Review

Prebiotics from seaweeds: an ocean of opportunity?

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**Abstract:** Seaweeds are an underexploited and potentially sustainable crop which offer a rich source of bioactive compounds including novel complex polysaccharides, polyphenols, fatty acids, and carotenoids. The purported efficacies of these phytochemicals have led to potential functional food and nutraceutical applications which aim to protect against cardiometabolic and inflammatory risk factors associated with non-communicable diseases such as obesity, type 2 diabetes, metabolic syndrome, cardiovascular disease, inflammatory bowel disease, and some cancers. Concurrent understanding that perturbations of gut microbial composition and metabolic function manifest throughout health and disease has led to dietary strategies such as prebiotics, which exploit the diet-host-microbe paradigm to modulate the gut microbiota, such that host health is maintained or improved. The prebiotic definition was recently updated to “a substrate that is selectively utilized by host microorganisms conferring a health benefit”, which, given that previous discussion regarding seaweed prebiotics has focused upon saccharolytic fermentation, an opportunity is presented to explore how non- complex polysaccharide components from seaweeds may be metabolized by host microbial populations to benefit host health. Thus, this review provides an innovative approach to consider how the gut microbiota may utilize seaweed phytochemicals such as polyphenols, polyunsaturated fatty acids, and carotenoids, and provides an updated discussion regarding the catabolism of seaweed-derived complex polysaccharides with potential prebiotic activity. Additional *in vitro* screening studies and *in vivo* animal studies are needed to identify potential prebiotics from seaweeds, alongside untargeted metabolomics to decipher microbial-derived metabolites from seaweeds. Furthermore, controlled human intervention studies with health-related end points to elucidate prebiotic efficacy are required.

**Keywords:** Seaweed;Gut Microbiota; Prebiotics; Dietary Fibre; Complex Polysaccharides; Polyphenols; Polyunsaturated Fatty Acids; Carotenoids; Phytochemicals

1. Introduction

Seaweeds are an underexploited and sustainable crop which offer a rich source of bioactive compounds, including novel dietary fibers, polyphenols, fatty acids, and carotenoids [1,2]. Epidemiological evidence comparing Japanese and Western diets have correlated seaweed consumption (5.3 g/day in Japan) with decreased incidence of chronic disease [3], while the purported efficacies of seaweed phytochemicals have led to potential functional food and nutraceutical applications which aim to protect against cardiometabolic and inflammatory risk factors associated with non-communicable diseases such as obesity, type two diabetes, metabolic syndrome, cardiovascular disease, inflammatory bowel disease, and some cancers [1].

Current understanding of mutualistic diet-host-microbe interactions has generated efforts to exploit diet to maintain health status, and to prevent or overcome non-communicable diseases, where an imbalance of gut microbiota composition and metabolic function manifests during the onset and pathophysiology of gastrointestinal, neurological, and cardio-metabolic diseases, often congruent with intestinal inflammation and compromised gut barrier function [4,5]. As such, it has become pertinent to explore dietary strategies which modulate gut microbial composition and function to improve host health. This includes the use of prebiotics as fermentable substrates to enable selective gut commensal metabolism.

The prebiotic definition was recently updated to “a substrate that is selectively utilised by host microorganisms conferring a health benefit”, which includes the inhibition of pathogens, immune system activation, and vitamin synthesis and provides opportunity to explore the prebiotic efficacy of non-complex polysaccharide components such polyphenols, phytochemicals, and polyunsaturated fatty acids (PUFAs) [6]. It is recognized that other microbial species have the potential to catabolize prebiotics, besides the classical examples of *Bifidobacterium* and *Lactobacillus* [6], courtesy of culture-independent techniques such as 16S rRNA next generation sequencing and whole genome shotgun metagenomic sequencing which have provided taxonomic classification to identify microbial abundance/diversity and inferred or identified metabolic function [7].

Given that previous discussion regarding the prebiotic potential of seaweed components has focused solely upon the saccharolytic fermentation of complex polysaccharides and the physiological effects of short chain fatty acid metabolites (SCFAs) [3,8,9], scope exists to explore the prebiotic potential of other phytochemical components derived from seaweeds, namely polyphenols, carotenoids, and PUFAs, applicable to both human and animal health.

This review aims to provide an updated discussion regarding the fermentation and potential prebiotic effect of seaweed polysaccharides and oligosaccharides, based on recent evidence from *in vitro* fermentation studies and *in vivo* animal models, and to postulate how other seaweed phytochemicals such as polyphenols, PUFAs, and carotenoids, may interact with the gut microbiota to manipulate microbial composition and/or function to elicit bioactivities pertained to a prebiotic. The latter provides new opportunities to complete prebiotic screening studies using *in vitro* techniques and pre-clinical animal models to understand how parent compound biotransformation into endogenously-derived or gut microbiota-derived metabolites impact bioaccessibility and bioavailability to influence gut microbial community structure and function, conducive to a prebiotic effect. Evidence from clinical trials with health-related end-points and mechanistic insight is imperative to substantiate health claims associated with a prebiotic effect.

# **2. Complex Polysaccharides**

Seaweeds contain 2.97% – 71.4% complex polysaccharides [2,3], which include alginate, fucoidan, and laminarin in brown seaweeds; xylan and sulphated galactans such as agar, carrageenan, and porphyran in red seaweeds; whilst ulvan and xylan are found in green seaweeds. The monosaccharide composition of the major brown, red, and green seaweed glycans are presented in **Table 1**, **Table 2**, and **Table 3,** respectively. Whilst no human study to date has explored prebiotic sources from seaweeds, several *in vitro* studies [10-13], and *in vivo* animal studies [14,15], have explored the prebiotic potential seaweeds and their polysaccharide components.

Seaweed polysaccharides are atypical in structure to terrestrial glycans, and are understood to resist gastric acidity, host digestive enzymes, and gastrointestinal absorption [8]. Seaweed glycans may therefore serve as fermentation substrates for specific gut microbial populations or facilitate substrate cross-feeding of partially broken-down intermediates such as oligosaccharides and metabolic cross-feeding of SCFAs to cause indirect proliferation of specific bacteria [16-20]. The physiological effects of SCFAs, primarily acetate, propionate, and butyrate, includes the reduction of luminal pH to inhibit pathogens, the provision of energy sources to colonocytes, and the activation of free fatty acid receptors; where acetate and propionate are ligands for anorexigenic signaling pathways in appetite regulation and can inhibit the rate limiting step of hepatic cholesterol synthesis via 3-hydroxy-3-methylglutaryl CoA reductase inhibition [21-23].

To facilitate saccharolytic fermentation in the colon, the gut microbiota must harbor functional carbohydrate active enzymes (CAZymes) to catabolise seaweed glycans as carbon sources within the colonic digesta. The repertoire of CAZymes expressed by the human gut microbiota includes glycoside hydrolase and polysaccharide lyase families to facilitate degradation via hydrolysis and elimination reactions, respectively [24-26]. Whole genome sequencing has previously identified gene clusters which encode the catabolic machinery responsible for the breakdown of prebiotics, which includes the CAZyme families responsible for the catabolism of inulin, lactulose, fructo-oligosaccharides, xylo-oligosaccharides, and galacto-oligosaccharides by human gut commensal strains, including *Bifidobacterium longum* NCC2705, *Bifidobacterium adolescentis* ATCC 15703, *Streptococcus thermophilus* LMD9, Eubacterium rectale ATCC 33656, Bacteroides vulgatus ATCC 8482, and Fecalibacterium prausnitzii KLE1255 [27].

Based on open source data from the Carbohydrate-Active enZYmes Database [28],Table 1, Table 2 and Table 3 detail the CAZyme families which may exert specificity for seaweed glycans and highlights the gut bacterial populations which have demonstrable evidence for seaweed glycan utilization. This is dominated by *Bacteroides*, which harbor extensive glycolytic versatility [26,29]. This may explain why *in vitro* batch culture fermentation data of seaweeds and seaweed glycans indicate the proliferation of *Bacteroides* spp.; whilst the degradation of complex seaweed glycans by *Bacteroides* spp. could also facilitate the cross-feeding of oligosaccharides, monosaccharides, and SCFAs for gut commensals deemed beneficial to health, including bifidobacteria.

*In vitro* fermentation studies are frequently used as screening tools to model colonic fermentation and determine substrate utilization by an *ex vivo* fecal inoculum, with seaweed as a sole carbon source. An overview of recent *in vitro* fermentation studies which have evaluated the fermentation of whole seaweeds or extracted complex polysaccharide components by the human gut microbiota is presented in Table 4 (brown seaweeds), Table 5 (red seaweeds), and Table 6 (green seaweeds). These tables include differences in study methodologies, for example, test substrate dosage; the use of an *in vitro* digestion before the fermentation experiment (declared within the methods section of the cited research paper); how the inoculum was prepared; duration of the fecal fermentation experiment; microbial enumeration method; and the analytical technique used to ascertain metabolite changes during the fermentation. The use of an *in vitro* digestion before *in vitro* fermentation is often used to determine whether a substrate is resistant to endogenous digestive enzymes and small intestinal absorption, and to provide the fraction of a dietary component which is bioaccessible in the colon [30]. The lack of an *in vitro* digestion before fermentation experiments may cause false positive results, given that low molecular weight components present in seaweed extracts, normally absorbed in the small intestine, are used as substrates for the *ex vivo* microbiota. Table 7 highlights data from *in vivo* rodent studies which have evaluated the potential prebiotic effect of seaweeds and seaweed glycans.

**Table 1: Potential degradation of brown seaweed glycans by the human gut microbiota**

|  |  |  |  |
| --- | --- | --- | --- |
| **Carbohydrate** | **Carbohydrate-Active Enzyme (CAZyme)** | **Evidenced Glycolytic Bacteria** | **Reference** |
| Alginate | 1,4-β-D-mannuronic acidα-L-guluronic acid | PL6 Alginate lyasePL6 MG-specific alginate lyase | *Bacteroides clarus**Bacteroides eggerthii* |  |
| PL15 Alginate lyasePL15 Oligoalginate lyase | *Bacteroides ovatus**Bacteroides thetaiotaomicron**Bacteroides xylanisolvens* | [31-36] |
| PL17 Alginate lyasePL17 Oligoalginate lyase | *Bacteroides clarus**Bacteroides eggerthii* |  |
| Fucoidan | Sulphated 1,2-1,3-1,4-α-L-fucose | GH29 α-L-fucosidaseGH29 α-1,3/1,4-L-fucosidase | Not determined | [18,37] |
| GH95 α-L-fucosidaseGH95 α-1,2-L-fucosidase |  |
| Laminarin | 1,3-1,6-β-glucose | GH16 β-glucanaseGH16 β-1,3-1,4-glucanaseGH16 endo-1,3-β-glucanase | *Bacteroides distasonis**Bacteroides fragilis**Bacteroides thetaiotaomicron* | [38,39] |
| PL, Polysaccharide Lyase family; GH, Glycoside Hydrolase family. Potential glycolytic bacteria were identified using the Carbohydrate-Active enZYmes Database [28].  |

**Table 2: Potential degradation of red seaweed glycans by the human gut microbiota**

|  |  |  |  |
| --- | --- | --- | --- |
| **Carbohydrate** | **Carbohydrate-Active Enzyme (CAZyme)** | **Evidenced Glycolytic Bacteria** | **Reference** |
| Agar (Galactan) | 1,3-β-D-galactose1,4-3,6-anhydro-α-L-galactose | GH2 β-galactosidase | *Bacteroidetes plebeius* |  |
| GH16 β-agarase | [40-43] |
| GH86 β-agarase |  |
| GH117 1,3-α-3,6-anhydro-L-galactosidase |  |
| Carrageenan (Galactan) | 1,4-β-D-galactose1,3-α-D-galactose3,6-anhydro-D-galactose | GH2 β-galactosidase | *Bacteroides plebeius* | [42,44] |
| GH117 1,3-α-3,6-anhydro-L-galactosidase |  |
| Porphyran (Galactan) | Sulphated 1,3-β-D-galactose1,4-α-L-galactose-6-sulfate3,6-anhydro-α-L-galactose | GH16 β-porphyranaseGH86 β-porphyranase | *Bacteroides plebeius* | [42,45,46] |
| Xylan | 1,3-1,4-β-D-xylose | GH3 xylan 1,4-β-xylosidaseGH5 endo-1,4-β-xylanaseGH10 endo-1,4-β-xylanaseGH10 endo-1,3-β-xylanaseGH11 endo-β-1,4-xylanaseGH11 endo-β-1,3-xylanaseGH43 β-xylosidaseGH43 xylanaseGH43 β-1,3-xylosidaseGH67 xylan α-1,2-glucuronidaseGH115 xylan α-1,2-glucuronidaseCE1 - CE7 and CE12 acetyl xylanesterases | Not determined | [47-50] |
| PL, Polysaccharide Lyase family; GH, Glycoside Hydrolase family. Potential glycolytic bacteria were identified using the Carbohydrate-Active enZYmes Database [28]. |

Table 3: Potential degradation of green seaweed glycans by the human gut microbiota

|  |  |  |  |
| --- | --- | --- | --- |
| **Carbohydrate**  | **Carbohydrate-Active Enzyme (CAZyme)** | **Evidenced Glycolytic Bacteria** | **Reference** |
| Ulvan | Sulphated 1,4-β-D-Glucuronic acidα-L-Rhamnose1,4-β-D-xyloglucan | GH78 α-L-rhamnosidase | Not determined | [51,52] |
| GH145 α-L-rhamnosidase  |   |
| Xylan | 1,3-β-D-xylose | GH10 endo-1,3-β-xylanase,  | Not determined |  |
| GH11 endo-β-1,3-xylanase | [53] |
| GH43 β-1,3-xylosidase  |   |
| PL, Polysaccharide Lyase family; GH, Glycoside Hydrolase family. Potential glycolytic bacteria were identified using the Carbohydrate-Active enZYmes Database [28]. |

2.1 Brown Seaweed Polysaccharides

Brown seaweeds are commonly used as food ingredients owing to their commercial abundance [54]. The anti-obesogenic effects of brown seaweeds is reported in mice, where supplementation of 5% (w/w) *Saccorhiza polyschides* extract, containing 12% dietary fibre, reduced body weight gain and fat mass of mice with diet-induced obesity [55]. The anti-obesogenic effect was attributed to the fermentation of alginate and fucoidan complex polysaccharide components, owing to reduced microbial bile salt hydrolase activity; however, no gut microbial compositional data were provided. Elsewhere, the *in vitro* evidence (Table 4) indicates that whole brown seaweeds and their extracted complex polysaccharide components are fermented by the *ex vivo* fecal microbiota, with increased production of acetate, propionate, butyrate, and total SCFAs reported during fermentation experiments. A corresponding increase in populations such as *Bifidobacterium, Bacteroides*, *Lactobacillus, Roseburia*, *Parasutterella, Fusicatenibacter*, *Coprococcus, Fecalibacterium* is also reported [56-58].

2.1.1 Alginate

Alginates are composed of 1,4-linked α-L-guluronic (G) and β-D-mannuronic acid (M) residues to form GM, GG and MM blocks, and represent 17 - 45% dry weight of brown seaweeds [59]. The colloidal properties of alginates have wide application in food processing, biotechnology, medicine and pharmaceutical industries [60], while degraded sodium alginate is an approved item of “foods with specified uses”, under the categories of “Foods that act on cholesterol plus gastrointestinal conditions” and “Foods that act on blood cholesterol levels” in Japan [61]. The presence of water soluble alginate oligosaccharides in the faeces of pigs fed alginate is indicative of alginate lyase activity by the luminal or mucus adherent gut microbiota [62], although an adaptation period of > 39 days is reported for the degradation of G blocks by the porcine microbiota whilst M blocks are readily degraded [63].

The capacity for alginate to modulate the gut microbiota of Japanese individuals was highlighted over 20 years ago [64], where alginate supplementation (30 kDa, 10 g/day, n = 8) significantly increased fecal bifidobacteria populations in healthy male volunteers after both one and two weeks, alongside significantly increased acetic and propionic acids after two weeks. Deleterious metabolites including fecal sulphide, phenol, p-cresol, indole, ammonia and skatole were significantly reduced compared to the control (free living) diet. Notably, fecal bifidobacteria counts and SCFA concentrations returned to baseline in the week after alginate diet cessation, which highlights the transient nature of the gut microbiota and the need for greater powered long-term human intervention studies.

Subsequent *in vitro* fermentation studies have indicated that alginate is fermented by the human gut microbiota, for example, a 24-hour *in vitro* fermentation of a 212 kDa alginate increased total bacterial populations, although no statistical increase in individual *Bifidobacterium*, *Bacteroides*/*Prevotella*, *Lactobacillus*/*Enterococcus*, *Eubacterium rectale*/*Clostridium coccoides*, or *Clostridium histolyticum* populations were observed [11]. Acetic acid, propionic acid and total SCFAs were significantly increased after 24 hours fermentation with the 212 kDa alginate, while an alginate of 97 kDa increased total SCFA and acetate production after 10 hours of fermentation. Alginates of 38 kDa, and 97 kDa did not change microbial abundance, although the authors could not correlate molecular weight with fermentation patterns.

Alginate oligosaccharides (AOS) (~3.5 kDa) can be obtained via acidic or enzymatic hydrolysis of alginate polysaccharides [59], and enzymatically derived AOS have promoted the growth of *Bifidobacterium bifidum* ATCC 29521, *Bifidobacterium longum* SMU 27001 and *Lactobacilli*, *in vitro* [65]. Supplementation of 2.5% AOS for two weeks significantly increased fecal bifidobacteria in rats compared to control and 5% FOS supplemented diets (13-fold and 4.7-fold increase, respectively), while fecal lactobacilli were 5-fold greater in rats who consumed AOS compared to FOS. Enterobacteriaceae and enterococci populations were significantly decreased following AOS supplementation. Elsewhere, the hydrolysis of alginate, mannuronic acid oligosaccharides (MO) and guluronic oligosaccharides (GO) during a 48-hour batch culture fermentation with the fecal microbiota of Chinese individuals demonstrated increased production of acetate, propionate, butyrate, and total SCFAs compared to the substrate-free control, where GO generated the greatest increase [36]. Subsequent strain isolation from the stools of individuals whom demonstrated alginate degradation during fermentation identified *Bacteroides xylanisovlens* G25, *Bacteroides thetaiotomicron* A12, *Bacteroides ovatus* A9, and *Bacteroides ovatus* G19 as strains capable of hydrolysing alginate and AOS, where *Bacteroides ovatus* G19 expressed α-1,4-guluronanlyase and β-1,4-mannuronanlyase CAZymes [34].

A *Bacteroides xylanisolvens* strain with 99% similarity to *Bacteroides xylanisolvens* XB1A was recently isolated from the stool of a Chinese individual and the alginate lyase gene expressed was 100% homologous to the alginate lyase of *Bacteroides ovatus* strain ATCC 8483 [33]. The preceding *in vitro* fermentation study demonstrated increased production of acetate, propionate, butyrate, and total SCFAs compared to the soluble starch control vessel following 72-hour fermentation of alginate.

Alginate lyase depolymerises alginate polysaccharides to lower molecular weight oligosaccharides via β-elimination, and is most commonly expressed by marine bacteria, including *Flammeovirga* spp., *Vibrio* spp., *Pseudoalteromonas* spp., *Glaciecola* *chathamensis* S18K6, and *Zobellia galactanivorans* [66-70], while the terrestrial bacteria *Paenibacillus* sp. Strain MY03 was recently reported to have alginate lyase and agarase genes [71]. The acquisition of alginate lyase genes by human gut *Bacteroides* is a suggested consequence of horizontal gene transfer from the marine environment [31,72], where seaweed consumption may have provided a vector to exert a selective pressure to induce diet-driven adaptations of the gut microbiota [35,73-77]. Recent work by Matthieu *et al.* [35] suggests that an alginate degradation system within the genome of human gut *Bacteroides* was a result of ancient acquisition, where the polysaccharide utilization loci encodes PL6 and PL17 alginate lyase gene pairs and hypothetical proteins responsible for alginate recognition, internalization, and catabolism, including bacterial ABC transporter proteins to facilitate alginate uptake across the bacterial membrane [78]. Nevertheless, *in vivo* rodent studies have demonstrated that seaweed glycans are fermented even though animals have never been exposed to dietary seaweeds before intervention, which suggests that the gut microbiome contains genes for CAZymes which can degrade seaweed glycans when expressed.

2.1.2 Laminarin

Laminarin is a water-soluble storage polysaccharide consisting of 1,3- or 1,6-β-glucose with an average molecular weight of 5 kDa [79] and accounts for 10 - 35% of the dry weight of brown seaweeds [59]. One *in vitro* batch culture fermentation of laminarin demonstrated increased *Bifidobacteria* and *Bacteroides* after 24 hours [80], while another demonstrated increased propionate and butyrate production after 24-hours [14]. A subsequent *in vivo* rat study (143 mg laminarin per kg body weight per day for 14 days) indicated that laminarin was not selectively fermented by *Lactobacillus* and *Bifidobacterium*, but could modify jejunal, ileal, caecal and colonic mucus composition, secretion, and metabolism to protect against bacterial translocation. The authors suggest that increased luminal acidity and/or catabolism of laminarin by mucolytic commensals could elicit such effects, which corroborates the evidence that a complex polysaccharide-rich diet maintains mucus layer integrity to promote gut barrier function [81,82]. Future studies regarding intestinal mucus modulation by laminarin may wish to characterize gut microbiota compositional changes following laminarin ingestion, to detect the abundance of glycan degraders such as *Bacteroides* spp. [83,84] or mucolytic species associated with health such as *Akkermansia muciniphila* or *Ruminococcus* spp*.* [85,86]. Elsewhere, laminarin increased L-cell GLP-1 secretion to attenuate diet-induced obesity in mice, and improved glucose homeostasis and insulin sensitivity [87]. The authors suggested that the observed cytosolic Ca2+ cascade caused GLP-1 secretion, which is in agreement with GPR41/43 receptor activation by SCFAs produced by gut microbial fermentation [88,89], however, data obtained to assess laminarin-induced changes to gut microbiota composition and metabolic output is needed to ascribe a prebiotic effect in this study.

The abundance of glycoside hydrolase and β-glucosidase enzymes expressed by the human gut microbiota may have the capacity to catabolise laminarin [24,90-92], for example, a *Bacteroides* *cellulosyliticus* WH2 human gut isolate was able to grow on laminarin-supplemented minimal media *in vitro*, (incidentally it did not grow on alginate, carrageenan, or porphyran) [93]; however, the molecular mechanisms by which human gut *Bacteroides* breakdown laminarin are likely distinct from those responsible for the degradation of mix linked β 1,3- 1,4- glucans such as those found in cereals (e.g. by BoGH16MLG) [94].

2.1.3 Fucoidan

Fucoidans are water soluble polysaccharides composed of sulphated 1,2- or 1,3- or 1,4-α-L-fucose which exist as structural polysaccharides in brown seaweeds and occupy 5-20% of algal dry weight [59,95]. The structural heterogeneity of fucoidan encompasses varying degrees of branching, sulphate content, polydispersity, and irregular monomer patterns, which can include fucose, uronic acid, galactose, xylose, arabinose, mannose, and glucose residues [9, 60,96].

A recent *in vitro* fermentation study of fucoidan (< 30 kDa) extracted from *Laminaria japonica* demonstrated a greater increase in bifidobacteria and lactobacilli following 24 and 48 hours fermentation relative to >30 kDa fucoidan [12], while fucoidan from *Ascophyllum nodosum* (1330 kDa) and *Laminaria japonica* (310 kDa) were shown to increase Lactobacillus and Ruminococcaceae, respectively, in the caecal microbiota of mice gavaged with 100mg/kg/day [98]. Fucoidan also reduced serum LPS-binding protein levels in this study - indicative of a reduced antigen load and reduced inflammatory response. In contrast, fucoidan with a fucose-rich and highly sulphated fucoidan extracted from *Cladosiphon okamuranus* was not fermented by the rat gut microbiota [99].

While the purported bioactivities of fucoidan include anti-obesogenic, anti-diabetic, anti-microbial, and anti-cancer properties [100], there is limited evidence to implicate a role for the gut microbiota with such bioactivities, and studies are needed to evaluate the structure-dependent fermentation of fucoidan to ascribe a prebiotic effect. For the latter, this is surprising given the myriad of α-fucosidase enzymes present in the human gut bacterial glycobiome.

Table 4: *in vitro* fermentation of brown seaweeds with human fecal inoculum

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Seaweed** | **Substrate** | **Dose** | **Use of an *in vitro* Digestion Before Fermentation?** | **Experimental Parameters** | **Microbial Enumeration** | **Microbial Changes** | **Metabolomics Analysis Technique** | **Metabolite Changes** | **Reference** |
| *Ecklonia radiata* | Water extract (WE)Acid extract (AE)Celluclast enzyme extract(CEE)Alcalase enzyme extract (AEE)Free sugar fraction (FF)Polysaccharide fraction (PF)Seaweed residue (SR)Seaweed powder (SP) | 1.5% (w/v) | No | 10% (w/v) pooled inoculum (n = 3)24-hours | qPCR | = *F. prausnitzii = C. leptum**= R. bromii* ↑ Total bacteria(CEE, AEE, WE, FF)↑ *Bifidobacterium ↑ Bacteroidetes**↑ Lactobacillus ↑ C. coccoides*(CEE)↑ *E. coli ↑ Enterocccus*(WE, AE, CEE, AEE, FF, PF, SP) | GC-FID | ↑ Acetate↑ Propionate↑ Butyrate(WE, AE, CEE, AEE, FF, PF, SP)↑ Total SCFA | [58] |
| *Ecklonia radiata* | Crude fraction (CF)Phlorotannin-enriched fraction (PF)Low-molecular weight polysaccharide fraction (LPF)High-molecular weight polysaccharide fraction (HPF) | 1.5% (w/v) | Yes | 10% (w/v) pooled inoculum(n = 3)24-hours | qPCR | *↑ Bifidobacterium ↑ Lactobacillus* (LPF)*↑ F. prausnitzii ↑ C. coccoides* *↑ Firmicutes*(CF, LPF)*↑ Bacteroidetes ↑E. coli*(CF, PF, LPF, HPF)*↓ Enterococcus* (CF, PF) | GC-FID | ↑Acetate(CF)↑Propionate(CF, LPF, HPF)↑ Butyrate(CF, LPF, HPF)↑ Total SCFA(CF, LPF, HPF) | [101] |
| qPCR, Quantitative PCR; GC-FID, Gas Chromatography with Flame Ionization Detector; SCFA, Short Chain Fatty Acid; =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Seaweed**  | **Substrate** | **Dose** | **Use of an *in vitro* Digestion Before Fermentation?** | **Experimental Parameters** | **Microbial Enumeration**  | **Microbial Changes** | **Metabolomics Analysis Technique** | **Metabolite Changes** | **Reference** |
| *Sargassum muticum*  | *Sargassum muticum* Alcalase enzyme extract (SAE) | 1% (w/v) | Yes | 10% (w/v) single inoculum24-hours | FISH | = *Bifidobacterium* *= Lactobacillus* *= Clostridium histolticum*↑ *Bacteroides/Prevotella* *↓ C.coccoides/E.rectale* | HPLC | ↑ Total SCFA | [102] |
| *Sargassum thunbergii* | Polysaccharide extract | 0.3% (w/v) | No | 20% (w/v) pooled inoculum (n=3) 24-hours | 16S rRNA NGS | ↑ *Bacteroidetes* ↑ *Bacteroidetes:Firmicutes* ratio ↑ *Bifidobacterium* ↑ *Roseburia* ↑ *Parasutterella*↑ *Fusicatenibacter* ↑ *Coprococcus* ↑ *Fecalibacterium* | GC-MS | ↑ Acetate ↑ Propionate ↑ Butyrate ↑ Valerate ↑ Total SCFA  | [56] |
| FISH, Flourescence in situ Hybridization; 16S rRNA NGS, 16S rRNA Next Generation Sequencing; HPLC, High Performance Liquid Chromaography; GC-MS, Gas Chromatography-Mass Spectrometry; SCFA, Short Chain Fatty Acid; =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |

**Table 4 continued**

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|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Seaweed**  | **Substrate** | **Dose** | **Use of an *in vitro* Digestion Before Fermentation?** | **Experimental Parameters** | **Microbial Enumeration**  | **Microbial Changes** | **Metabolomics Analysis Technique** | **Metabolite Changes** | **Reference** |
| *-* | Alginate | 5% (w/v) | No | 10% (w/v) single inoculum72-hours | 16S rRNA DGGE16S rRNA NGS | ↑ *Bacteroides* | GC-FID | ↑ Propionate↑ Butyrate↑ Total SCFA | [33] |
| - | Alginate (A)Mannuronic acid oligosaccharides (MO)Guluronic acid oligosaccharides (GO) Propylene glycol alginate sodium sulphate (PSS) | 5g/L (A)8g/L (MO GO PSS) | No | 10% (w/v) single inoculum48-hours | 16S rRNA DGGE | Detection of *Bacteroides xylanisolvens, Clostridium clostridioforme/Clostridium symbiosum, Bacteroides finegoldii, Shigella flexneri/E.coli, E.fergusonii,* and *Bacteroides ovatus* | HPLC | A, MO, GO: ↑ Acetate ↑ Propionate ↑ Butyrate ↑ Total SCFA  | [103] |
| *Ascophyllum nodosum* | Sulphated polysaccharide extract | 9 mg/ml | Yes | 10% (w/v) pooled inoculum (n = 4)24 hours | 16S rRNA NGS | ↑ *Bacteroides*↑ *Phascolarctobacterium**↑ Oscillospira**↑ Fecalibacterium* | GC-FID | ↑ Acetate ↑ Propionate↑ Butyrate↑ Total SCFA | [104] |
| 16S rRNA DGGE, 16S rRNA Denaturing Gradient Gel Electrophoresis; 16S rRNA NGS, 16S rRNA Next Generation Sequencing; GC-FID, Gas Chromatography with Flame Ionization Detector; HPLC, High Performance Liquid Chromaography; SCFA, Short Chain Fatty Acid; =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |

**Table 4 continued**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Seaweed**  | **Substrate** | **Dose** | **Use of an *in vitro* Digestion Before Fermentation?** | **Experimental Parameters** | **Microbial Enumeration**  | **Microbial Changes** | **Metabolomics Analysis Technique** | **Metabolite Changes** | **Reference** |
| *Laminaria digitata* | Crude polysaccharide extract (CE)Depolymerized crude polysaccharide extract (DE) | 1% (w/v) | Yes | 20% (w/v) pooled inoculum (n = 3)48 hours | 16S rRNA NGS | ↑ *Parabacteroides* (CE, DE)↑ *Fibrobacter* (CE)*↓ Streptococcus**↓ Ruminococcus*↑ *Lachnospiraceae* uc (DE)↓ *Peptostreptococcaceae* IS (DE)↑ *Dialister* (CE, DE)↑ γ B38UC (CE) | GC-FID | ↑ Acetate (CE, DE)↑ Propionate (CE, DE)↑ Butyrate (CE, DE)↑ Total SCFA (CE, DE) | [105] |
| - | Laminarin | 1% (w/v) | No | 10% (w/v) pooled inoculum (n = 5)24 hours | qPCR | ↑ *Bifidobacterium*↑ *Bacteroides* | HPLC | ↑ Acetate↑ Propionate↑ Total SCFA | [80] |
| 16S rRNA NGS, 16S rRNA Next Generation Sequencing; qPCR, Quantitative PCR; GC-FID, Gas Chromatography with Flame Ionization Detector; HPLC, High Performance Liquid Chromatography; SCFA, Short Chain Fatty Acid; =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |

2.2 Red Seaweed Polysaccharides

2.2.1 Galactans (Carrageenan, Agar, and Porphyran)

Red seaweeds such as *Gelidium* spp and *Gracilaria* spp. are used in the commercial production of agar and carrageenan food additives, including thickening, stabilizing and encapsulation agents [54]. A summary of evidence from recent *in vitro* fermentation experiments using red seaweed-derived substrates is presented in Table 5.

Carrageenans are composed of sulphated 1,4-β-D-galactose, 1,3-α-D-galactose, and 3,6-anhydro-D-galactose [33, 35], and constitutes 30 - 75% dry weight of red seaweeds [59]. In rats fed 2.5% Chondrus crispus, of which carrageenan is a major polysaccharide component, fecal Bifidobacterium breve, and acetate, propionate, and butyrate SCFAs were significantly increased alongside a significant decrease in the pathogens Clostridium septicum and Streptococcus pneumonia, as compared to the basal diet [15]. Furthermore, a 1:1 mixture of polysaccharide extracts from *Kappaphycus alvarezii* (containing carrageenan) and *Sargassum polycystum* (brown seaweed) has lowered serum lipids in rats [40]. In a study by Li *et al.* [34], β-carrageenase activity in a Bacteroides uniforms 38F6 isolate complex of Bacteroides xylanisolvens and Escherichia coli hydrolysed κ-carrageenan oligosaccharides into 4-O-sulfate-d-galactose, κ-carratriose, κ-carrapentaose, and κ-carraheptaose, which could facilitate cross-feeding to promote the growth of Bifidobacterial populations.

Agar is composed of sulphated 1,3-β-D-galactose and 1,4- 3,6-anhydro-α-L-galactose [31-34] and can be fractionated into agarose and agaropectin [8]. Low molecular weight agar of 64.64 kDa has demonstrated a bifidogenic effect alongside increased acetate and propionate SCFA concentrations after 24 hours *in vitro* fermentation with human stool inoculum [11], while mice fed with 2.5% (w/v) neoagarose oligosaccharides for 7 days demonstrated increased caecal and fecal *Lactobacillus* and *Bifidobacterium* [106]. The utilization of agaro-oligosaccharides was noted *in vitro* by Bacteroides uniforms L8, isolated from Chinese individuals, which secreted a β-agarase CAZyme to breakdown agarooligosaccharides into agarotriose and subsequently facilitated the growth of *Bifidobacterium infantis* and *Bifidobacterium adolescentis* via the cross feeding of agarotriose [107].

Porphyran is made up of sulphated 1,3-β-D-galactose, 1,4-α-L-galactose-6-sulfate and 3,6-anhydro-α-L-galactose [42,108,109]. An *in vitro* fecal fermentation study indicated that porphyran did not significantly increase SCFAs, but stimulated *Lactobacilli* and *Bacteroides* populations [80]. While pure cultures of *Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium adolescentis,* but not *Bifidobacterium bifidum*, were able to ferment dried *Porphyra yezoensis* (Nori), containing a low protein content (25%), whereas Nori with a high protein content (41%) was not fermented [110]. It is likely that carbohydrate content was highest in the low protein Nori, thus seasonal- and species-variation and in seaweed macronutrient content should be considered a determinant factor for the fermentability of whole seaweeds [111-114].

Evidence for the horizontal transfer of genes for porphyranase and agarase CAZymes from the marine bacteria, *Zobellia galactanivorans*, to *Bacteroides plebeius* of Japanese individuals is indicative of diet-driven adaptations of the human gut microbiome [42,108]; however, the North American counterparts in this study did not consume seaweeds and did not express such CAZymes. This may mean that the fermentation of seaweed polysaccharides such as porphyran and agar requires exposure to, and acquisition of, specific CAZymes usually present in the marine environment [74]. Red seaweed galactans are emerging prebiotic candidates given the commercial availability of red seaweed hydrocolloids and the potential gut modulatory effects of oligosaccharides obtained from red seaweeds. Nevertheless, further *in vivo* evidence is needed, given the purported pro-inflammatory effects of low molecular weight carrageenan [115-117].

2.2.2 Xylan

Xylan, composed of 1,3 – 1, 4- linked xylose, is a major constituent of red seaweeds such as *Palmaria palmata*. A previous *in vitro* fecal fermentation study of xylan derived from *P*. *palmata*, reported that xylose was fermented after six hours alongside a 58:28:14 ratio of acetate, propionate, and butyrate SCFAs (total SCFAs were 107 mM/L) [118]. This study did not ascertain bacterial compositional data, and thus a knowledge gap is presented given that xylans and xylooligosaccharides (XOS) extracted from terrestrial plants such as wheat husks and maize, are mooted as potential prebiotics owing to evidence of bifidogenesis, improved plasma lipid profile, and positive modulation of immune function markers in healthy adults [119,120]. Given that human gut *Bacteroides* spp. express a repertoire of xylanases and xylosidases [121], investigations regarding the capacity of the human gut microbiota to catabolise red seaweed xylans and XOS are suggested.

Table 5: *in vitro* fermentation of red seaweeds with human fecal inoculum

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Seaweed**  | **Substrate** | **Dose** | **Use of an *in vitro* Digestion Before Fermentation?** | **Experimental Parameters** | **Microbial Enumeration**  | **Microbial Changes** | **Metabolomics Analysis Technique** | **Metabolite Changes** | **Reference** |
| *Kappaphycus alvarezii* | Whole Seaweed (WS) | 1% (w/v) | Yes | 10% (w/v) single inoculum24-hours | FISH | ↑ *Bifidobacterium* ↓ *Clostridium coccoides/Eubacterium rectale* | HPLC | ↑ Total SCFA | [13] |
| *Osmundea pinnatifida* | *Osmundea pinnatifida* Viscozyme extract (OVE) | 1% (w/v) | Yes | 10% (w/v) single inoculum 24-hours | FISH | = *Bifidobacterium* *= Lactobacillus* *= Clostridium histolticum* | HPLC | ↑Total SCFA | [102] |
| *Gracilaria rubra* | Polysaccharide extract (PE) | 1%(w/v) | Yes | 10% (w/v) pooled inoculum (n=4)24-hours | 16S rRNA NGS | ↑ Bacteroides↑Prevotella ↑Phascolarctobacterium↓Firmicutes:Bacteroidetes | GC-FID | ↑ Acetate↑ Propionate↑ Isobutyrate↑ Total SCFA | [122] |
| - | Porphyran | 1% (w/v) | No | 10% (w/v) pooled inoculum (n = 5)24 hours | qPCR | ↑ *Bifidobacterium*↑ *Bacteroides* | HPLC | = Acetate= Propionate= Butyrate= Total SCFA | [80] |
| FISH, Flourescence in situ Hybridization; 16S rRNA NGS, 16S rRNA Next Generation Sequencing; qPCR, Quantitative PCR; GC-FID, Gas Chromatography with Flame Ionization Detector; HPLC, High Performance Liquid Chromatography; SCFA, Short Chain Fatty Acid; =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |

2.3 Green Seaweed Polysaccharides

2.3.1 Ulvan

Ulvans are water-soluble cell wall polysaccharides that account for 8 - 29% dry weight of green seaweeds, and are composed of sulphated 1,3-α-L-rhamnose, 1,4-β-D-glucuronic acid, and 1,4-β-D-xyloglucan [52]. Previous reports indicate that *Ulva lactuca* and ulvan polysaccharides are poorly fermented by the human gut microbiota [8,96,123], while an *in vitro* fermentation study of *Enteromorpha* spp. with a human fecal inoculum reported no difference in *Enterococcus, Lactobacillus*, and *Bifidobacterium* populations compared to the control; only an increase in *Enterobacter* after 24 and 48 hours of fermentation (Table 6) [12]. In contrast, a recent *in vitro* fecal fermentation study indicated that Ulvan stimulated the growth of *Bifidobacterium* and *Lactobacillus* populations and promoted the production of lactate and acetate [80]. Further, a murine study showed that *Enteromorpha* (EP) and *Enteromorpha* polysaccharides (PEP) ameliorated inflammation associated with Loperamide-induced constipation in mice [124], where alpha diversity, Firmicutes, and Actinobacteria were increased in the fecal microbiota of seaweed-supplemented mice compared to the constipated control. Bacteroidetes and Proteobacteria were decreased, while Bacteroidales S24-7 and Prevotellaceae were increased in EP and PEP, respectively. Current evidence for the fermentation of green seaweeds and their polysaccharides is limited and fermentation may require specific α-L-rhamnosidase activity by gut commensals [51]. More experimental evidence is needed to understand the impact of ulvans and ulvan-oligosaccharides in the human and animal diet.

2.4 Future Prospective – Obtaining Oligosaccharides

Enzyme technologies are reported to increase the extraction yield and reduce the molecular weight of bioactive components obtained from seaweeds, with examples of enhanced prebiotic activity when commercially available cellulases or seaweed-specific enzymes were used to hydrolyse polysaccharides [101]. Despite limited commercially availability of seaweed-specific enzymes, an avenue for functional oligosaccharide production is presented if efforts to develop commercially viable saccharolytic enzymes from microorganisms (primarily marine). Examples of such glycoside hydrolases include fucoidanase from *Sphingomonas paucimobilis* PF-1 [125]; ulvan lyase from *Alteromonas* sp. [126] and the family Flavobacteriaceae [127]; β-agarase from *Cellulophaga omnivescoria* W5C [128] and *Cellvibrio* spp. PR1 [129]; alginate lyase from *Flammeovirga* spp. [130], and *Paenibacillus* spp. [71]; and laminarinase [131]. Factors which influence the stability and efficacy of such hydrolytic enzymes may include metal ion interaction, or thermostability at the high temperatures needed to prevent gelling of polysaccharides. Recent insight into the production of agarooligosaccharides and neoagarooligosaccharides from agar exemplify this [132].

Table 6: *in vitro* fermentation of green seaweeds with human fecal inoculum

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Seaweed**  | **Substrate** | **Dose** | **Use of an *in vitro* Digestion Before Fermentation?** | **Experimental Parameters** | **Microbial Enumeration**  | **Microbial Changes** | **Metabolomics Analysis Technique** | **Metabolite Changes** | **Reference** |
| *Enteromorpha prolifera* | Polysaccharide extract (PE) | 0.2g in 9.5ml 0.8g in 9.5ml | Yes |  10.5% (w/v) pooled inoculum (n = 3)12, 24, and 48-hours | Microbial culture | ↑ *Enterobacter* (0.2 PE and 0.8 PE at 24h and 48h)= *Enterococcus* *= Lactobacillus* *= Bifidobacterium* | GC-FID | = Acetate= Butyrate= Lactate | [12] |
| - | Ulvan | 1% (w/v) | No | 10% (w/v) pooled inoculum (n = 5)24 hours | qPCR | ↑ *Bifidobacterium*↑ *Lactobacillus* | HPLC | ↑ Acetate↑ Lactate | [80] |
| qPCR, Quantitative PCR; GC-FID, Gas Chromatography with Flame Ionization Detector; HPLC, High Performance Liquid Chromatography; =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Animal** | **Substrate** | **Dose** | **Duration** | **Biological Sample** | **Microbiota Changes** | **Metabolite Changes** | **Reference** |
| 30 Male Sprague-Dawley Rats | *Chondrus crispus* Whole Seaweed (WS) | 0.5% (w/w) 2.5% (w/w)  | 21 days | Faeces | ↑*Bifidobacterium* ↑ *Legionella* ↑ *Sutterella* ↑ *Blautia* ↑ *Holdemania* ↑ *Shewanella* ↑ *Agarivorans* ↓ *Streptococcus* ↑ *Bifidobacterium* *breve* (2.5% WS) | ↑ Acetate ↑ Propionate (2.5% WS)↑ Butyrate ↑ Total SCFA | [77] |
| 24 Male Sprague-Dawley Rats | *Ecklonia radiata* Whole Seaweed (WS)Ecklonia radiata Polysaccharide Fraction (PF) | 5% (w/w) WS5% (w/w) PF | 7 days | Caecum | ↑ *F. prausnitzii ↑ E. coli* (PF)*↓ Enterococcus* (WS)↓ *Lactobacillus* ↓ *Bifidobacterium* ↓ Firmicutes:Bacteroidetes  | ↑ Acetate ↑ Propionate ↑ Butyrate (PF)↓ Valerate↓ Hexanoate↑ Total SCFA ↓ i-Butyrate ↓ i-Valerate ↓ phenol ↓ p-cresol | [57] |
| 18 Male Wistar Rats | Alginate (A)Laminarin (L)Fucoidan (F) | 2% (w/w) | 14 days | Caecum | A: *↑ Bacteroides* (*Bacteroides* *capillosus*)Presence of *Enterorhabdus*L: ↑ Proteobacteria. Presence of *Lachnospiracea, Parabacteroides* (*Parabacteroides distasonis*) & *Parasutterella*F: Not fermented | ↑ Propionate (L)↑ Total SCFA (A, L) | [99] |
| SCFA, Short Chain Fatty Acid; =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |

 Table 7: Impact of seaweeds on the rodent gut microbiota

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Animal** | **Substrate** | **Dose** | **Duration** | **Biological Sample** | **Microbiota-Associated Changes** | **Metabolite Changes** | **Reference** |
| 16 MaleC57 BL/6 Mice | *Saccorhiza polyschides* extract (BAE) | High fat diet + 5% (w/w) BAE | 8 months | Faeces | ↓ Fecal bile salt hydrolase activity | ↓ Secondary bile acids | [55] |
| 18 Male Wistar Rats | Alginate (A)Laminarin (L) | 2% (w/w)  | 14 days | Caecum | ↑ *Lactobacillus* ↑ *Porphyromonas* ↑*Coprobacillus*↑ *Oscillibacter valencigenes* ↓ *Parabacteroides* (L)↑ *Catabacter honkongensis* ↑ *Stomatobaculum longum* ↓ *Adlercreuzia* (A)↓ *Helicobacter* (A, L) | ↑ Lactic acid (L)= Acetate= Propionate = Butyrate = Total SCFA ↓ Indole | [133] |
| 18 Male C57BL/6 mice | *Ascophyllum nodosum* Fucoidan (FuA)*Laminaria**japonica* Fucoidan (FuL) | 100mg/kg/day | 6 weeks | Caecum | ↑ *Lactobacillus* ↑*Anaeroplasma* ↑ *Thalassospira*  (FuA)↑ *Ruminococcaceae ↓ Alistipes, ↓ Clostridiales* *↓ Akkermansia* (FuL)*↓ Candidatus* *↓ Arthromitus ↓ Peptococcus* *↓ Lachnospiraceae Incertae Sedis* (FuA, FuL) | - | [98] |
| SCFA, Short Chain Fatty Acid; =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |

**Table 7 continued**

**Table 7 continued**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Animal** | **Substrate** | **Dose** | **Duration** | **Biological Sample** | **Microbiota-Associated Changes** | **Metabolite Changes** | **Reference** |
| 32 Female Kunming mice | *Enteromorpha prolifera* (EP)*Enteromorpha* polysaccharide extract (PEP) | 1:5 (w/w) | 7 days | Faeces | ↑Alpha diversity (EP)↑ Bacteroidales S24-7 (EP)↑ Prevotellaceae (PEP)↑ Firmicutes ↑Actinobacteria (EP, PEP)↓ Bacteroidetes ↓ Proteobacteria (EP, PEP) | - | [124] |
| 15 Male Wistar rats | *Ascophyllum nodosum* seaweed crude polysaccharide (SCP)SCP *Lactobacillus plantarum* hydrolysate (SCPH Lp)SCP *Enterococcus fecalis* hydrolysate (SCPH Ef)Alginate (A)Hydrolysed Alginate (HA) | 0.2g per 180 – 200g rat weight | 4 days | Faeces | - | ↑ Acetate (HA > A > SCPH Lp > SCPH Ef)↑ Propionate (HA = A = SCPH Lp = SCPH Ef)↑ Butyrate (HA = A = SCPH Lp = SCPH Ef)(relative to day zero) | [134] |
| =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |

# 3. Polyphenols

Seaweeds are rich in polyphenols such as catechins, flavonols, and phlorotannins. Red and green seaweeds are a source of bromophenols, phenolic acids, and flavonoids [135], while phlorotannins are the most abundant polyphenol in brown seaweeds. Most research to date concerns the bioactivity of phlorotannins, a class of polyphenol unique to brown algae comprised of phloroglucinol monomers and categorised as eckols, fucols, fuhalols, ishofuhalols, phloroethols, or fucophloroethols [135]. The purported bioactivities of seaweed polyphenols are associated with the mitigation of risk factors pertained to type 2 diabetes and cardiovascular disease including hyperglycemia, hyperlipidemia, inflammation and oxidative stress [136-140], and also anti-microbial activity [135,141]. Owing to heterogeneity in both molecular weight and the level of isomerization, characterization of polyphenols is difficult [142-144], and a paucity of information exists regarding the endogenous digestion and microbial catabolism of seaweed polyphenols, with scarce mechanistic understanding of how they may exert health benefits via the gut microbiota.

Most polyphenols of plant origin must undergo intestinal biotransformation by endogenous enzymes and the gut microbiota prior to absorption across enterocytes. These enzymatic transformations include elimination of glycosidic bonds, for example, flavonoids are converted to glycones (sugars) and aglycones (non-sugars – polyphenols) by endogenous β-glucosidases in the small intestine [145]. The transport of aglycones to the liver via the portal vein results in phase II biotransformation (coupling reactions, chiefly hepatic conjugation to O-glucuronides and O-sulfates) to facilitate urinary and biliary elimination. Phase II metabolites are absorbed into the systemic circulation, or excreted in bile and re-enter the duodenum (hepatic recycling), where subsequent glucuronidase, glycosidase, or sulphatase-mediated deconjugation by the colonic microbiota may favour aglycone reabsorption [146].

Approximately 90-95% of dietary polyphenolsreach the colon intact [147], where biotransformation and metabolism by the gut microbiota occurs via hydrolysis, reduction, decarboxylation, demethylation, dehydroxylation, isomerisation, and fission [148], to produce low-molecular weight compounds with less chemical heterogeneity than the polyphenol parent compound [145].It is suggested that a complex network of gut microbial species is necessary for full biotransformation of polyphenols, whereas simple reactions such as deglycosylation can be achieved by individual gut strains. Furthermore, the bioactivities associated with dietary polyphenol intake may be dependent on the catabolic capacity and composition of the gut microbiota, owing to biological activity of metabolites rather than the parent polyphenol compound present in food [149,150], while a synergistic effect between prebiotic polyphenols and probiotic bacteria may occur [6].

The identification of bacteria which possess the metabolic capabilities to utilize polyphenols was previously identified in *Eubacterium oxidoreducens* spp., which could catabolise gallate, pyrogallol, phloroglucinol and quercetin [151]. Quercetin biotransformation by *Eubacterium ramulus* has also been identified [152], and multiple human gut microbes which possess phenolic enzymes capable of breaking down glycosides, glucuronides, sulphates, esters, and lactones was summarised by Selma *et al*. [148]. Such microorganisms included *E. coli* with β-glucuronidase activity; *Eubacterium* spp., *Bacteroides* spp., and *Clostridium* spp. with β-glucosidase activity; *Lactobacilli*, *Eubacterium* spp., *Clostridium* sp, *Butyrbacterium*, *Streptococcus*, and *Methylotrophicum* with demethylase activity; and *E. coli*, *Bifidobacterium*, *Lactobacilli*, *Bacteroides*, *Streptococci*, *Ruminococci*, and *Enterococci* with esterase activity. There is also evidence for α-L-Rhamnosidase mediated hydrolysis of rutinose, present on glycosylated polyphenols (rhamnoglycosides), to produce aglycones, by species such as *Bacteroides thetaiotaomicron* [51], *Bifidobacterium dentium* [153], *Bifidobacterium catenulatum* [154], *Bifidobacterium pseudocatenulatum* [154], and *Lactobacillus plantarum* [155].

Current knowledge regarding the fate of seaweed polyphenols in the human gastrointestinal tract is scarce; however, it is understood that the limited absorption of *Ascophyllum nodosum* polyphenols from small intestinal enterocytes to the portal vein may facilitate the conjugation of polyphenols to methylated, glucuronidated, or sulphated forms rather than hydrolysis to aglycones [156,157]. Subsequently, unabsorbed conjugated polyphenols are available for biotransformation by the colonic microbiota, then potentially absorbed across the colonocytes. Indeed, Corona *et al*. [158], observed a reduction of total polyphenol contents of an *Ascophyllum nodosum* polyphenol extract, high molecular weight fraction (>10 kDa), and low molecular weight fraction (1-10 kDa) following *in vitro* digestion and batch culture fermentation; although anti-genotoxic activity against H2O2 induced DNA damage of HT-29 cells was increased (to a greater extent by the high molecular weight fraction). This study did not assess the microbiota composition, however, elsewhere, an *in vitro* fermentation of an *Ecklonia radiata* phlorotannin extract significantly increased *Bacteroidetes*, *Clostridium coccoides*, *E. coli*, and *Fecalibacterium prausnitzii*, but decreased *Bifidobacterium* and *Lactobacillus* populations after 24 hours fermentation [57]. More *in vitro* digestion studies would be useful to understand the stability of seaweed polyphenols as extracts or within the seaweed matrix [159,160]. These studies may be complemented by studies which use ileostomy patient cohorts to determine structural changes to seaweed polyphenols following upper GI digestion *in vivo* to indicate polyphenol bioaccessibility in the colon [161].

A recent review highlighted the potential for dietary polyphenols to modulate the gut microbiota by increasing *Bifidobacterium, Lactobacillus, Bacteroides*, *Enterococcus,* *Akkermansia muciniphila*, and *Fecalibacterium prausnitzii* populations [156]. This review did not include any studies which assessed modulation of the gut microbiota by seaweed polyphenols and therefore a research opportunity is presented. Inter-individual variation of gut microbiota composition and function is the key determinant for gut microbiota-mediated biotransformation of phenolic compounds to bioactive metabolites [162,163]. Therefore, identification of bacterial species or strains with the ability to catabolise seaweed polyphenols and their respective catabolic machinery is needed to understand how seaweed polyphenols could be prebiotic [164,165]. Moreover, considering that gut microbiota-derived secondary metabolites reach a peak plasma concentration much later than the original aglycone or hepatic conjugates, controlled nutrikinetic studies could elude how dietary polyphenols from seaweeds interact with host-microbiota metabolism [166,167]. Identification of fecal, urinary, serum, or tissue biomarkers via untargeted and targeted metabolomics approaches, and the use of stable isotope studies, may also indicate variation in synthesis, bioavailability, metabolism, and excretion of polyphenols and associated metabolites. While an integration of dose response studies alongside metagenomics and metabolomics analyses, akin to those conducted for berry and wine polyphenols, could elude how much seaweed polyphenol is required to have an impact, if any, on gut microbial composition, metabolic function, and host health [168,169].

# 4. Other Seaweed Phytochemicals

**4.1 Carotenoids**

Carotenoids are lipid soluble compounds which function within the photosynthetic machinery of seaweeds to produce pigments. Fucoxanthin is the predominant carotenoid in brown seaweeds [170], while lutein, β-carotene, astaxanthin, echinenone, violaxanthin, neoxanthin, and zeaxanthin are found in red and green seaweeds. Carotenoids are used as food colouring additives, while the application of fucoxanthin as functional food ingredients is suggested, owing to putative anti-oxidant, anti-inflammatory, anti-cancer, anti-obesity, and anti-diabetic bioactivities [171-176].

While some carotenoids are absorbed by enterocytes and converted into vitamin A and retinoid derivatives by endogenous beta‐carotene oxygenase 1 (BCO1) and beta‐carotene dioxygenase 2 (BCO2) enzymes [177,178], the bioavailability of carotenoids in the blood is reported as 10% - 40% [179], which has led to suggestions that carotenoids could be fermented by the gut microbiota [177,180]. The only evidence to date has demonstrated that male C57BL/6J mice supplemented with 0.04% (w/w) astaxanthin during an eight-week pilot study had increased abundance of caecal *Bifidobacterium* [181], whereas *Proteobacteria* and *Bacteroides* were significantly increased in the caecum of BCO2 knockout mice. Analysis of health biomarkers were not reported. Given the differences in microbiota composition between wild type and BCO2 knockout mice in this study, there is scope to investigate how carotenoids and their endogenous derivatives interact with the gut microbiota. Looking ahead, the use of *in vitro* models of gastrointestinal digestion and colonic fermentation would be useful to assess whether there is a direct substrate to microbiota effect or a host–microbe effect [182].

**4.2 Polyunsaturated Fatty Acids (PUFAs)**

The lipid content of seaweed ranges from 1-5% dry weight, which includes n-3 PUFAs such as eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) [183,184]. The n-3 PUFA are associated with anti-inflammatory activity to reduce cardiovascular disease risk and may also exert beneficial effects on brain function and behavior, as mediated by the microbiota-gut-brain axis [185]. Dietary EPA and DHA intake is reported to improve microbial diversity, reduce the Firmicutes/Bacteroidetes ratio, reduce LPS-producing bacteria, and increase populations of Bifidobacteria, Lachnospiraceae, and lipopolysaccharide (LPS)-suppressing bacteria in both humans and animal models [185-187]. Although the evidence to date has focused on fish-derived n-3 PUFA, great scope exists to evaluate the prebiotic effect of n-3 PUFA obtained from seaweeds.

# 5. Fermented foods

Fermented foods are understood to have improved nutritional and functional properties owing to bioactive or bioavailable components [188]. Seaweeds (mainly kelp) are a common vegetable ingredient in the fermented food, Kimchi. The microbial content of kimchi provides a source of probiotics, nutrients, and bioactive metabolites, which are reported to have anti-microbial, anti-oxidant, and anti-obesogenic activities [189-191]. One randomized controlled trial (RCT) observed that consumption of a seaweed Kimchi made from *L. japonica* for four weekspromoted the growth and survival of gut microbial lactic acid bacteria in humans [192], whilst another RCT concluded that consumption of 1.5g/day fermented *L. japonica* containing 5.56% γ-aminobutyric acid (GABA) (*Lactobacillus brevis* BJ2 culture) was associated with a reduction in oxidative stress in healthy adults over four weeks, indicated by decreased serum γ-glutamyltransferse (GGT) and malondialdehyde, and increased antioxidant activity of superoxide dismutase and catalase compared to the placebo [193]. The latter study indicates that foods containing fermented brown seaweeds such as *L. japonica* may offer a novel source of GABA enriched ingredients, which are associated with hypotensive and anti-inflammatory effects [191]. Anti-oxidant, anti-diabetic, and anti-hypertensive efficacies are also reported for Korean rice wine fermented with *L. japonica* [194], while *Sargassum* spp. fermented with a starter culture of *Enterococcus faecium* was reported to contain higher soluble polyphenol and mannuronic acid-rich alginate contents [195], which may increase the provision of microbiota accessible components for colonic fermentation.

Reports of the functional properties of fermented foods containing red seaweeds is scarce; however, examples of red seaweed fermented foods include a fermented *Porphyra yezoensis* seaweed sauce, which used the marine halophilic lactic acid bacteria, *Tetragenococcus halophilus*, as a starter culture [196]; a *Gracilaria domingensis* aqueous extract applied as a texture modifier in fermented milks as a non-animal alternative to gelatin [197]; and carrageenan as a salt replacer in the production of fat-free cheese [198].

Given the availability of red, brown, and green seaweeds both commercially and locally [199], the production of seaweed-containing fermented foods could be a cost-effective alternative to bioactive component extraction. Nevertheless, an understanding of how both the live bacteria and the metabolites presented in the fermented food contribute towards health is required [200].

# 6. Seaweeds and Animal Health

Seaweeds also have an historical use as animal feed ingredients [201]. The capacity for seaweeds to modulate the gut microbiota of monogastrics such as pigs and hens is presented in Table 8 and Table 9, respectively, which complements the recent evidence for the application of seaweed bioactives in monogastric animal feed [202]. Table 8 shows limited evidence that the β-glucan, laminarin, may increase *Lactobacillus* populations but not *Bifidobacterium* populations. While there is scarce evidence for the selective stimulation of health-associated bacteria in pigs by the sulphated fucose, fucoidan. Only one recent study has evaluated the effect of dietary alginate on the porcine microbiota, where the genera *Ruminococcus*, *Roseburia* and *Lachnospira*, and an unclassified bacterium of the F16 family were increased, alongside a significant decrease in the genus *Blautia,* the family Clostridiaceae, and an unclassified bacterium of RF39 family [203]. In Table 9, recent evidence indicates that hens fed *Chondrus crispus* and *Sarcodiotheca gaudichaudii* red seaweeds may increase ceacal SCFA concentrations and modulate populations of *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Streptococcus salivarius*, and *Clostridium perfringens* [204,205]; however, a bidirectional change in microbial composition was dose dependent and has only been assessed in two studies to date. Given the use of pigs as an animal model of humans [206], data from *in vivo* monogastric studies which are designed to evaluate the prebiotic potential of dietary seaweeds and seaweed-derived components could provide insight into the potential for human applications.

Table 10 and Table 11 summarise recent studies which have examined the impact of seaweed diets on the ruminant microbiota of cows and sheep, respectively, with potential application of reducing methane production. Despite demonstrating decreased methane production, the cow rumen *in vitro* fermentation studies presented in Table 10 did not assess microbiota compositional changes, thus a knowledge gap is presented to understand which bacteria (if any), are increased or decreased, and are associated with a reduction in methane production. Table 11 shows that methanogenic bacteria and methane production were significantly decreased compared to the basal grass substrate control following the *in vitro* fermentation of sheep rumen with the red seaweed *Asparagopsis taxiformis* [207]. While sheep given an *ad* libitum diet of *Ascophyllum nodosum* brown seaweed (1%, 3%, or 5% w/w) for 21 days demonstrated a dose-dependent decrease in propionate and butyrate SCFAs and a dose-dependent increase in acetate, while several bacteria were significantly decreased, including *Prevotella copri*, *Roseburia* spp.*,* and *Coprococcus* spp., while *Blautia producta* and the family Veillonellaceae were significantly increased compared to the basal diet. Moreover, the specific case of seaweed-fed North Ronaldsay sheep highlights how isolated organisms of the ruminant microbiome, such as *Prevotella* spp., *Clostridium butyricum*, *Butyrivibrio fibrisolvens*, and Spirochaetes have adapted to hydrolyse alginate laminarin, and fucoidan [208,209]. However, there is a paucity of evidence to implicate any health benefits attributed to a seaweed diet in these animals.

Table 8: Impact of seaweeds on the porcine gut microbiota

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Animal** | **Seaweed Component** | **Dose** | **Duration** | **Biological Sample** | **Microbiota Changes** | **Metabolite Changes** | **Reference** |
| 20 pregnant gilts and 48 piglets | Laminarin/Fucoidan Extract | 10g/day  | Gestation (day 83) to weaning (day 28) | Faeces (Sow)Colonic digesta (Piglet) | Sows (parturition): *↓ Enterobacteriaceae* *= Lactobacilli*Piglets (birth, 48h after birth, weaning):= *Enterobacteriaceae = Lactobacilli* | - | [210] |
| 200 pigs | *Ecklonia cava* Whole Seaweed | 0.05% (w/w)0.1% (w/w) 0.15% (w/w)  | 28 days | Caecum | ↑ *Lactobacillus spp.* *↓ E. coli*= Total Anaerobes | - | [211] |
| 24 pigs | Laminarin/FucoidanExtract (SD)Laminarin/Fucoidan Wet Seaweed (WS) | 5.37 Kg/tonne SD26.3 Kg/tonne WS | 21 days | Ileum Caecum Colon | *= Bifidobacteria* *= Lactobacillus**= Enterobacterium* (SD, WS)↑ *Lactobacillus agilis* (colon) | - | [212] |
| 48 pigs | Laminarin Extract | 300ppm  | 32 days | Faeces | ↑ *Lactobacillus* *= Bifidobacteria* | = Acetate↓ Propionate = Butyrate= Valerate = i-Butyrate= i-Valerate | [213] |
| 48 pigs | β-glucan | 250 g/tonne150g/tonne | 29 days | Ileum CaecumProximal and Distal colon | = *Lactobacilli* = *Bifidobacteria.*↑ *Lactobacillus* diversity | - | [212] |
| =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |

**Table 8 continued**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Animal** | **Seaweed Component** | **Dose** | **Duration** | **Biological Sample** | **Microbiota Changes** | **Metabolite Changes** | **Reference** |
| 168 pigs | Laminarin (L) Fucoidan (F) |  240 mg/kg F150 mg/kg L300 mg/kg L150 mg/kg L and 240mg/kg F300 mg/kg L and 240 mg/kg F | 35 days | Faeces | = *E. coli* = *Bifidobacteria*  *↑ Lactobacilli* | = Acetate= Propionate= Butyrate= Valerate= i-Butyrate= i-Valerate= Total SCFA | [214] |
| 9 pigs | Alginate | 5.14% (w/w)  | 84 days | Faeces | = Diversity↑ Unclassified F16 family ↓ Clostridiaceae ↓ Unclassified RF39 (Mollicutes)↑ Ruminococcus ↑ Roseburia ↑ unclassified F16 genus (TM7) ↑ Lachnospira ↓ Blautia | - | [203] |
| =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |

Table 9: Impact of seaweeds on the hen gut microbiota

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Animal** | **Seaweed Component** | **Dose** | **Duration** | **Biological Sample** | **Microbiota Changes** | **Metabolite Changes** | **Reference** |
| 160 laying hens | *Chondrus crispus* Whole Seaweed (CC)*Sarcodiotheca gaudichaudii* Whole Seaweed (SG) | 0.5% (w/w) 1% (w/w) 2% (w/w)  | 30 days | IleumCaecal digesta | ↑ *Bifidobacterium longum* (CC2, SG1, SG2)↑ *Streptococcus salivarius* (CC1, CC2, SG2)↓ *Clostridium perfringen*s (CC1, CC2, SG1, SG2)↓ *Lactobacillus acidophilus* (CC1, CC2) | ↑ Acetate (CC1, SG1)↑ Propionate (CC2)↑ Butyrate (SC2) | [204] |
| 96 laying hens | *Chondrus crispus* Whole Seaweed (CC)*Sarcodiotheca gaudichaudii* Whole Seaweed (SG) | Control diet + 2% (w/w) seaweed Control diet + 4% (w/w) seaweed | 28 days | Caecum | ↑ *Lactobacillus acidophilus* (CC4)↓ *Bifidobacterium longum* (SG2, SG4, CC4)↓ *Streptococcus salivarius* (SG2, SG4, CC2, CC4)↑ Bacteroidetes (SG4, CC2, CC4) | ↑ Propionate (CC4) | [205] |
| =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |

Table 10: *in vitro* fermentation of seaweeds with cow rumen inoculum

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Seaweed** | **Substrate** | **Experimental Parameters** | **Dose (w/v)** | **Microbial Enumeration**  | **Microbial Changes** | **Metabolomics Analysis Technique** | **Metabolite Changes** | **Reference** |
| *Ascophyllum nodosum* (AN) *Laminaria digitata* (LD)  | Whole Seaweed | 50% pooled inoculum (n = 4) 24-hours | 0.5g/L1g/L 2g/L | -  | **-** | GC-FID | ↑Propionate ↑Butyrate (LD)↓ BCFA↓Methane | [103] |  |  |
| *Asparagopsis taxiformis* | Whole Seaweed | 20% pooled inoculum (n = 4)72-hours | 0.5%1%2%5%10% | -  | **-** | GC-FID | ↓ Total gas production ↓ Methane ↓ Acetate ↑ Propionate↑ Butyrate (2%, 10%)↓ Total SCFA (5%, 10%) | [215] |
| *Ulva sp.**Laminaria ochroleuca**Saccharina latissima**Gigartina sp.**Gracilaria vermiculophylla* | Whole Seaweed | 20% pooled inoculum (n = 2)24-hours | 25% | **-** | **-** | GC-FID | ↓ Methane | [216] |
| Brown seaweed by-products (BSB) | - | 50% (v/v)single inoculum0, 3, 6, 9, 12, & 24-hours | 2%4% | **-** | **-** | GC-FID | ↓ Ammonia (3, 9, 12 & 24h)↓ Total SCFA (24h) | [217] |
| GC-FID, Gas Chromatography; =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |

Table 11: Impact of seaweeds on the sheep rumen microbiota

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Seaweed** | **Dose** | **Experimental Parameters** | **Microbial Enumeration** | **Microbial Changes** | **Metabolomics Analysis Technique** | **Metabolite Changes** | **Reference** |  |  |
| *Asparagopsis taxiformis*Whole Seaweed | 2% | *in vitro* batch culture fermentation20% (v/v) pooled sheep rumen fluid inoculum (n = 4)48 and 72-hours | 16S rRNA NGSqPCR | ↓Methanogens↓Bacteroidetes/Firmicutes ratio↓ mcrA gene expression | GC-MS | ↓ Total Gas↓ Methane↑ Hydrogen | [207] |  |  |
| *Ascophyllum nodosum*Whole Seaweed | 1%3%5% | Rams (n = 8)21 days *ad libitum* | 16S rRNA NGS | ↓ undefined TM7-1↓undefined Coriobacteriaceae ↓Roseburia↓Coprococcus sp.↓Prevotella copri↑Blautia producta↑ Entodinium species 1↑Veillonellaceae | GC-FID | Dose dependent:↑ Acetate↓ Propionate↓ ButyratePICRUSt:↑Butanoate metabolism↑ Fatty acid metabolism↓Glycerophospholipid metabolism | [218] |  |  |
| 16S rRNA NGS, 16S rRNA Next Generation Sequencing; qPCR, Quantitative PCR; GC-FID, Gas Chromatography; =, no statistical difference compared to the control; ↑, significant increase compared to the control; ↓ significant decrease compared to the control. Microbial and metabolite changes with abbreviations in parenthesis indicate the substrate(s) which exerted the effect. If no abbreviations in parenthesis are presented, then all of the seaweed substrates tested exerted the effect. |  |  |

7. Conclusions

Current evidence regarding the prebiotic effects of seaweeds is dominated by complex polysaccharide components. This is because prebiotic research was previously focused on saccharolytic fermentation by the gut microbiota. Accumulating evidence from *in vitro* and *in vivo* animal studies provides encouraging data regarding the utilisation of red seaweed galactans and brown seaweed glycans such as alginates and laminarins, with minor evidence for fucoidan and the green seaweed polysaccharide, ulvan.

Given that the most recent definition of a prebiotic places non- complex polysaccharide components in vogue, an opportunity is presented to explore how other seaweed phytochemicals, including polyphenols, carotenoids, and PUFAs, are metabolized by host microbial populations to benefit host health. Future investigations should consider the use of *in vitro* screening studies and *in vivo* animal studies to identify putative prebiotic compounds from seaweeds via the identification of host organisms which utilize seaweed components and the bioactive metabolites produced (via untargeted metabolomics). Furthermore, controlled human intervention studies with health-related end points to elucidate prebiotic efficacy are required.

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