wakame-fed groups as compared to the cellulose control group (Table 2).

Table 2. Effect of Cellulose, Nori (*P. tenera*), and Wakame (*U. pinnatifida*) on Body Weight Gain, Food Intake, and Food Efficiency in Rats (Mean Values ± SD; $n = 10$)

group	body wt gain (g)	food intake (g)	food efficiency[a]
cellulose	57.3 ± 10.1	234.6 ± 13.7	0.24 ± 0.0
nori	49.7 ± 8.2	209.6 ± 10.4	0.24 ± 0.0
wakame	56.9 ± 10.7	235.0 ± 21.5	0.24 ± 0.1

[a] Food efficiency = weight gain × food intake^{-1}.

Table 3. Effect of Cellulose, Nori (*P. tenera*), and Wakame (*U. pinnatifida*) on Cecal Parameters in the Rat: Total Cecal Weight and Weight, pH, and SCFA of Intestinal Contents (Mean Values ± SD; $n = 10$)[a]

group	total cecal wt (g)	cecal content (g of fresh matter)	cecal pH	cecal SCFA[b] (μmol/mL)
cellulose	2.49 ± 0.67[a]	1.87 ± 0.60[a]	6.93 ± 0.03[a]	3.50 ± 0.55[a]
nori	3.50 ± 0.87[b]	2.71 ± 0.77[b]	7.64 ± 0.04[b]	2.45 ± 0.55[b]
wakame	3.50 ± 0.73[b]	2.73 ± 0.18[b]	7.39 ± 0.01[c]	1.80 ± 0.20[c]

[a] Different following letters in the same column denote statistically significant differences ($p \leq 0.05$). [b] Short-chain fatty acid (SCFA) concentration of cecal contents.

The addition of seaweeds to the diet, however, caused a significant increase in the weight of the cecum and its contents in comparison with cellulose (Table 3). This increase was probably due to a higher amount of water in the cecal contents of animals consuming nori and wakame, because both algae showed a high in vitro water holding capacity (*10*, *18*).

Similarly, the pH of cecal contents was significantly higher in the alga-fed groups than in the control one, with nori causing the highest increase (Table 3). Cecal pH is influenced by the accumulation of fermentation products such as SCFA (acetate, propionate, butyrate, etc.) and organic acids (lactate, succinate, pyruvate, etc.) and also by the composition of the nondigested material in the gut (*4*). SCFA concentration in the cecal contents was highest in the cellulose group, which might account for the lower pH values. On the other hand, although the cecal SCFA concentration was higher in the nori group than in the wakame-fed animals, cecal contents were more acidic in the latter. These differences might reflect the different polysaccharide compositions of the soluble dietary fiber fractions in both seaweeds. Nori is mainly composed of neutral (agar) and variably acidic (carrageenans) polysaccharides, whereas wakame is very rich in acidic polysaccharides (alginates, rich in mannuronic and guluronic acids) that could have an important pH-lowering effect.

Although this experiment was not designed to study the fermentability of algal polysaccharides in vivo, SCFA concentrations in rat cecal contents can be considered as a measure of such in vivo fermentation. Cellulose is a poorly fermentable substrate, and yet the cecal SCFA concentration in this group was higher than in the two algae groups (Table 3), which suggests a poor fermentability of seaweeds even after 12 days of adaptation to the diet. There are no data in the literature concerning the in vivo fermentative behavior of edible seaweeds. Only scarce data on the degradation of certain algal soluble polysaccharides (alginates, carrageenans and laminarans) in rats are available. These data show a high resistance to intestinal degradation of alginates and carrageenans from brown and red seaweeds, respectively (*19*−*21*), whereas laminarans appeared to be extensively degraded (*22*).

Table 4. Percentage of Dry Matter Disappearance after in Vitro Fermentation of Different Substrates Using Inocula from Rats Fed Cellulose, Nori (*P. tenera*), and Wakame (*U. pinnatifida*) (Mean Values ± SD; $n = 3$)[a]

	inoculum from dietary group		
substrate	cellulose	nori	wakame
lactulose	100.0 ± 0.0[a]	41.6 ± 2.8[b]	77.2 ± 1.1[c]
cellulose	24.6 ± 0.7[a]	10.1 ± 1.5[b]	10.5 ± 1.0[b]
citrus pectin	97.8 ± 0.8[a]	89.9 ± 5.1[b]	104.2 ± 0.8[a]
nori	75.5 ± 0.6[a]	86.0 ± 1.2[b]	nd[b]
wakame	57.2 ± 1.3[a]	nd	52.3 ± 0.72[b]

[a] Different superscript letters in a row indicate statistically significant differences ($p \leq 0.05$). [b] nd, not determined.

Rat cecal contents from each cellulose-, nori-, and wakame-fed group were used as inoculum to ferment different substrates in vitro. Lactulose, used as a reference substrate that is easily and totally fermentable, and citrus pectins and cellulose, used as highly and poorly fermentable substrates, respectively, were separately fermented along with nori and wakame.

DMD after 24 h of in vitro fermentation is shown in Table 4. When cecal contents from the cellulose control group were used as inoculum, the percentage of DMD was almost 100% for lactulose and pectins and only 25% for cellulose, whereas nori and wakame showed substrate disappearances of 75 and 57%, respectively. These results suggest that the microflora of nonadapted animals is capable of only a partial degradation of algal dietary fiber, with an apparent higher utilization of nori than wakame seaweeds. Michel et al. (*23*) showed that up to 76% of wakame was degraded after 24 h of in vitro fermentation with human fecal inoculum. The high degradation values reported by these authors might be due either to a different ability of the human microflora to degrade this subtract or to a different composition of the brown algae, which is highly variable depending on factors such as season, geographical location, and harvesting time.

When the cecal contents of rats adapted to diets containing algal DF were used as inocula, the profile of DMD was significantly changed. Degradation of lactulose was considerably decreased, mainly in rats adapted to the nori diet, which points toward a reduced fermentative ability of the microflora after the consumption of red and brown seaweeds. This seems also valid for cellulose, with a significantly reduced percentage of DMD in the residues from fermentation with nori and wakame inocula (10% DMD) as compared with the cellulose ones (25%). However, citrus pectins showed a high percentage of DMD (104%) when fermented in the presence of cecal contents from the wakame-fed animals. This is in contradiction with the reduced degradability of lactulose observed with the same inoculum. Degradation of citrus pectins by the microflora of the nori group was lower than with the cellulose one (90 versus 98%), which agrees with the reduced degradability of lactulose by this algal inoculum.

When seaweeds were fermented with the inocula from animals that consumed nori or wakame in the diet, a higher percentage of DMD was observed for nori, suggesting an apparent adaptation of the microflora to this substrate. In contrast, Wakame degradation was slightly, yet significantly, lower with the inoculum from the corresponding dietary group than with the cellulose one. Therefore, it is difficult to conclude from these results if adaptation of the colonic microflora to the diet takes place.

Table 5. Total Short-Chain Fatty Acid Production (Micromoles per Milligram of Dry Matter) after 24 h of Fermentation of Different Substrates with Inoculum from Rats Fed Cellulose, Nori (*P. tenera*), and Wakame (*U. pinnatifida*) (Mean Values ± SD; $n = 3$)[a]

	inoculum from dietary group		
substrate	cellulose	nori	wakame
lactulose	$19.9 \pm 1.2^{a,\alpha}$	$5.0 \pm 0.6^{a,\beta}$	$15.2 \pm 1.2^{a,\gamma}$
cellulose	$5.0 \pm 0.5^{b,c,\alpha}$	$3.7 \pm 0.1^{b,\beta}$	$2.1 \pm 0.4^{b,\gamma}$
citrus pectin	$14.4 \pm 0.7^{d,\alpha}$	$11.1 \pm 0.3^{c,\beta}$	$11.0 \pm 0.7^{c,\beta}$
nori	$5.6 \pm 0.4^{c,e,\alpha}$	$15.4 \pm 0.7^{d,\beta}$	nd[b]
wakame	$5.9 \pm 0.2^{e,\alpha}$	nd	$5.7 \pm 0.2^{d,\beta}$

[a] Different superscript Roman letters in a column and Greek characters in a row indicate statistically significant differences ($p \leq 0.05$). [b] nd, not determined.

Results for DMD show the importance of the origin of the inoculum and its influence modifying substrate degradability. Nevertheless, these are gravimetric results that should be considered with caution because they may be affected by errors derived from partial solubilization of DF polysaccharides and/or elimination of soluble, low molecular weight oligosaccharides produced during bacterial degradation of the substrates. These compounds could be lost during the isolation of nonfermented residues and would escape gravimetric quantification.

Determination of SCFA production after 24 h of in vitro fermentation in the presence of the cellulose, nori, or wakame inocula provided an estimation of the degree of substrate metabolism by the colonic microflora. As was expected, highly fermentable substrates such as lactulose and pectins yielded the highest SCFA levels and a poorly fermentable substrate such as cellulose, the lowest, when cecal contents from cellulose fed rats were used as inoculum (Table 5). Nori and wakame were poorly fermented, with SCFA levels similar to those produced from cellulose. Considering that previous analysis in our laboratory showed that DF in both seaweeds was very rich in soluble polysaccharides (44 and 52% of the total DF content in nori and wakame, respectively) (*10*), a higher SCFA production could have been expected because soluble polysaccharides are easily and completely fermentable in land plants (*5*). However, the chemical structure and composition of algal soluble polysaccharides differ from that in land vegetables, which could affect their fermentative behavior.

Soluble DF polysaccharides in red alga are mainly composed of sulfated polymers of 1,3-β-D-galactose and 1,4-α-3,6-anhydrogalactose, the latter with a characteristic L or D conformation in agars and carrageenans, respectively (*24*). Both polysaccharides have been shown to be highly resistant to in vitro fermentation, with low yields of SCFA production (*25*), although they may be depolymerized and desulfated by the colonic bacteria (*26*, *27*). On the other hand, there are three different types of soluble polysaccharides in brown seaweeds: alginates (polymers of β-1,4-D-mannuronate and α-1,4-L-guluronate), laminarans (β-1,3 glucans with different proportions of β-1,6 linkages and mannitol residues on the reducing sugars), and fucans (heterogeneous sulfated polymers composed of variable proportions of fucose, galactose, xylose, mannose, glucuronic acid, and mannuronic acid) (*8*, *24*, *28*). Whereas laminarans are highly fermentable polysaccharides, alginates and fucans are resistant to bacterial metabolism, although they can undergo partial depolymerization and/or desulfation by the intestinal microflora (*23*, *25*). β-Elimination is considered to be the primary degradation route of alginate, the main polysaccharide in wakame (*23*). Therefore, the low SCFA production after in vitro fermentation of the studied seaweeds with the cellulose inoculum was due to the differential composition of their soluble dietary fiber fractions.

Fermentability values, expressed as a percentage of the SCFA produced by lactulose with the cellulose inoculum (reference value for 100% fermentability), indicate that only ~30% of the algae was metabolized to SCFA (Figure 1). These values are even lower that those obtained for the percentage of DMD (Table 4). Such discrepancy between substrate disappearance and SCFA production had previously been reported by other authors for brown seaweed DF and isolated soluble polysaccharides (*23*). As mentioned above, some algal polysaccharides can suffer partial depolymerization by bacterial enzymes, rendering oligomers that are no further metabolized by the intestinal microflora, which would explain the low SCFA production from these seaweeds. Alternatively, degradation products could be metabolized through different pathways, rendering fermentation products other than SCFA. This possibility has not been investigated because it fell outside the aims of this study. However, no acidification was observed during algal fermentation (Table 6), which would rule out the possible production of organic acids as final fermentation products. In all cases, SCFA production was paralleled by pH values of the fermentation media, with little acidification in samples with reduced substrate fermentation.

Consumption of nori during 12 days caused a marked decrease in the fermentative capacity of intestinal microorganisms. SCFA levels were 75% of those produced by the cellulose inoculum for pectin and cellulose substrates and only 26% for lactulose (Table 5). This resulted in a significantly reduced fermentability for all of the substrates as compared with the lactulose reference (Figure 1). Both fermentability and percentage of DMD (Table 4) were lower in this batch as compared with the cellulose inoculum, although the reduction was more marked for the percentage of fermentability. This implies that substrate depolymerization was higher than metabolism to SCFA. Therefore, an apparent loss of metabolic activity by the intestinal microflora takes place in the presence of red algal polysaccharides as the only source of DF.

Interestingly, when nori was fermented with the cecal contents from animals receiving this alga in the diet, SCFA production was significantly increased, with SCFA production 3 times that observed with the cellulose inoculum (Table 5). Therefore, nori was the most fermented substrate by the microorganisms from nori-fed rats. In this case, fermentability data are close to those observed for DMD, indicating that soluble polysaccharides in nori were extensively degraded and metabolized to SCFA. These results suggest an adaptation of the intestinal flora to this substrate. This adaptation involves an induction of bacterial enzyme systems capable of degrading and metabolizing red algal polysaccharides (agar and carrageenan), modifying their ability to ferment other substrates of different chemical structure and composition such as lactulose, pectins, or cellulose.

This was not the case, however, with the brown alga. SCFA production with the wakame inoculum was reduced for all substrates as compared with the cellulose

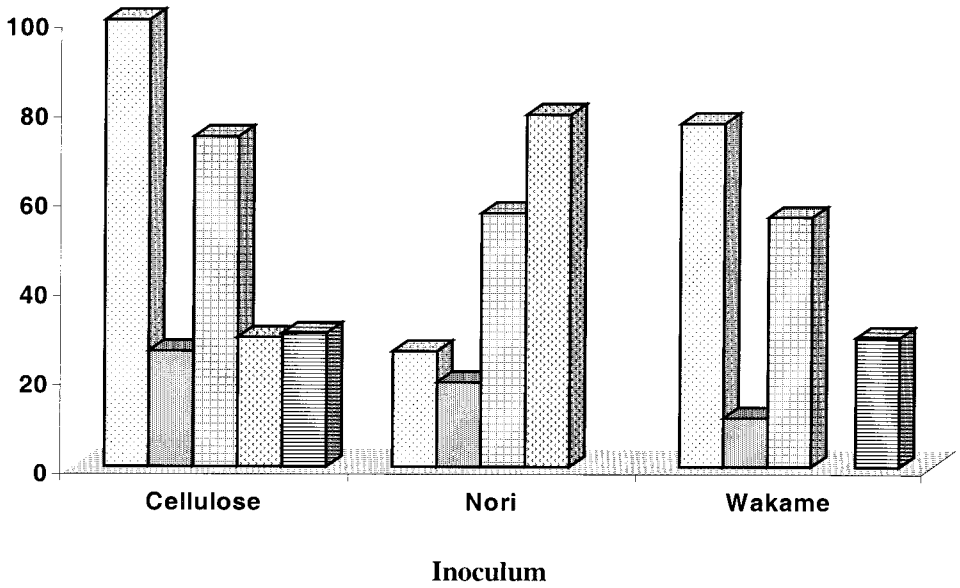

Figure 1. Percentage of fermentability of different substrates with inoculum from rats fed cellulose, nori (*P. tenera*), or wakame (*U. pinnatifida*). Substrates: (dotted bars) lactulose; (finely cross-hatched bars) cellulose; (checkerboard bars) citrus pectins; (arrowhead bars) nori; (striped bars) wakame. Lactulose fermented with the cellulose inoculum (lactulose-C) was considered as a reference value of 100% fermentability. % fermentability = $(SCFA_{substrate}/SCFA_{lactulose-C}) \times 100$.

Table 6. pH Values of Fermentation Media after 24 h of Fermentation of Different Substrates with Inoculum from Rats Fed Cellulose, Nori (*P. tenera*), and Wakame (*U. pinnatifida*) (Mean Values ± SD; *n* = 3)[a]

	inoculum from dietary group		
substrate	cellulose	nori	wakame
lactulose	$6.08 \pm 0.08^{a,\alpha}$	$6.95 \pm 0.03^{a,\alpha}$	$6.17 \pm 0.03^{a,\beta}$
cellulose	$7.02 \pm 0.02^{b,\alpha}$	$6.99 \pm 0.04^{a,\alpha}$	$6.98 \pm 0.02^{b,\alpha}$
citrus pectin	$6.56 \pm 0.05^{c,\alpha}$	$6.54 \pm 0.08^{b,\alpha}$	$6.53 \pm 0.04^{c,\alpha}$
nori	$6.94 \pm 0.02^{b,\alpha}$	$6.23 \pm 0.52^{c,\beta}$	nd[b]
wakame	$6.92 \pm 0.06^{b,\alpha}$	nd	$6.91 \pm 0.01^{d,\alpha}$

[a] Different superscript Roman letters in a column and Greek characters in a row indicate statistically significant differences ($p \leq 0.05$). [b] nd, not determined.

inoculum (Table 5). Fermentability percentages of 77 and 11% for lactulose and cellulose, respectively, agreed with the DMD values (Table 4). On the contrary, citrus pectins were extensively degraded (104%), but they suffered only partial metabolism to SCFA (56% fermentability as compared with the lactulose reference). Finally, even after 12 days of microflora adaptation to brown algal polysaccharides, the fermentability of Wakame was not improved as compared with the cellulose inoculum (Figure 1), with only marginally higher SCFA production in the latter (5.93 versus 5.69 μmol of SCFA/mg of brown alga fermented by the cellulose or wakame inocula, respectively). Again, substrate disappearance was higher than the percentage of metabolism to SCFA, suggesting partial depolymerization of wakame polysaccharides by the intestinal microflora.

Michel (*29*) studied the inducibility of brown seaweed alginate fermentation by measuring gas production after incubation of sodium alginate with fermentation media from previous in vitro fermentations of alginates with human fecal inoculum. Total gas production was accelerated in the presence of preadapted bacteria, starting after 5 h of incubation as compared with the 12 h induction time required by the nonadapted flora. However, net gas production after 24 h of fermentation did not increase in comparison with non-preadapted bacteria, indicating a similar degree of alginate utilization. These results suggest that induction of the bacterial capacity to ferment alginate results in a higher rate but similar extent of substrate metabolism. Therefore, no differences between adapted (wakame) and nonadapted (cellulose) bacteria were to be expected in our study, because fermentations were always studied after 24 h of incubation of wakame with the different inocula. Although wakame feeding did not induce a higher bacterial utilization of this brown seaweed's polysaccharides, wakame like nori modified the fermentative behavior of the microflora toward other substrates such as lactulose, pectins, and cellulose.

In summary, red and brown seaweeds were poorly fermented by the intestinal microflora of nonadapted rats consuming cellulose, with low metabolism of partly degraded algal polysaccharides. Adaptation to diets containing nori or wakame led to changes in microbial activity that involved a decreased fermentative degradation of usually totally fermentable substrates such as lactulose and pectins. Red and brown seaweeds differed in that while nori induced a metabolic adaptation that increased bacterial utilization of the red algae polysaccharides, wakame failed to do so. The different behaviors of both algae might be a consequence of their distinct chemical compositions. High-fidelity molecular approaches should be applied to assess the modulation of microflora composition and enzymatic activity through dietary supplementation. Because of the physiological importance of the colonic metabolism of nondigested compounds and their fermentation products, the impact of algal polysaccharides' modifying the intestinal microflora activity requires further research.

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LITERATURE CITED

(1) Gibson, G. R. Dietary modulation of the human gut microflora using prebiotics. *Br. J. Nutr.* **1998**, *80*, S209−S212.

(2) Martín-Carrón, N.; Goñi, I. Prior exposure of cecal microflora to grape pomaces does not inhibit in vitro fermentation of pectin. *J. Agric. Food Chem*. **1998**, *46*, 1064−1070.
(3) Macfarlane, G. T.; Gibson, G. R. Metabolic interactions between colonic bacteria and the host. In *Human Health: The Contribution of Microorganism*; Gibson, S. A. W., Ed.; Springler-Verlag: London, U.K., 1994; pp 17−52.
(4) Cummings, J. H.; Macfarlane, G. T. The control and consequences of bacterial fermentation in the human colon. *J. Appl. Bacteriol.* **1991**, *70*, 443−459.
(5) Gibson, G. R.; Roberfroid, H. B. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotic. *J. Nutr.* **1995**, *25*, 1401−1412.
(6) Koopman, J. Kolonisatie resistentie van het maagdarmkanaarl (Colonic resistance of the alimentary tract). *Tijdschr. Diergeneeskd.* **1984**, *109*, 1017−1026.
(7) Lahaye, M.; Thibault, J. F. Chemical and physicochemical properties of fibres from algal extraction byproducts. In *Dietary Fibre: Chemical and Biological Aspects*; Southgate, D. A. T., Waldron, K., Johnson, I. T., Fenwick, G. R., Eds.; The Royal Society of Chemistry Press: Cambridge, U.K., 1990; pp 68−72.
(8) Fleury, N.; Lahaye, M. Chemical and physicochemical characterization of fibres from *Laminaria digitata* (Kombu breton): a physiological approach. *J. Sci. Food Agric.* **1991**, *55*, 389−400.
(9) Lahaye, M. Marine algae as sources of fibres: determination of soluble and insoluble dietary fiber contents in some sea vegetables. *J. Sci. Food Agric.* **1991**, *54*, 587−594.
(10) Rupérez, P.; Saura-Calixto, F. Dietary fiber and physicochemical properties of edible Spanish seaweeds. *Eur. Food Res. Technol.* **2000**, in press.
(11) Lahaye, M.; Kaeffer, B. Seaweed dietary fibres: structure, physicochemical and biological properties relevant to intestinal physiology. *Sci. Aliments* **1997**, *17*, 563−584.
(12) Lebet, V.; Arrigoni, E.; Amadò, R. Measurement of fermentation products and substrate disappearance during incubation of dietary fiber sources with human fecal flora. *Lebensm. Wiss. -Technol.* **1998**, *31*, 473−479.
(13) Reeves, P. G.; Nielsen, F. H.; Fahey, G. C. AIN-93 purified rodent diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76 rodent diet. *J. Nutr.* **1993**, *123*, 1939−1951.
(14) Barry, J.-L.; Hoebler, C.; Macfarlane, G. T.; Macfarlane, S.; Mathers, J. C.; Reed, K. A.; Mortensen, P. B.; Nordgaard, I.; Rowland, I. R.; Rumney, C. J. Estimation of the fermentability of dietary fibre in vitro: a European interlaboratory study. *Br. J. Nutr.* **1995**, *74*, 303−322.
(15) Goering, H. K.; Van Soest, P. J. Forage fiber analysis (apparatus, reagents, procedures and some applications). In *Agricultural Handbook 379;* U.S. Department of Agriculture; U.S. Government Printing Office: Washington, DC, 1979.
(16) Guillon, F.; Renard, C.; Hospers, J.; Thibault, J.-F.; Barry, J.-L. Characterization of residual fibres from fermentation of pea and apple fibres by human fecal bacteria. *J. Sci. Food Agric.* **1995**, *68*, 521−529.
(17) Spiller, G. A.; Chernoff, M. C.; Hill, R. A.; Gates, J. E.; Nassar, J. J.; Shipley, E. A. Effect of purified cellulose, pectin and low-residue diet on fecal volatile fatty acids, transit time and fecal weight in humans. *Am. J. Clin. Nutr.* **1980**, *33*, 754−759.
(18) Suzuki, T.; Ohsugi, Y.; Yoshie, Y.; Shirai, T.; Hirano, T. Dietary fiber content, water-holding capacity and binding capacity of seaweeds. *Fish. Sci.* **1996**, *62*, 454−461.
(19) Humphreys, E. R.; Triffitt, J. T. Absorption by the rat of alginate labelled with carbon-14. *Nature* **1968**, *219*, 1172−1173.
(20) Dewar, E. T.; Maddy, M. L. Faecal excretion of degraded and native carrageenan by the young rat. *J. Pharm. Pharmacol.* **1970**, *22*, 791−793.
(21) Elsehans, B.; Blume, R.; Caspary, W. F. Long-term feeding of unavailable carbohydrate gelling agents. Influence of dietary concentration and microbial degradation on adaptive responses in the rat. *Am. J. Clin. Nutr.* **1981**, *34*, 1837−1848.
(22) Kuda, T.; Fujii, T.; Saheki, K.; Hasegawa, A.; Okuzumi, M. Effects of brown algae on fecal flora of rats. *Nippon Suisan Gakkaishi* **1992**, *58*, 307−314.
(23) Michel, C.; Lahaye, M.; Bonnet, C.; Mabeau, S.; Barry, J.-L. *In vitro* fermentation by human faecal bacteria of total and purified dietary fibres from brown seaweeds. *Br. J. Nutr.* **1996**, *75*, 263−280.
(24) McCandless, E. L.; Graigie, J. S. Sulfated polysaccharides in red and brown algae. *Annu. Rev. Plant Physiol.* **1979**, *30*, 41−53.
(25) Michel, C.; Macfarlane, G. Digestive fates of soluble polysaccharides from marine macroalgae: involvement of the colonic microflora and physiological consequences for the host. *J. Appl. Microbiol.* **1996**, *80*, 349−369.
(26) Stokes, R. L.; Long, W. F.; Williamson, F. B. Degradation of dietary carrageenan in the guinea pig. *Biochem. Soc. Trans.* **1981**, *9*, 164P.
(27) Gibson, G. R.; Macfarlane, G.; Cummings, J. H. The fermentability of polysaccharides by mixed human faecal bacteria in relation to their suitability as bulk-forming laxatives. *Lett. Appl. Microbiol.* **1990**, *11*, 251−254.
(28) Percival, E. Algal polysaccharides. In *The Carbohydrates: Chemistry and Biochemistry*, 2nd ed.; Pigman, W., Horton, D., Herp, A., Eds.; Academic Press: London, U.K., 1970; pp 537−567.
(29) Michel, C. Etudes in vitro de la fermentation des fibres algales par une flore digestive humaine. Ph.D. Thesis, Ecole Nationale Superieure Agronomique de Rennes, 1993.

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