doi:10.20944/preprints202110.0010.v1

Title: Macroalgae microbiomes may hold the key to reduce methane emissions from ruminant livestock

Author: Janja Ceh

Author details: Max Planck Tandem Group in Plant Microbial Ecology at the Universidad del Valle, Calle 13 100-00, Building E20, 760032 Cali, Valle del Cauca, Colombia.

Correspondence: janja.m.ceh@gmail.com, ORCID ID https://orcid.org/0000-0002-2418-3628

Abstract

Ruminant mammals extract nutrients from plant-based food through fermentation in the rumen; fiber and starch are pre-digested by microorganisms and methane is produced as a by-product, which released into the atmosphere acts as a potent greenhouse gas. In an effort to reduce enteric methanogenesis, dietary additives for ruminants have been investigated, and marine macroalgae have proven particularly promising, e.g., the inclusion of 0.2% dry matter of the red alga *A. taxiformis* into cow feed decreased *in vivo* methane production by up to 98%. Thus, if globally applied, the addition of algae in ruminant diets could revolutionize the management of greenhouse gas emissions across the livestock sector. However, the ozone-depleting nature of halogen compounds produced in *Asparagopsis* sp. and the reported adverse health impacts on humans, along with impracticability issues and the difficulty to produce, commercialize and distribute algae widely, has sown some doubt on the feasibility of using macroalgae as methane mitigation instruments. To circumvent such obstacles, and taking into account the paradigm that eukaryotic hosts cannot be understood without considering interactions with their associated microbiome, the exploration of marine algae associated microorganisms is anticipated. Following the notion that in the close and intimate relationships between algae-hosts and their microbiota the origin of chemical response mechanisms is often unclear, and that compounds initially assigned to algae have previously been shown to stem from host-associated microbes, it is not unreasonable to think that these may be involved in the antimethanogenic effects of marine algae in the rumen. Once identified, such microorganisms could lead to antimethanogenic feed additives, and reduce enteric methanogenesis from livestock ruminants substantially. This review is three-fold: it provides a brief, historic overview of macroalgae as feed supplements for ruminants, sums up the difficulties related to using whole-macroalgae as large-scale antimethanogenic feed additives, and describes the macroalga microbiome, including its potential to serve as an antimethanogen for enteric fermentation.

Keywords

Greenhouse gases, methanogenesis, meat production, algae, microorganisms

1. Introduction

Greenhouse gases warm the Earth by absorbing and trapping heat in the atmosphere. The powerful greenhouse gas (GHG) – methane - represents 14% of the total GHG emitted and has increased in atmospheric concentration by about 150% since 1750 (Solomon, Manning, Marquis, & Qin, 2007). Methane has a heat-absorbing potential of about 28 times greater than carbon dioxide (over a 100-year period) and accounts for about 20% of the heating effects of all GHGes combined (Maasakkers et al., 2019; Solomon et al., 2007). With ~44% of its gas emissions released in form of methane (Rojas-Downing, Nejadhashemi, Harrigan, & Woznicki, 2017), livestock production systems significantly contribute to anthropogenic GHGes; 34% of released livestock-associated GHGes can be attributed to deforestation, 31% to manure management, and 35% to enteric methanogenesis - an anaerobic fermentation process in the foregut of ruminants that produces carbon dioxide and hydrogen, which is then transformed into methane by methanogenic microorganisms (Morgavi, Forano, Martin, & Newbold, 2010). This process makes up for the largest methane source within the agricultural sector (Patra, 2012), and equals an 18% of its total anthropogenic emissions, a value that even exceeds the global transportation sector (Solomon et al., 2007).

According to the Food and Agriculture Organization of the United Nations (FAO), the demand for milk and meat in industrial countries has risen by 2.4%, and 1.3% per annuum, respectively, since 2005. The further expected global population growth to an estimated 9.7 billion by 2050 (Vollset et al., 2020), is likely to cause an increase of 70% in meat and dairy production to meet nutritional demands (Steinfeld et al., 2006), hence will inevitably result in a surge of GHGes and other harmful environmental impacts.

The immense pressure to manage the climate and achieve emission targets calls for the identification of practical strategies to reduce the production of GHGes. and mitigating methane release from the livestock industry is particularly crucial to meet such goals. The marine red alga *A. taxiformis* has been shown to reduce methanogenesis in ruminants dramatically (Machado et al., 2018), and Mizrahi and colleagues (2021) identified the red alga as one of two current most promising strategies to reduce methanogenesis from ruminants. However, the production and global distribution of whole-plant macroalgae as livestock feed has its associated environmental risks and has proven to be challenging and impractical (Abbott et al., 2020; Regal et al., 2020). Just over the last year alone various reviews have been published, assessing nutritional and anti-methanogenic aspects of macroalgae feed for ruminants (Morais et al. 2020, Honan et al. 2021, Pandey et al. 2021), however, none has yet addressed the microbial partners, algae live in close association with. Microbiome studies have revealed essential interdependencies between multicellular organisms and their associated microbes and eukaryotes are no longer considered one organism, but rather a sum of themselves and all microorganisms associated with them (Bordenstein & Theis, 2015; McFall-Ngai et al., 2013; Rosenberg & Zilber-Rosenberg, 2016; van der Loos, Eriksson, & Salles, 2019), with each member of the consortium fulfilling essential roles. Though algae are known for their chemical response mechanisms resulting in potent bioactive compounds, the producers and the mechanisms triggering the biosynthesis of these compounds remain largely unclear, and in a few cases metabolites initially assigned to algae have been shown to originate from microorganisms (Goecke, Labes, Wiese, & Imhoff, 2010). Likewise, it is entirely possible that members of the macroalgal microbiome are involved in, or even responsible for the methanogenesis-reducing capacities of algae in the rumen, which is the core of this review article. After a brief historic overview of macroalgae use as ruminant feed, and the difficulties associated with the production of whole-alga feed additives, the macroalga-associated microbiome is reviewed and the potential of microorganisms as antimethanogens explored, with the ultimate goal to encourage the identification of such microbes for the development of practical and applicable antimethanogenic live microbial feed-additives, that - if globally distributed and used - could positively affect the climate through inhibiting enteric methanogenesis in ruminants.

2. Our knowledge to date

2.1 Ruminal methanogenesis

The rumen can be understood as an anaerobic and methanogenic fermentation chamber, where a phylogenetically diverse microbial consortium interacts in a well-orchestrated way (Brulc et al., 2009; McCann, Wickersham, & Loor, 2014; Mizrahi, Wallace, & Moraïs, 2021), to break down complex polysaccharides such as cellulose and hemicellulose from plant feed before it enters the true stomach and the rest of the digestive system of the animal (Morgavi et al., 2010). Methane is generated in the final step of the microbial food-chain, combining the end products from fiber-degradation by bacteria (carbon dioxide, methanol or methylated amines), and hydrogen produced by protozoans. These are utilised by methanogenic bacteria and archaea to generate methane (Costa & Leigh, 2014; Liu & Whitman, 2008), and while both, archaebacteria and methanogenic archaea are involved in ruminal methanogenesis (Wood, Kennedy, & Wolfe, 1968), the overwhelming majority is carried out by archaea of the genus *Methanobrevibacter* (Wirth et al., 2018). Species can vary between individual animals and/or animal species (Rebecca Danielsson et al., 2017), and specific groups of *Methanobrevibacter* species are correlated with higher/lower CH_4 emissions, which can be favoured by certain feed additives (R Danielsson, Schnürer, Arthurson, & Bertilsson, 2012; Rebecca Danielsson et al., 2014). While two different types of bacterial communities were linked with low CH_4 production in sheep (Kittelmann et al., 2014), a similar study on dairy cows did not find statistically significant correlations between specific microbial communities (archaea and bacteria), and high or low methane production, but found a tendency for particular species of microbes, for example, proteobacteria and unclassified archaea, to be more abundant in low CH_4 emitters (Rebecca Danielsson et al., 2017).

2.2 Marine macroalgae – the key to reducing ruminal methanogenesis?

Extensive research has been carried out to investigate the potential of feed supplements for livestock as inhibitors of methanogenesis within the rumen (reviewed in Gerber, Henderson, & Makkar, 2013); amongst those, the supplementation of animal rations with marine macroalgae have been standing out as particularly effective (Mizrahi et al., 2021; Ripple et al., 2014).

2.2.1 Secondary metabolites in marine algae

Marine macroalgae are abundant in all oceans and play vital roles in marine ecosystems as habitat forming ecosystem engineers. The diverse paraphyletic group contains thousands of species (Rindi, Soler-Vila, & Guiry, 2012) and consists of three main phyla, the Phaeo- (brown algae), Rhodo- (red algae), and Chlorophyta (green algae). To withstand extreme competition in marine environments, marine organisms have developed strategic, adaptive coping mechanisms; one such strategy is the synthesis of highly bioactive compounds (Abdul, Choi, Jung, & Choi, 2016; Corona et al., 2016; Yang, Nam, Kong, & Kim, 2010). Since all elements existing can be found in the sea in stable and constant quantities (Gomez-Zavaglia, Prieto Lage, Jimenez-Lopez, Mejuto, & Simal-Gandara, 2019), they provide the building blocks for an almost unlimited variety of metabolites for marine organisms. Marine macroalgae in particular absorb elements from their environment, containing 10-20 times more than land plants (Gaillard et al., 2018), and produce a wide array of bioactive compounds, referred to as secondary metabolites (Cimino & Ghiselin, 2001). Opposed to the somewhat misleading term, they are often involved in primary ecological processes like growth, reproduction and defence (Cabrita, Vale, & Rauter, 2010), and some contain antibacterial, antiviral, antioxidant, anti-inflammatory, and anti-carcinogenic properties (Chandini, Ganesan, & Bhaskar, 2008; Kang et al., 2008; Val et al., 2001; Yuan & Walsh, 2006). Red algae in particular have been reported to produce all major classes of algal natural products, except phlorotannins (Blunt, Copp, Keyzers, Munro, & Prinsep, 2016), and over 90% of the compounds they produce, contain bromine or chlorine, reflecting the abundance of these ions in seawater (Cabrita et al., 2010). The use of halogens increases the bioactivity of secondary metabolites, that serve as defence mechanisms against surface colonisation (antifoulants) by macro- and microorganisms (Paul, de Nys, & Steinberg, 2006a), and against herbivore consumers (Hay & Fenical, 1988). Genera within the Rhodophyta, known to produce large quantities of organobromines, are *Laurencia* (order Ceramiales), *Delisea*, *Asparagopsis*, *Bonnemaisonia* and *Ptilonia*, (order Bonnemaisoniales), (Davis & Vasanthi, 2011; Nash, Rindi, & Guiry, 2005).

2.2.2 The red alga genus *Asparagopsis*

The red alga *Asparagopsis* has been recognized for its strong antimethanogenic effect in the rumen. It belongs to the order Bonnemaisoniales, family Bonnemaisoniaceae and contains two recognized medium-sized species, *A. armata* Harvey and *A. taxiformis* (Delile) Trevisan. While *A. armata* is a temperate species, native to southern Australia and New Zealand (Horridge, 1951), but also found near the British Isles, the Canary and Salvage Islands (Andreakis, Procaccini, & Kooistra, 2004), *A. taxiformis* has a rather tropical to warm temperate distribution through parts of the Atlantic and the Indo-Pacific (Andreakis et al., 2004). Both species are considered introduced in the Mediterranean Sea (Boudouresque & Verlaque, 2002). Like many red algae, *Asparagopsis* are characterized by a complex triphasic diplohaplontic lifecycle, alternating between an erect, plumose gametophyte (haploid), a microscopic carposporophyte (diploid), and a filamentous tetrasporophyte (diploid), with each phase appearing morphologically distinct. The two species differ morphologically by long stolons that bear harpoon-like hooks in *A. armata*, but are absent in *A. taxiformis* (Andreakis et al., 2004).

Asparagopsis is considered a rich source of natural products with more than a hundred described; these include halomethanes, haloalkanes, haloketones and haloacids (Burreson, Moore, & Roller, 1975; McConnell & Fenical, 1977; Moore, 1977; Woolard, Moore, & Roller, 1979). The most abundant secondary metabolite in *A. taxiformis* is bromoform (Paul et al., 2006a; Vergés, Paul, & Steinberg, 2008), followed by lower concentrations of dibromocholoromethane, bromochloroacetic acid and dibromoacetic acid (Machado et al., 2016b), whereas *A. armata* contains mainly bromoform and dibromoacetic acid (Paul et al., 2006a). These compounds are produced and accumulated within the vacuoles of specialised gland cells. and act as antiherbivore (Paul, De Nys, & Steinberg, 2006b; Vergés et al., 2008) and antimicrobial (Paul et al., 2006a) defence mechanisms when released. Deprived of a bromine source, and thus unable to produce halogenated metabolites *A. armata* displayed significantly higher densities of epiphytic bacteria than unmanipulated conspecifics (Paul et al., 2006a), and bromide-positive algae deterred consumption by mesograzers (Paul et al., 2006b).

2.2.3 Macroalgae as feed supplement in ruminants – a brief historic overview

Macroalgae are a stable component in the human diet in many cultures (Nanri et al., 2017), and their use in both, human- and animal nutrition has a long standing history in coastal areas. In Bellum Africum, written 45 BC, an unnamed author wrote: “in times of scarcity, they [the Greeks] collected seaweed from the shore and, having washed it in fresh water, gave it to their cattle and thus prolonged their lives” (Newton, 1951). Also, the Icelandic sagas, reported the use of algae as human food since at least the year 961. For example, the red alga *Palmaria palmata* (local name “sol”), collected on the beach at low tide, washed in fresh water and air-dried, served as food for humans and animals alike when supplies were scarce due to extended periods of harsh climate (Chapman, 1970).

At the turn of the twentieth century early scientific studies erroneously claimed that kelp was of no value in animal feed, putting a relative halt on further investigations of algae as a feed supplement for livestock (Morrison & Morrison, 1954), however, undeterred animal breeders held on to including algae in animal rations, most commonly using the brown alga *Ascophyllum nodosum* (Evans & Critchley, 2014). It was not until the early 1970’s that algae were recognized as a rich source for micro-elements (Lunde, 1970), but a clear explanation for the productivity and health enhancement in animals by kelp meal was only unveiled through a detailed analysis of alga-associated polysaccharides in the early 2000s. Low inclusion levels of macroalgae in the ruminant diet exerted a potent prebiotic activity (Ye Wang, Han, Hu, Li, & Yu, 2006), resulting in improved gastro-intestinal health and productivity, and a renewed interest in the use of macroalgae in commercial animal agriculture emerged.

In 2015 a study evaluating dietary benefits of algae in cows, and also measuring methane emissions, found that storm-toss shoreweed mixed *in vitro* with rumen microbiota caused a reduction in methane emissions by 12-16% (Kinley & Fredeen, 2015), an interesting result, considering that methanogenesis causes losses in metabolic energy in the animal (Gerber et al., 2013), and also adds to GHG emissions. The prospect of algae serving as a promotor for higher growth rates in cattle, while simultaneously improving the sustainability of cattle production systems through the mitigation of methane (Machado et al., 2016b), sparked immense interest to further investigate the topic (Dubois et al., 2013; Machado, Magnusson, Paul, de Nys, & Tomkins, 2014; Machado et al., 2016a; Y Wang, Xu, Bach, & McAllister, 2008). At about the same time, another study compared twenty species of tropical macroalgae on *in vitro* fermentation parameters and revealed that all investigated algae reduced the production of methane, with the red algae *A. taxiformis* being most effective (Machado et al., 2014). In a next step, an *in vivo* study reported unprecedented effects of including 0.10% or 0.20% of *A. taxiformis* into steer feed, lowering methane emissions by 40 - 98%, while demonstrating a weight gain in steers of 42 - 53%. No negative effects on the daily feed intake, feed conversion efficiencies, or rumen function were detected, and neither residues nor changes in meat quality were observed (Kinley et al., 2020). A second *in vivo* study evaluating *Asparagopsis* as a functional feed ingredient for lactating dairy cows, reported a decrease in methane emissions by 67% with no effect on milk quality (Roque, Salwen, Kinley, & Kebreab, 2019), again confirming the efficacy of the alga. Not surprisingly, Kinley and colleagues (2020) pointed out that low inclusion rates of *A. taxiformis* in ruminant diets could revolutionize the management of GHG emissions across the livestock sector.

3. The bottleneck

The incorporation of the strongly antimethanogenic alga *A. taxiformis* into ruminant feed appears to be a promising strategy to mitigate the large share of global GHGes from ruminant animals. However, for this to be a practical application, an effective, reliable and consistent product must be guaranteed, and a multitude of associated challenges have to be considered, including a sustainable supply of raw material, the variability in quantities of bioactives produced by algae (seasonality, geographic distribution), and ultimately the production- associated costs.

Macroalgae can vary in composition, depending on the species, season of collection, habitat and environmental factors, for example, the bromoform concentration in *Asparagopsis* differs between the two species (Machado et al., 2016b; Paul et al., 2006a), depends on the environment (e.g., the availability of carbon and nitrogen) (Mata, Gaspar, & Santos, 2012), the life stages and differs between sexes (Vergés et al., 2008). The major metabolite bromoform varied in concentration between sampling times and life-stages (filamentous tetrasporophyte vs. plumose gametophyte stage), and a persistently lower secondary metabolite content in male than in female gametophytes. Also, levels of dibromoacetic acid, the second most abundant metabolite in *A. armata*, were consistently higher in the tetrasporophyte than in the gametophyte, with little temporal variation. Not all life-history stages were simultaneously available for sampling throughout the year, with tetrasporophytes present year-round while gametophytes were only found between late winter and summer (Vergés et al., 2008).

Variation in biochemical composition and bioactivity does not only depend on the natural variability of the seaweed but also on preservation processes, as shown in the case of *A. taxiformis* (Regal et al., 2020). These affect the concentration, composition and bioactivity of algal metabolites (Jiménez-Escrig, Jiménez-Jiménez, Pulido, & Saura-Calixto, 2001; Le Lann, Jegou, & Stiger-Pouvreau, 2008; Stévant, Rebours, & Chapman, 2017), highlighting the critical importance of appropriate drying and storage conditions to preserve biomass quality and ensure product safety (Gupta, Cox, & Abu-Ghannam, 2011; Le Lann et al., 2008; Regal et al., 2020).

A sufficient supply of *A. taxiformis* for industrial agriculture from wild harvest can largely be excluded, thus farming the algae would be required. Research initiatives around the globe have taken on the challenge to develop methods for growing *A. taxiformis*, however, there is little knowledge to date about cultivating the species; for example, its life-cycle has not yet been closed under culture conditions. Moreover, volatile halocarbons produced by marine algae (Carpenter & Liss, 2000; Carpenter, Malin, Liss, & Küpper, 2000), form a major source of halogen radicals in the atmosphere, and when transported into the troposphere and the stratosphere, they contribute to ozone depletion and thus to climate change (Aschmann, Sinnhuber, Atlas, & Schauffler, 2009; Phang et al., 2015). Examples are brominated and chlorinated halocarbons (Piers & Joshi, 2005), which are released at higher rates by tropical than temperate algae (Abrahamsson, Ekdahl, Collen, Pedersen, & Oceanography, 1995). Considering that *A. taxiformis* produces significant amounts of bromoform and other halocarbons, large scale aquaculture of this species may contribute to climate change, which defeats the original idea of its use for the reduction of GHG emissions. Additionally, *A. taxiformis'* invasive potential (Boudouresque & Verlaque, 2002), may represent an ecological risk to marine ecosystems when deployed in open ocean culture systems.

4. Changing direction

4.1 Bromoform and other antimethanogenic agents in algae

The occurrence of large quantities of brominated compounds in coastal areas (mostly bromoform and dibromomethane) is mainly attributed to the enzymatic activity of peroxidases in marine macroalgae (reviewed in Wever et al., 1993), among which the Rhodophyta are considered the main producers. While secondary metabolites in green and brown algae contain as little as 7%, and less than 1% of bromine or chlorine, red algae in comparison comprise of more than 90% (Harper et al., 2001). The red alga *A. taxiformis*, for example, bioaccumulates large quantities of bromoform which is believed to be the main active compound in reducing ruminal methanogenesis. Acting as an enzymatic inhibitor in the rumen, bromoform reacts with reduced cobamide vitamin B_{12}, and thus lowers the efficiency of the cobamide-dependent enzymatic methyl-transferase step that is essential for the biosynthesis of methane (Wood et al., 1968). It is interesting, though, that neither highly concentrated synthetic bromoform, bromochloromethane or chloroform were as effective in reducing methane production in ruminants as *A. taxiformis* whole-plant feed (Chalupa, 1977; Lanigan, 1972), which Machado and colleagues (2018) suggested to be based on the synergistic effect of multiple bioactives contained in the alga.

If halobromides are the active compounds causing the reduction of ruminal methanogenesis by *A. taxiformis*, it is somewhat surprising that other red algae, which also synthesize substantial amounts of halobromides, are less efficient in doing so. In an *in vitro* study including three freshwater (green), and 17 marine algae (7 green, 6 brown, and 4 red), the strongest antimethanogenic effect was recorded for the red alga *A. taxiformis*, reducing methane by 99.8%, followed by the brown alga *Dictyota* (98.6%), and the green alga *Cladophora patentiramea* (93.9%), (Machado et al., 2014). However, the red alga *Laurencia*, which is particularly well known for its highly abundant halogenated metabolites (Fenical, 1981), ranked only 11 out of 20 (89.1%), and the remaining two red algae *Hypnea* and *Halymenia*, 10 (89.6%), and 17 (86.7%), respectively. Even species from freshwater, which contains substantially lower concentrations of halogens than marine environments, reduced ruminal methanogenesis by at least 83%. Most studies, investigating the ability of algae to reduce methane production in the rumen, have focussed on the red alga *A. taxiformis* (Machado et al., 2016b), with bromoform as the main active compound. However, other macroalgae that contain little or no bromoform exhibit the same capacity, even if at lower levels (Machado et al., 2014).

4.2 The alga microbiome

A common denominator in all alga species, as in all eukaryotes, is an associated microbiome, comprising archaea, bacteria, fungi, protozoa and viruses. Together, the host organism and its microbes form a functional entity in which the microbial consortium plays crucial roles in the hosts development, health, survival and fitness (Bordenstein & Theis, 2015; McFall-Ngai et al., 2013; Rosenberg & Zilber-Rosenberg, 2016; van der Loos et al., 2019), and disruptions to these host–microbiome interactions can have significant and often negative impacts on the host (Rosenberg, Koren, Reshef, Efrony, & Zilber-Rosenberg, 2007).

Marine macroalgae evolved in a microbial world; 1 mL of seawater contains up to 10^7 viruses, 10^6 bacteria, 10^3 fungi, 10^3 microalgae, and 10 to 100 microscopic larvae and spores (Cole, 1982; Engel, Jensen, & Fenical, 2002; Harder, 2009; Jensen & Fenical, 1994). Algal surfaces act as the primary physiological and ecological interface with the environment where the uptake and exchange of nutrients, and the detection and communication of chemical cues and signals take place (Wahl et al., 2010). An abundant secretion of metabolic organic compounds forms a favorable micro-niche for a structurally and functionally diverse microbiota (Barott et al., 2011; Egan et al., 2013), that is significantly different in composition to the bacterial community found in the surrounding seawater (Egan et al., 2013). These microbial epiphytes consist of prokaryotes and eukaryotes, though bacteria (generalists and specialists) are considered the dominant active group (Brodie et al., 2016; de Oliveira et al., 2012), followed by diatoms and fungi (Qian, Lau, Dahms, Dobretsov, & Harder, 2007). Generally, the host-microbe association in macroalgae is considered stable (Kong & Chan, 1979), however, the microbial composition can vary in different parts of the thallus (Staufenberger, Thiel, Wiese, & Imhoff, 2008), and also over different seasons, and over the lifespan of an alga (Sakami, 1996). While the alga supplies microorganisms with organic nutrients, the microbiota provides the alga host with important services (Egan et al., 2013; Harder, Campbell, Egan, & Steinberg, 2012), including the supply of carbon dioxide, minerals, vitamins, growth factors, fixed nitrogen and detoxifying compounds (Armstrong, Yan, Boyd, Wright, & Burgess, 2001; Chisholm, Dauga, Ageron, Grimont, & Jaubert, 1996; Croft, Lawrence, Raux-Deery, Warren, & Smith, 2005; Dimitrieva, Crawford, & Yüksel, 2006; Goecke et al., 2010; Riquelme, Rojas, Flores, & Correa, 1997; Singh, Bijo, Baghel, Reddy, & Jha, 2011), and can furthermore shape the host's morphology and life cycle (Ghaderiardakani, Coates, & Wichard, 2017).

An estimated half of all microalgae depend on their associated bacterial epiphytes to provide them with the essential micronutrient vitamin B_{12}, which is acquired through the direct interaction with bacteria (Croft et al., 2005; Tang, Koch, & Gobler, 2010), and some macroalgae, like the freshwater/marine species *Chladophora glomerata* depends on exogenous vitamin B_{12} for growth and reproduction (Gerloff & Fitzgerald, 1976; Hoffmann & Graham, 1984; Hoflmann, 1990). Genome sequences of 72 cultivable bacterial strains associated with the surface of the brown alga *Ectocarpus* revealed gene clusters related to vitamin B_{12}, to detoxification (degeneration of superoxide radicals, reactive oxygen species, cyanate), and secondary metabolite synthesis pathways, suggesting that the bacterial metabolism contributes towards the host's wellbeing (Karimi et al., 2020). Algae associated bacteria have also shown to influence developmental transitions and growth of alga hosts (Pedersén, 1968; Tapia, González, Goulitquer, Potin, & Correa, 2016). For example, in *Ulva* spp. (Chlorophyta) and *Ectocarpus* spp. (Phaeophyta), the removal of microorganisms from the algae resulted in an atypical morphotype, which, upon adding the appropriate microbes, recovered their typical form (Ghaderiardakani et al., 2017; Tapia et al., 2016). A similar phenomenon was observed in the two Rhodophyta species *Dasya pedicellata* and *Polysiphonia urceolata* (Provasoli & Pintner, 1972). Bacteria associated with *Ectocarpus* are involved in the acclimation process as the alga undergoes the transition from seawater to freshwater (Dittami et al., 2016). Inferred functions from the *Cladophora* bacteriobiome include methane and cellulose degradation, nitrogen fixation (Zulkifly et al., 2012), and bacterial phosphorous acquisition (Young, Tucker, & Pansch, 2010). Many algal host-microbiome alliances have been reported to be species-specific, for example bacterial communities derived from the red macroalgal species *Bonnemaisonia asparagoides*, *Lomentaria clavellosa* and *Polysiphonia stricta* were more similar in the same species originating from different localities, than in different species from the same habitat (Nylund et al., 2009). On the other hand, highly variable bacterial assemblages between individuals of *Ulva australis*, suggested that the bacterial community composition in this host is rather driven by functional genes and transcriptomes, than by the taxonomy or phylogeny of specific bacteria strains (Burke, Steinberg, Rusch, Kjelleberg, & Thomas, 2011; Burke, Thomas, Lewis, Steinberg, & Kjelleberg, 2011).

Marine macroalgae are continuously exposed to potentially harmful microorganisms, nonetheless, despite not possessing a cell-based immune system (Potin, Bouarab, Küpper, & Kloareg, 1999), diseases are rare in algae (Goecke et al., 2010). This phenomenon has been assigned to the bioactive secondary metabolites produced by algae that act as antimicrobial defence mechanisms (Engel et al., 2002), however antimicrobial activity is also known from algae associated bacteria (Wiese, Thiel, Nagel, Staufenberger, & Imhoff, 2009), and macroalgae are equipped with physiological and biochemical properties to predetermine the composition of their associated epiphytic bacterial communities. For example, algal cell wall components can trigger specific interactions between macroalgae and beneficial bacteria (Engel et al., 2002; Lachnit, Meske, Wahl, Harder, & Schmitz, 2011), with microbes colonizing the outer surface layer of algae selected for by the secondary metabolites produced and exuded by individual alga species, with each alga possessing a specific chemical fingerprint. The red alga *Delisea pulchra*, for instance, excretes halogenated furanones that shape their epibiome (Longford et al., 2019), and also in the brown alga *Fucus vesiculosus* metabolites are involved in biofilm composition (Lachnit, Wahl, & Harder, 2010). The red alga *Agarophyton vermiculophyllum* is capable of chemically mediated recruitment of protective microbes, while deterring the settlement of pathogens through exuded metabolites, a phenomenon called microbial “gardening” (Saha & Weinberger, 2019). Various macroalgae have been shown to produce quorum sensing inhibitors to disrupt bacteria communication (e.g., Joint, Tait, & Wheeler, 2007), and also algae associated epiphytic bacteria suppress the settlement of other competitive bacteria by producing quorum sensing inhibitors and thus contributing to algal defences (Kanagasabhapathy, Yamazaki, Ishida, Sasaki, & Nagata, 2009).

Other than epiphytes, macroalgae harbor endophytic bacteria in their vacuolar systems or in the cytoplasm, as observed in various siphonous green algae, including *Bryopsis*, *Halimeda*, and *Caulerpa* (Goecke et al., 2010; Hollants, Leliaert, De Clerck, & Willems, 2013; Hollants, Leliaert, Verbruggen, Willems, & De Clerck, 2013), where the bacterial community promotes host growth and protects the host against pathogens. In *Bryopsis* endobacteria can either be recruited from the environment, or vertically inherited (Hollants, Decleyre, Leliaert, De Clerck, & Willems, 2011). Bacteria have also been reported inside algal galls (i.e. abnormal tissue growths) in red and brown macroalgae (Ashen & Goff, 1998).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 October 2021 doi:10.20944/preprints202110.0010.v1

4.3 Bioactive compounds and the alga holobiont

It has been suggested that algae devoid of their own chemical defences can rely on secondary metabolites produced by their associated bacteria (Egan, Thomas, Holmström, & Kjelleberg, 2000; Goecke et al., 2010; Holmström, Rittschof, & Kjelleberg, 1992); the green alga *Ulva reticulata*, for example, partly depends on antifouling compounds released by epibiotic bacteria (Dobretsov & Qian, 2002).

Any marine alga ever tested for its bioactive compounds was not assessed as an isolated organism, but consisted of itself and its associated microbial partners, many of which are known for their metabolic capacities (Goecke et al., 2010). Thus, the origin of active agents is not always clear and metabolites initially believed to be produced by eukaryotes, have been shown to originate from a microbe. For example, Murakami et al. (2004) identified the source of chlorophyll d - that was originally considered a genuine pigment in several species of marine red algae – to be an epiphytic cyanobacterium living on the thalli of red algae, and concluded that chlorophyll d is not a constituent of red algae, and that its biosynthesis does not even occur in eukaryotes.

Likewise, it is entirely possible that members of the macroalgal microbial consortium are involved in, or even responsible for the methanogenesis inhibiting properties of marine algae in ruminants. If true, and if such microorganisms are indeed capable to synthesize reactive metabolites independently from the alga host, their identification and isolation could lead to the development of a potent methanogenesis inhibitor via live microbiota inclusion in ruminant feed, and would thus present an elegant solution for circumventing the difficulties associated with the production and global distribution of alga whole-plant feed.

The single biggest known responsible factor for organohalogens in coastal areas is the enzymatic activity carried out in marine macroalgae (reviewed in Wever et al., 1993), however, the enzyme vanadium haloperoxidase that mediates the production of biogenic organohalogens, is not only found in brown, red and green algae, but also in diatoms, in bacteria and in fungi (Wever & van den Horst 2013), all of which have representatives in the alga microbiome. This suggests that the production of organobromides in macroalgae may not only stem from the alga-host, but may also involve members of its associated microbes.

For example, members of the genera *Erythrobacter* and *Pseudomonas*, both of which contain bromomethane-producing affiliates (Fujimori et al., 2012), have been identified in the *A. taxiformis* microbiome (Aires, Serrão, & Engelen, 2016). Moreover, *A. taxiformis* harbours an endophytic fungus *Nemania bipapillata* belonging to the Ascomycota phylum, from which novel compounds have recently been isolated (Medina et al., 2019). Other microbes associated with red algae (Williams, Yoshida, Moore, & Paul, 2003) are the cyanobacteria, which are considered amongst the most promising groups for the isolation of natural halogenated compounds (Borowitzka, 1995; Burja, Banaigs, Abou-Mansour, Burgess, & Wright, 2001).

Also, halomethanes may only be indirectly involved in the methane reduction capacity of algae, meaning that algae exude halobromides or other halomethanes that select for specific epibionts, which are involved in the antimethanogenic algal activity, rather than the halomethanes *per se*. For example, methanotrophic bacteria and archaea that use methane as a source of energy have been shown to be associated with various marine organisms, like corals, sponges, jellyfish, and others (Hestetun, Dahle, Jørgensen, Olsen, & Rapp, 2016; Lee, Kling, Araya, & Ceh, 2018; Siboni, Ben-Dov, Sivan, & Kushmaro, 2008), and the methanotrophs *Methylotenera*, *Methylibium*, and *Methylobacter* are symbiotic partners of the freshwater alga *Chladophora glomerata* (Zulkifly et al., 2012). Methanotrophs require oxygen and vitamin B_{12} (Iguchi, Yurimoto, Sakai, & microbiology, 2011), and may thus be attracted to algae by emitted oxygen from photosynthesis, and by the presence of vitamin B_{12} producers in algal bio films (Graham, Wilcox, & Knack, 2015; Knack et al., 2015). Oxygen and vitamin B_{12} are also abundant in the rumen. Thus, algal-associated methanotrophs may play a role in the algae-associated methane reduction in ruminants.

4.4 Future perspectives

Algae associated microorganisms are almost certain to be involved in the alga-feed associated inhibition of methanogenesis in ruminants, either by participating in the production of metabolites, or otherwise, and may thus harbour a great potential to lead to an easy-to-produce-and-distribute microbial feed additive for ruminants. Culture-based, as well as molecular- and chemistry techniques can be used to identity responsible key members in algae associated microbial consortia. Algae associated microbial assemblages, including prokaryotic (16S rDNA) and eukaryotic species (18S, 28S rDNA, ITS), need to be identified, functional genes of interest targeted, and distinct microbial functions unveiled, to provide baseline information and insight into biosynthetic pathways.

If only endophytes that are targeted, optimal surface sterilization of algae is necessary to exclude possible epibiont contamination (Flewelling et al., 2013; Hollants, Leliaert, De Clerck, & Willems, 2010). Specialized gland cells that have been identified as bromomethane production and accumulation sites in the red algae *A. taxiformis* and *Laurencia* sp., could be investigated to clarify if microorganisms are present, and possibly involved in the production of halogens.

Fluorescence *in situ* Hybridisation (FISH), using solid semithin algal sections and a triple band filter will allow the discrimination between microbial FISH signals and the autofluorescence of algal pigments (Hollants et al., 2010). If bacteria were localized in these gland cells, specific cells or entire areas of algal tissue could be isolated by laser dissection microscopy. These sections could then be further investigated using molecular biological methods (genomics, transcriptomics, proteomics, etc.), to elucidate the identity, abundance and possible function of the microorganism in question, and chemical methods could identify the bioactive compounds produced. Mass spectrometry techniques, such as desorption electrospray ionization mass spectrometry (DESI-MS), used to detect natural products on tissue surfaces, can help to localize and identify the origin of natural compounds from macroalgae (Lane et al., 2009).

Lastly, the isolation and identification of cultivable microbes from marine algae is crucial for bioprospection efforts. A reliable feed additive for the inhibition of methanogenesis in ruminants could consist of a probiotic (live microorganisms), and a prebiotic substrate, selectively favouring the probiotic organism.

5. Conclusions

Meeting global greenhouse gas emission targets is a pressing issue of our times, and mitigating methane release from the livestock sector is crucial to meet these goals. Due to the potent capacity to inhibit methanogenesis in ruminants, marine macroalgae qualify as valuable feed additives to reduce methane emissions. However, the production and global distribution of whole-plant macroalgae as livestock feed is challenging and impractical. Here we described the potential of macroalgal associated microbial consortia and suggest an in-depth exploration effort of these microbiota, with the ultimate goal to encourage the identification of such microbes for the development of practical and applicable antimethanogenic live microbial feed-additives, that - if globally distributed and used - could positively affect the climate through inhibiting enteric methanogenesis in ruminants.

Acknowledgements

Many thanks go to Jose Marin Riascos for commenting on an early version of this manuscript.

Funding

No funding was received for this work

Conflict of Interest Statement

There is no conflict of interest.

References

Abbott, D. W., Aasen, I. M., Beauchemin, K. A., Grondahl, F., Gruninger, R., Hayes, M., . . . Kirwan, S. F. (2020). Seaweed and seaweed bioactives for mitigation of enteric methane: Challenges and opportunities. *Animals, 10*(12), 2432. doi:10.3390/ani10122432

Abdul, Q. A., Choi, R. J., Jung, H. A., & Choi, J. S. (2016). Health benefit of fucosterol from marine algae: a review. *Journal of the Science of Food and Agriculture, 96*(6), 1856-1866. doi:10.1002/jsfa.7489.

Abrahamsson, K., Ekdahl, A., Collen, J., Pedersen, M., & Oceanography. (1995). Marine algae-a source of trichloroethylene and perchloroethylene. *Limnology, 40*(7), 1321-1326. doi:10.4319/lo.1995.40.7.1321

Aires, T., Serrão, E. A., & Engelen, A. H. (2016). Host and environmental specificity in bacterial communities associated to two highly invasive marine species (genus *Asparagopsis*). *Frontiers in microbiology, 7*, 559. doi:10.3389/fmicb.2016.00559.

Andreakis, N., Procaccini, G., & Kooistra, W. H. (2004). *Asparagopsis taxiformis* and *Asparagopsis armata* (Bonnemaisoniales, Rhodophyta): genetic and morphological identification of Mediterranean populations. *European journal of phycology, 39*(3), 273-283. doi:10.1080/0967026042000236436

Armstrong, E., Yan, L., Boyd, K. G., Wright, P. C., & Burgess, J. G. (2001). The symbiotic role of marine microbes on living surfaces. *Hydrobiologia, 461*(1-3), 37-40. doi:10.1023/A:1012756913566

Aschmann, J., Sinnhuber, B., Atlas, E. L., & Schauffler, S. (2009). Modeling the transport of very short-lived substances into the tropical upper troposphere and lower stratosphere. *Atmos. Chem. Phys, 9*(23), 9237-9247. doi:10.5194/acp-9-9237-2009

Ashen, J. B., & Goff, L. J. (1998). Galls on the marine red alga Prionitis lanceolata (Halymeniaceae): specific induction and subsequent development of an algal–bacterial symbiosis. *American Journal of Botany, 85*(12), 1710-1721. doi:10.2307/2446505

Barott, K. L., Rodriguez-Brito, B., Janouškovec, J., Marhaver, K. L., Smith, J. E., Keeling, P., & Rohwer, F. L. (2011). Microbial diversity associated with four functional groups of benthic reef algae and the reef-building coral *Montastraea annularis*. *Environmental microbiology, 13*(5), 1192-1204. doi:10.1111/j.1462-2920.2010.02419.x.

Blunt, J. W., Copp, B. R., Keyzers, R. A., Munro, M. H., & Prinsep, M. R. (2016). Marine natural products. *Natural product reports, 33*(3), 382-431. doi:10.1039/c7np00052a.

Bordenstein, S. R., & Theis, K. R. (2015). Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *Plos Biology, 13*(8), e1002226. doi:10.1371/journal.pbio.1002226.

Borowitzka, M. A. (1995). Microalgae as sources of pharmaceuticals and other biologically active compounds. *Journal of Applied Phycology, 7*(1), 3-15. doi:10.1007/BF00003544

Boudouresque, C. F., & Verlaque, M. (2002). Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. *Marine pollution bulletin, 44*(1), 32-38. doi:10.1016/S0025-326X(01)00150-3

Brodie, J., Williamson, C., Barker, G. L., Walker, R. H., Briscoe, A., & Yallop, M. (2016). Characterising the microbiome of Corallina officinalis, a dominant calcified intertidal red alga. *FEMS microbiology ecology, 92*(8), fiw110. doi:10.1093/femsec/fiw110

Brulc, J. M., Antonopoulos, D. A., Miller, M. E. B., Wilson, M. K., Yannarell, A. C., Dinsdale, E. A., . . . Wacklin, P. (2009). Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. *Proceedings of the National Academy of Sciences, 106*(6), 1948-1953. doi:10.1073/pnas.0806191105

Burja, A. M., Banaigs, B., Abou-Mansour, E., Burgess, J. G., & Wright, P. G. (2001). Marine cyanobacteria—a prolific source of natural products. *Tetrahedron, 57*(46), 9347-9377. doi:10.1016/S0040-4020(01)00931-0

Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S., & Thomas, T. (2011). Bacterial community assembly based on functional genes rather than species. *Proceedings of the National Academy of Sciences, 108*(34), 14288-14293. doi:10.1073/pnas.1101591108

Burke, C., Thomas, T., Lewis, M., Steinberg, P., & Kjelleberg, S. (2011). Composition, uniqueness and variability of the epiphytic bacterial community of the green alga Ulva australis. *The ISME journal, 5*(4), 590-600. doi:10.1038/ismej.2010.164

Burreson, B., Moore, R. E., & Roller, P. (1975). Haloforms in the essential oil of the alga *Asparagopsis taxiformis* (Rhodophyta). *Tetrahedron Letters, 16*(7), 473-476. doi:10.1016/S0040-4039(00)71897-1

Cabrita, M. T., Vale, C., & Rauter, A. P. (2010). Halogenated compounds from marine algae. *Marine Drugs, 8*(8), 2301-2317. doi:10.3390/md8082301

Carpenter, L., & Liss, P. (2000). On temperate sources of bromoform and other reactive organic bromine gases. *Journal of Geophysical Research: Atmospheres, 105*(D16), 20539-20547. doi:10.1029/2000JD900242

Carpenter, L., Malin, G., Liss, P., & Küpper, F. (2000). Novel biogenic iodine-containing trihalomethanes and other short-lived halocarbons in the coastal east Atlantic. *Global Biogeochemical Cycles, 14*(4), 1191-1204. doi:10.1029/2000GB001257

Chalupa, W. (1977). Manipulating rumen fermentation. *Journal of Animal Science, 45*(3), 585-599. doi:10.2527/jas1977.453585x

Chandini, S. K., Ganesan, P., & Bhaskar, N. (2008). In vitro antioxidant activities of three selected brown seaweeds of India. *Food chemistry, 107*(2), 707-713. doi:10.1016/j.foodchem.2007.08.081

Chapman, V. (1970). Seaweeds as animal fodder and manure. In *Seaweeds and their uses* (2nd edition ed., pp. 63-85): Camelot, London.

Chisholm, J., Dauga, C., Ageron, E., Grimont, P., & Jaubert, J. (1996). 'Roots' in mixotrophic algae. *Nature, 381*(6581), 382-382. doi:10.1038/381382a0

Cimino, G., & Ghiselin, M. (2001). Marine natural products chemistry as an evolutionary narrative. *Marine chemical ecology*, 115-154. doi:10.1201/9781420036602

Cole, J. J. (1982). Interactions between bacteria and algae in aquatic ecosystems. *Annual Review of Ecology Systematics, 13*, 291-314. doi:10.1146/annurev.es.13.110182.001451

Corona, G., Ji, Y., Anegboonlap, P., Hotchkiss, S., Gill, C., Yaqoob, P., . . . Rowland, I. (2016). Gastrointestinal modifications and bioavailability of brown seaweed phlorotannins and effects on inflammatory markers. *British Journal of Nutrition, 115*(7), 1240-1253. doi:10.1017/S0007114516000210.

Costa, K. C., & Leigh, J. A. (2014). Metabolic versatility in methanogens. *Current opinion in biotechnology, 29*, 70-75. doi:10.1016/j.copbio.2014.02.012

Croft, M. T., Lawrence, A. D., Raux-Deery, E., Warren, M. J., & Smith, A. G. (2005). Algae acquire vitamin B 12 through a symbiotic relationship with bacteria. *Nature, 438*(7064), 90-93. doi:10.1038/nature04056

Danielsson, R., Dicksved, J., Sun, L., Gonda, H., Müller, B., Schnürer, A., & Bertilsson, J. (2017). Methane production in dairy cows correlates with rumen methanogenic and bacterial community structure. *Frontiers in microbiology, 8*, 226. doi:10.3389/fmicb.2017.00226

Danielsson, R., Schnürer, A., Arthurson, V., & Bertilsson, J. (2012). Methanogenic population and CH4 production in Swedish dairy cows fed different levels of forage. *Applied environmental microbiology, 78*(17), 6172-6179. doi:10.1128/AEM.00675-12

Danielsson, R., Werner-Omazic, A., Ramin, M., Schnürer, A., Griinari, M., Dicksved, J., & Bertilsson, J. (2014). Effects on enteric methane production and bacterial and archaeal communities by the addition of cashew nut shell extract or glycerol—An in vitro evaluation. *J Journal of Dairy Science, 97*(9), 5729-5741. doi:10.3168/jds.2014-7929

Davis, G. D. J., & Vasanthi, A. H. R. (2011). Seaweed metabolite database (SWMD): A database of natural compounds from marine algae. *Bioinformation, 5*(8), 361. doi:10.6026/97320630005361

de Oliveira, L. S., Gregoracci, G. B., Silva, G. G. Z., Salgado, L. T., Amado Filho, G., Alves-Ferreira, M., . . . Thompson, F. L. (2012). Transcriptomic analysis of the red seaweed Laurencia dendroidea (Florideophyceae, Rhodophyta) and its microbiome. *BMC genomics, 13*(1), 487. doi:10.1186/1471-2164-13-487

Dimitrieva, G., Crawford, R., & Yüksel, G. (2006). The nature of plant growth-promoting effects of a pseudoalteromonad associated with the marine algae Laminaria japonica and linked to catalase excretion. *Journal of Applied Microbiology, 100*(5), 1159-1169. doi:10.1111/j.1365-2672.2006.02831.x

Dittami, S. M., Duboscq-Bidot, L., Perennou, M., Gobet, A., Corre, E., Boyen, C., & Tonon, T. (2016). Host–microbe interactions as a driver of acclimation to salinity gradients in brown algal cultures. *The ISME journal, 10*(1), 51-63. doi:10.1038/ismej.2015.104

Dobretsov, S. V., & Qian, P.-Y. (2002). Effect of bacteria associated with the green alga Ulva reticulata on marine micro-and macrofouling. *Biofouling, 18*(3), 217-228. doi:10.1080/08927010290013026

Dubois, B., Tomkins, N. W., Kinley, R. D., Bai, M., Seymour, S., Paul, N. A., & de Nys, R. (2013). Effect of tropical algae as additives on rumen in vitro gas production and fermentation characteristics. *American Journal of Plant Sciences, 4*, 34-43. doi:10.4236/ajps.2013.412A2005

Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S., & Thomas, T. (2013). The seaweed holobiont: understanding seaweed–bacteria interactions. *FEMS Microbiology Reviews, 37*(3), 462-476. doi:10.1111/1574-6976.12011.

Egan, S., Thomas, T., Holmström, C., & Kjelleberg, S. (2000). Phylogenetic relationship and antifouling activity of bacterial epiphytes from the marine alga *Ulva lactuca*: brief report. *Environmental microbiology, 2*(3), 343-347. doi:10.1046/j.1462-2920.2000.00107.x

Engel, S., Jensen, P. R., & Fenical, W. (2002). Chemical ecology of marine microbial defense. *Journal of Chemical Ecology, 28*(10), 1971-1985.

Evans, F., & Critchley, A. (2014). Seaweeds for animal production use. *J Journal of applied phycology, 26*(2), 891-899. doi:10.1007/s10811-013-0162-9

Fenical, W. (1981). Natural halogenated organics. In *Elsevier oceanography series* (Vol. 31, pp. 375-393): Elsevier.

Flewelling, A. J., Ellsworth, K. T., Sanford, J., Forward, E., Johnson, J. A., & Gray, C. A. (2013). Macroalgal endophytes from the Atlantic Coast of Canada: a potential source of antibiotic natural products? *Microorganisms, 1*(1), 175-187. doi:10.3390/microorganisms1010175

Fujimori, T., Yoneyama, Y., Taniai, G., Kurihara, M., Tamegai, H., Hashimoto, S., & oceanography. (2012). Methyl halide production by cultures of marine proteobacteria *Erythrobacter* and *Pseudomonas* and isolated bacteria from brackish water. *Limnology, 57*(1), 154-162. doi:10.4319/lo.2012.57.1.0154

Gaillard, C., Bhatti, H. S., Novoa-Garrido, M., Lind, V., Roleda, M. Y., & Weisbjerg, M. R. (2018). Amino acid profiles of nine seaweed species and their in situ degradability in dairy cows. *Animal Feed Science Technology, 241*, 210-222. doi:10.1016/j.anifeedsci.2018.05.003

Gerber, P. J., Henderson, B., & Makkar, H. P. (2013). *Mitigation of greenhouse gas emissions in livestock production: a review of technical options for non-CO_2 emissions*: Food and Agriculture Organization of the United Nations (FAO).

Gerloff, G. C., & Fitzgerald, G. P. (1976). *The nutrition of Great Lakes Cladophora*: U S. Environmental Protection Agency, Office of Research and Development ….

Ghaderiardakani, F., Coates, J. C., & Wichard, T. (2017). Bacteria-induced morphogenesis of *Ulva intestinalis* and *Ulva mutabilis* (Chlorophyta): a contribution to the lottery theory. *FEMS microbiology ecology, 93*(8). doi:10.1093/femsec/fix094

Goecke, F., Labes, A., Wiese, J., & Imhoff, J. (2010). Chemical interactions between marine macroalgae and bacteria. *Marine Ecology Progress Series, 409*, 267-299. doi:10.3354/meps08607

Gomez-Zavaglia, A., Prieto Lage, M. A., Jimenez-Lopez, C., Mejuto, J. C., & Simal-Gandara, J. (2019). The Potential of Seaweeds as a Source of Functional Ingredients of Prebiotic and Antioxidant Value. *Antioxidants, 8*(9), 406. doi:10.3390/antiox8090406

Graham, L. E., Wilcox, L. W., & Knack, J. J. (2015). Why we need more algal metagenomes 1. *Journal of phycology, 51*(6), 1029-1036. doi:10.1111/jpy.12344

Gupta, S., Cox, S., & Abu-Ghannam, N. (2011). Effect of different drying temperatures on the moisture and phytochemical constituents of edible Irish brown seaweed. *LWT-Food Science Technology, 44*(5), 1266-1272. doi:10.1007/s10811-019-01846-1

Harder, T. (2009). Marine epibiosis: concepts, ecological consequences and host defence. In *Marine and industrial biofouling* (pp. 219-231): Springer.

Harder, T., Campbell, A. H., Egan, S., & Steinberg, P. D. (2012). Chemical mediation of ternary interactions between marine holobionts and their environment as exemplified by the red alga Delisea pulchra. *Journal of Chemical Ecology, 38*(5), 442-450. doi:10.1007/s10886-012-0119-5

Harper, M., Bugni, T., Copp, B., James, R., Lindsay, B., Schnabel, P., . . . Ireland, C. (2001). Introduction to the chemical ecology of marine natural products. In J. McClintock & B. Baker (Eds.), *Marine chemical ecology* (pp. 3-71): Boca Raton, FL, USA.

Hay, M. E., & Fenical, W. (1988). Marine plant-herbivore interactions: the ecology of chemical defense. *Annual review of ecology systematics, 19*(1), 111-145. doi:10.1146/annurev.ecolsys.19.1.111

Hestetun, J. T., Dahle, H., Jørgensen, S. L., Olsen, B. R., & Rapp, H. T. (2016). The Microbiome and occurrence of methanotrophy in carnivorous sponges. *Frontiers in microbiology, 7*, 1781. doi:10.3389/fmicb.2016.01781

Hoffmann, J. P., & Graham, L. E. (1984). Effects of selected physicochemical factors on growth and Zoosporogenesis of Cladophora glomerata (Chlorophyta) 1. *Journal of phycology, 20*(1), 1-7. doi:10.1111/j.0022-3646.1984.00001.x

Hoflmann, J. P. (1990). Dependence of photosynthesis and vitamin B12 uptake on cellular vitamin B12 concentration in the multicellular alga Cladophora glomerata (Chlorophyta). *Limnology Oceanography, 35*(1), 100-108. doi:10.4319/lo.1990.35.1.0100

Hollants, J., Decleyre, H., Leliaert, F., De Clerck, O., & Willems, A. (2011). Life without a cell membrane: challenging the specificity of bacterial endophytes within Bryopsis (Bryopsidales, Chlorophyta). *BMC microbiolog, 11*(1), 255. doi:10.1186/1471-2180-11-255

Hollants, J., Leliaert, F., De Clerck, O., & Willems, A. (2010). How endo-is endo-? Surface sterilization of delicate samples: a Bryopsis (Bryopsidales, Chlorophyta) case study. *Symbiosis, 51*(1), 131-138. doi:10.1007/s13199-010-0068-0

Hollants, J., Leliaert, F., De Clerck, O., & Willems, A. (2013). What we can learn from sushi: a review on seaweed–bacterial associations. *FEMS microbiology ecology, 83*(1), 1-16. doi:10.1111/j.1574-6941.2012.01446.x

Hollants, J., Leliaert, F., Verbruggen, H., Willems, A., & De Clerck, O. (2013). Permanent residents or temporary lodgers: characterizing intracellular bacterial communities in the siphonous green alga Bryopsis. *Proceedings of the Royal Society B: Biological Sciences, 280*(1754), 20122659. doi:10.1098/rspb.2012.2659

Holmström, C., Rittschof, D., & Kjelleberg, S. (1992). Inhibition of settlement by larvae of Balanus amphitrite and Ciona intestinalis by a surface-colonizing marine bacterium. *Applied environmental microbiology, 58*(7), 2111-2115. doi:10.1128/AEM.58.7.2111-2115.1992

Horridge, G. A. (1951). Occurrence of *Asparagopsis armata* Harv. on the Scilly Isles. *Nature, 167*(4253), 732-733. doi:10.1038/167732c0

Iguchi, H., Yurimoto, H., Sakai, Y. J. A., & microbiology, e. (2011). Stimulation of methanotrophic growth in cocultures by cobalamin excreted by rhizobia. *Applied environmental microbiology, 77*(24), 8509-8515. doi:10.1128/AEM.05834-11

Jensen, P. R., & Fenical, W. (1994). Strategies for the discovery of secondary metabolites from marine bacteria: ecological perspectives. *Annual review of microbiology, 48*(1), 559-584. doi:10.1146/annurev.mi.48.100194.003015

Jiménez-Escrig, A., Jiménez-Jiménez, I., Pulido, R., & Saura-Calixto, F. (2001). Antioxidant activity of fresh and processed edible seaweeds. *Journal of the Science of Food Agriculture, 81*(5), 530-534. doi:10.1002/jsfa.842

Joint, I., Tait, K., & Wheeler, G. (2007). Cross-kingdom signalling: exploitation of bacterial quorum sensing molecules by the green seaweed Ulva. *Philosophical Transactions of the Royal Society B: Biological Sciences, 362*(1483), 1223-1233. doi:10.1098/rstb.2007.2047

Kanagasabhapathy, M., Yamazaki, G., Ishida, A., Sasaki, H., & Nagata, S. (2009). Presence of quorum-sensing inhibitor-like compounds from bacteria isolated from the brown alga Colpomenia sinuosa. *Letters in applied microbiology, 49*(5), 573-579. doi:10.1111/j.1472-765X.2009.02712.x

Kang, J., Khan, M., Park, N., Cho, J., Lee, M., Fujii, H., & Hong, Y. (2008). Antipyretic, analgesic, and anti-inflammatory activities of the seaweed *Sargassum fulvellum* and *Sargassum thunbergii* in mice. *Journal of ethnopharmacology, 116*(1), 187-190. doi:10.1016/j.jep.2007.10.032

Karimi, E., Geslain, E., KleinJan, H., Tanguy, G., Legeay, E., Corre, E., . . . evolution. (2020). Genome Sequences of 72 Bacterial Strains Isolated from Ectocarpus subulatus: A Resource for Algal Microbiology. *J Genome biology, 12*(1), 3647-3655. doi:10.1093/gbe/evz278

Kinley, R. D., & Fredeen, A. (2015). In vitro evaluation of feeding North Atlantic stormtoss seaweeds on ruminal digestion. *Journal of Applied Phycology, 27*(6), 2387-2393. doi:10.1007/s10811-014-0487-z

Kinley, R. D., Martinez-Fernandez, G., Matthews, M. K., de Nys, R., Magnusson, M., & Tomkins, N. W. (2020). Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed. *Journal of Cleaner Production*, 120836. doi:10.1016/j.jclepro.2020.120836

Kittelmann, S., Pinares-Patino, C. S., Seedorf, H., Kirk, M. R., Ganesh, S., McEwan, J. C., & Janssen, P. H. (2014). Two different bacterial community types are linked with the low-methane emission trait in sheep. *PLoS One, 9*(7), e103171. doi:10.1371/journal.pone.0103171

Knack, J., Wilcox, L., Delaux, P.-M., Ané, J.-M., Piotrowski, M., Cook, M., . . . Graham, L. (2015). Microbiomes of streptophyte algae and bryophytes suggest that a functional suite of microbiota fostered plant colonization of land. *International Journal of Plant Sciences, 176*(5), 405-420. doi:10.1086/681161

Kong, M., & Chan, K.-y. (1979). A study on the bacterial flora isolated from marine algae. *Botanica Marina, 22*(2), 83-98. doi:10.1515/botm.1979.22.2.83

Lachnit, T., Meske, D., Wahl, M., Harder, T., & Schmitz, R. (2011). Epibacterial community patterns on marine macroalgae are host-specific but temporally variable. *Environmental microbiology, 13*(3), 655-665. doi:10.1111/j.1462-2920.2010.02371.x

Lachnit, T., Wahl, M., & Harder, T. (2010). Isolated thallus-associated compounds from the macroalga Fucus vesiculosus mediate bacterial surface colonization in the field similar to that on the natural alga. *Biofouling, 26*(3), 247-255. doi:10.1080/08927010903474189

Lane, A. L., Nyadong, L., Galhena, A. S., Shearer, T. L., Stout, E. P., Parry, R. M., . . . Fernandez, F. M. (2009). Desorption electrospray ionization mass spectrometry reveals surface-mediated antifungal chemical defense of a tropical seaweed. *Proceedings of the National Academy of Sciences, 106*(18), 7314-7319. doi:10.1073/pnas.0812020106

Lanigan, G. (1972). Metabolism of pyrrolizidine alkaloids in the ovine rumen. IV. Effects of chloral hydrate and halogenated methanes on rumen methanogenesis and alkaloid metabolism in fistulated sheep. *Australian Journal of Agricultural Research, 23*(6), 1085-1091. doi:10.1071/AR9721085

Le Lann, K., Jegou, C., & Stiger-Pouvreau, V. (2008). Effect of different conditioning treatments on total phenolic content and antioxidant activities in two Sargassacean species: comparison of the frondose *Sargassum muticum* (Yendo) Fensholt and the cylindrical *Bifurcaria bifurcata* R. Ross. *Phycological Research, 56*(4), 238-245. doi:10.1111/j.1440-1835.2008.00505.x

Lee, M. D., Kling, J. D., Araya, R., & Ceh, J. (2018). Jellyfish life stages shape associated microbial communities, while a core microbiome is maintained across all. *Frontiers in microbiology, 9*, 1534. doi:10.3389/fmicb.2018.01534

Liu, Y., & Whitman, W. B. (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Annals of the New York Academy of Sciences, 1125*(1), 171-189. doi:10.1196/annals.1419.019.

Longford, S. R., Campbell, A. H., Nielsen, S., Case, R. J., Kjelleberg, S., & Steinberg, P. D. (2019). Interactions within the microbiome alter microbial interactions with host chemical defences and affect disease in a marine holobiont. *Scientific Reports*(9), 1-13. doi:10.1038/s41598-018-37062-z

Lunde, G. (1970). Analysis of trace elements in seaweed. *Journal of the Science of Food Agriculture, 21*(8), 416-418. doi:10.1002/jsfa.2740210806

Maasakkers, J. D., Jacob, D. J., Sulprizio, M. P., Scarpelli, T. R., Nesser, H., Sheng, J.-X., . . . Physics. (2019). Global distribution of methane emissions, emission trends, and OH concentrations and trends inferred from an inversion of GOSAT satellite data for 2010–2015. *Atmospheric Chemistry Physics, 19*(11), 7859-7881. doi:10.5194/acp-19-7859-2019

Machado, L., Magnusson, M., Paul, N. A., de Nys, R., & Tomkins, N. (2014). Effects of marine and freshwater macroalgae on in vitro total gas and methane production. *PLoS One, 9*(1). doi:10.1371/journal.pone.0085289

Machado, L., Magnusson, M., Paul, N. A., Kinley, R., de Nys, R., & Tomkins, N. (2016a). Dose-response effects of *Asparagopsis taxiformis* and *Oedogonium* sp. on in vitro fermentation and methane production. *Journal of Applied Phycology, 28*(2), 1443-1452. doi:10.1007/s10811-015-0639-9

Machado, L., Magnusson, M., Paul, N. A., Kinley, R., de Nys, R., & Tomkins, N. (2016b). Identification of bioactives from the red seaweed *Asparagopsis taxiformis* that promote antimethanogenic activity in vitro. *Journal of Applied Phycology, 28*(5), 3117-3126. doi:10.1007/s10811-016-0830-7

Machado, L., Tomkins, N., Magnusson, M., Midgley, D. J., de Nys, R., & Rosewarne, C. P. (2018). In vitro response of rumen microbiota to the antimethanogenic red macroalga *Asparagopsis taxiformis*. *Microbial ecology, 75*(3), 811-818. doi:10.1007/s00248-017-1086-8

Mata, L., Gaspar, H., & Santos, R. (2012). Carbon/nutrient balance in relation to biomass production and halogenated compound content in the red alga *Asparagopsis taxiformis* (Bonnemaisoniaceae). *Journal of phycology, 48*(1), 248-253. doi:10.1111/j.1529-8817.2011.01083.x

McCann, J. C., Wickersham, T. A., & Loor, J. J. (2014). High-throughput methods redefine the rumen microbiome and its relationship with nutrition and metabolism. *Bioinformatics biology insights, 8*, BBI. S15389. doi:10.4137/BBI.S15389

McConnell, O., & Fenical, W. (1977). Halogen chemistry of the red alga *Asparagopsis*. *Phytochemistry, 16*(3), 367-374. doi:10.1016/0031-9422(77)80067-8

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C., Carey, H. V., Domazet-Lošo, T., Douglas, A. E., . . . Gilbert, S. F. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences, 110*(9), 3229-3236. doi:10.1073/pnas.1218525110

Medina, R. P., Araujo, A. R., Batista, J. M., Cardoso, C. L., Seidl, C., Vilela, A. F., . . . Silva, D. H. (2019). Botryane terpenoids produced by Nemania bipapillata, an endophytic fungus isolated from red alga Asparagopsis taxiformis-Falkenbergia stage. *Scientific Reports, 9*(1), 1-11. doi:10.1038/s41598-019-48655-7

Mizrahi, I., Wallace, R. J., & Moraïs, S. (2021). The rumen microbiome: balancing food security and environmental impacts. *Nature Reviews Microbiology*, 1-14. doi:10.1038/s41579-021-00543-6

Moore, R. E. (1977). Volatile compounds from marine algae. *Accounts of Chemical Research, 10*(2), 40-47. doi:10.1021/ar50110a002

Morgavi, D., Forano, E., Martin, C., & Newbold, C. (2010). Microbial ecosystem and methanogenesis in ruminants. *Animal, 4*(7), 1024-1036. doi:10.1017/S1751731110000546

Morrison, F. B., & Morrison, E. B. (1954). *Feeds and feeding, abridged: the essentials of the feeding, care, and management of farm animals, including poultry*: Morrison.

Murakami, A., Miyashita, H., Iseki, M., Adachi, K., & Mimuro, M. (2004). Chlorophyll d in an epiphytic cyanobacterium of red algae. *Science, 303*(5664), 1633-1634. doi:10.1126/science.1095459

Nanri, A., Mizoue, T., Shimazu, T., Ishihara, J., Takachi, R., Noda, M., . . . Tsugane, S. (2017). Dietary patterns and all-cause, cancer, and cardiovascular disease mortality in Japanese men and women: The Japan public health center-based prospective study. *PLoS One, 12*(4). doi:10.1371/journal.pone.0174848.

Nash, R., Rindi, F., & Guiry, M. D. (2005). Optimum conditions for cultivation of the Trailliella phase of *Bonnemaisonia hamifera* Hariot (Bonnemaisoniales, Rhodophyta), a candidate species for secondary metabolite production. *Botanica Marina, 48*(4), 257-265. doi:10.1515/BOT.2005.035

Newton, L. (1951). Animal and human nutrition from seaweed resources in Europe: uses and potential. In *Seaweed utilization.* (pp. 31-57): Sampson Low, London.

Nylund, G. M., Persson, F., Lindegarth, M., Cervin, G., Hermansson, M., & Pavia, H. (2009). The red alga Bonnemaisonia asparagoides regulates epiphytic bacterial abundance and community composition by chemical defence. *FEMS microbiology ecology, 71*(1), 84-93. doi:10.1111/j.1574-6941.2009.00791.x

Patra, A. K. (2012). Enteric methane mitigation technologies for ruminant livestock: a synthesis of current research and future directions. *Environmental Monitoring Assessment, 184*(4), 1929-1952. doi:10.1007/s10661-011-2090-y

Paul, N. A., de Nys, R., & Steinberg, P. D. (2006a). Chemical defence against bacteria in the red alga *Asparagopsis armata*: linking structure with function. *Marine Ecology Progress Series, 306*, 87-101. doi:10.3354/MEPS306087

Paul, N. A., De Nys, R., & Steinberg, P. D. (2006b). Seaweed–herbivore interactions at a small scale: direct tests of feeding deterrence by filamentous algae. *Marine Ecology Progress Series, 323*, 1-9. doi:10.3354/meps323001

Pedersén, M. (1968). Ectocarpus fasciculatus: marine brown alga requiring kinetin. *Nature, 218*(5143), 776-776. doi:10.1038/218776a0

Phang, S.-M., Keng, F. S.-L., Singh, P.-K. M., Lim, Y.-K., Abd Rahman, N., Leedham, E. C., . . . Sturges, W. T. (2015). Can seaweed farming in the tropics contribute to climate change through emission of short-lived halocarbons? *MJS, 34*(1), 8-19. doi:10.22452/mjs.vol34no1.2

Piers, M. D. F., & Joshi, M. (2005). The role of halocarbons in the climate change of the troposphere and stratosphere. *Climatic Change, 71*(1-2), 249-266. doi:10.1007/s10584-005-5955-7

Potin, P., Bouarab, K., Küpper, F., & Kloareg, B. (1999). Oligosaccharide recognition signals and defence reactions in marine plant-microbe interactions. *Current opinion in microbiology, 2*(3), 276-283. doi:10.1016/S1369-5274(99)80048-4

Provasoli, L., & Pintner, I. (1972). Effect of bacteria on seaweed morphology. *J Phycology, 8*(10).

Qian, P.-Y., Lau, S. C., Dahms, H.-U., Dobretsov, S., & Harder, T. (2007). Marine biofilms as mediators of colonization by marine macroorganisms: implications for antifouling and aquaculture. *Marine Biotechnology, 9*(4), 399-410. doi:10.1007/s10126-007-9001-9

Regal, A., Alves, V., Gomes, R., Matos, J., Bandarra, N., Afonso, C., . . . Technology. (2020). Drying process, storage conditions, and time alter the biochemical composition and bioactivity of the anti-greenhouse seaweed *Asparagopsis taxiformis*. *European Food Research*, 1-13. doi:10.1007/s00217-020-03445-8

Rindi, F., Soler-Vila, A., & Guiry, M. D. (2012). Taxonomy of marine macroalgae used as sources of bioactive compounds. In *Marine bioactive compounds* (pp. 1-53): Springer.

Ripple, W. J., Smith, P., Haberl, H., Montzka, S. A., McAlpine, C., & Boucher, D. H. (2014). Ruminants, climate change and climate policy. *Nature climate change, 4*(1), 2-5. doi:10.1038/nclimate2081

Riquelme, C., Rojas, A., Flores, V., & Correa, J. A. (1997). Epiphytic bacteria in a copper-enriched environment in northern Chile. *Marine pollution bulletin, 34*(10), 816-820. doi:10.1016/S0025-326X(97)00086-6

Rojas-Downing, M., Nejadhashemi, A., Harrigan, T., & Woznicki, S. (2017). Climate change and livestock: impacts, adaptation, and mitigation. *Climate Risk Management (internet), 16*, 145-163. doi:10.1016/j.crm.2017.02.001

Roque, B. M., Salwen, J. K., Kinley, R., & Kebreab, E. (2019). Inclusion of *Asparagopsis armata* in lactating dairy cows' diet reduces enteric methane emission by over 50 percent. *Journal of Cleaner Production, 234*, 132-138. doi:10.1016/j.jclepro.2019.06.193

Rosenberg, E., Koren, O., Reshef, L., Efrony, R., & Zilber-Rosenberg, I. (2007). The role of microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology, 5*(5), 355-362. doi:10.1038/nrmicro1635

Rosenberg, E., & Zilber-Rosenberg, I. (2016). Microbes drive evolution of animals and plants: the hologenome concept. *J MBio, 7*(2), e01395-01315. doi:10.1128/mBio.01395-15

Saha, M., & Weinberger, F. (2019). Microbial "gardening" by a seaweed holobiont: surface metabolites attract protective and deter pathogenic epibacterial settlement. *Journal of Ecology, 107*(5), 2255-2265. doi:10.1111/1365-2745.13193

Sakami, T. (1996). Effects of algal excreted substances on the respiration activities of epiphytic bacteria on the brown alga, Eisenia bicyclis Kjellman. *Fisheries science, 62*(3), 394-396. doi:10.2331/fishsci.62.394

Siboni, N., Ben-Dov, E., Sivan, A., & Kushmaro, A. (2008). Global distribution and diversity of coral-associated Archaea and their possible role in the coral holobiont nitrogen cycle. *Environmental microbiology, 10*(11), 2979-2990. doi:10.1111/j.1462-2920.2008.01718.x

Singh, R. P., Bijo, A., Baghel, R. S., Reddy, C., & Jha, B. (2011). Role of bacterial isolates in enhancing the bud induction in the industrially important red alga Gracilaria dura. *FEMS microbiology ecology, 76*(2), 381-392. doi:10.1111/j.1574-6941.2011.01057.x

Solomon, S., Manning, M., Marquis, M., & Qin, D. (2007). *Climate change 2007-the physical science basis: Working group I contribution to the fourth assessment report of the IPCC* (Vol. 4): Cambridge university press.

Staufenberger, T., Thiel, V., Wiese, J., & Imhoff, J. F. (2008). Phylogenetic analysis of bacteria associated with Laminaria saccharina. *FEMS microbiology ecology, 64*(1), 65-77. doi:10.1111/j.1574-6941.2008.00445.x

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., De Haan, C., & Shadow, L. s. L. (2006). Environmental issues and options. *Food Agriculture Organization, Rome, Italy*.

Stévant, P., Rebours, C., & Chapman, A. (2017). Seaweed aquaculture in Norway: recent industrial developments and future perspectives. *Aquaculture International, 25*(4), 1373-1390. doi:10.1007/s10499-017-0120-7

Tang, Y. Z., Koch, F., & Gobler, C. J. (2010). Most harmful algal bloom species are vitamin B1 and B12 auxotrophs. *Proceedings of the National Academy of Sciences, 107*(48), 20756-20761. doi:10.1073/pnas.1009566107

Tapia, J. E., González, B., Goulitquer, S., Potin, P., & Correa, J. A. (2016). Microbiota Influences Morphology and Reproduction of the Brown Alga *Ectocarpus* sp. *Frontiers in microbiology, 7*, 197. doi:10.3389/fmicb.2016.00197

Val, A., Platas, G., Basilio, A., Cabello, A., Gorrochategui, J., Suay, I., . . . Reina, G. (2001). Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *International Microbiology, 4*(1), 35-40. doi:10.1007/s101230100006

van der Loos, L. M., Eriksson, B. K., & Salles, J. F. (2019). The macroalgal holobiont in a changing sea. *Trends in microbiology, 27*(7), 635-650. doi:10.1016/j.tim.2019.03.002

Vergés, A., Paul, N. A., & Steinberg, P. D. (2008). Sex and life-history stage alter herbivore responses to a chemically defended red alga. *Ecology, 89*(5), 1334-1343. doi:10.1890/07-0248.1

Vollset, S. E., Goren, E., Yuan, C.-W., Cao, J., Smith, A. E., Hsiao, T., . . . Chalek, J. (2020). Fertility, mortality, migration, and population scenarios for 195 countries and territories from 2017 to 2100: a forecasting analysis for the Global Burden of Disease Study. *The Lancet*. doi:10.1016/S0140-6736(20)30677-2

Wahl, M., Shahnaz, L., Dobretsov, S., Saha, M., Symanowski, F., David, K., . . . Weinberger, F. (2010). Ecology of antifouling resistance in the bladder wrack Fucus vesiculosus: patterns of microfouling and antimicrobial protection. *Marine Ecology Progress Series, 411*, 33-48. doi:10.3354/meps08644

Wang, Y., Han, F., Hu, B., Li, J., & Yu, W. (2006). In vivo prebiotic properties of alginate oligosaccharides prepared through enzymatic hydrolysis of alginate. *J Nutrition Research, 26*(11), 597-603. doi:10.1016/j.nutres.2006.09.015

Wang, Y., Xu, Z., Bach, S., & McAllister, T. (2008). Effects of phlorotannins from *Ascophyllum nodosum* (brown seaweed) on in vitro ruminal digestion of mixed forage or barley grain. *Animal Feed Science Technology, 145*(1-4), 375-395. doi:10.1016/j.anifeedsci.2007.03.013

Wever, R., Tromp, M., Van Schijndel, J., Vollenbroek, E., Olsen, R., & Fogelqvist, E. (1993). Bromoperoxidases: their role in the formation of HOBr and bromoform by seaweeds. In *Biogeochemistry of global change* (pp. 811-824): Springer.

Wiese, J., Thiel, V., Nagel, K., Staufenberger, T., & Imhoff, J. F. (2009). Diversity of antibiotic-active bacteria associated with the brown alga Laminaria saccharina from the Baltic Sea. *Marine Biotechnology, 11*(2), 287-300. doi:10.1007/s10126-008-9143-4

Williams, P. G., Yoshida, W. Y., Moore, R. E., & Paul, V. J. (2003). Novel iodinated diterpenes from a marine cyanobacterium and red alga assemblage. *Organic letters, 5*(22), 4167-4170. doi:10.1021/ol035620u

Wirth, R., Kádár, G., Kakuk, B., Maróti, G., Bagi, Z., Szilágyi, Á., . . . Kovács, K. L. (2018). The planktonic core microbiome and core functions in the cattle rumen by next generation sequencing. *Frontiers in microbiology, 9*, 2285. doi:10.3389/fmicb.2018.02285

Wood, J., Kennedy, F. S., & Wolfe, R. (1968). Reaction of multihalogenated hydrocarbons with free and bound reduced vitamin B_{12}. *Biochemistry, 7*(5), 1707-1713. doi:10.1021/bi00845a013

Woolard, F. X., Moore, R. E., & Roller, P. P. (1979). Halogenated acetic and acrylic acids from the red alga *Asparagopsis taxiformis*. *Phytochemistry, 18*(4), 617-620. doi:10.1016/S0031-9422(00)84271-5

Yang, Y. J., Nam, S.-J., Kong, G., & Kim, M. K. (2010). A case–control study on seaweed consumption and the risk of breast cancer. *British Journal of Nutrition, 103*(9), 1345-1353. doi:10.1017/S0007114509993242.

Young, E. B., Tucker, R. C., & Pansch, L. A. (2010). ALKALINE PHOSPHATASE IN FRESHWATER CLADOPHORA-EPIPHYTE ASSEMBLAGES: REGULATION IN RESPONSE TO PHOSPHORUS SUPPLY AND LOCALIZATION 1. *Journal of phycology, 46*(1), 93-101. doi:10.1111/j.1529-8817.2009.00782.x

Yuan, Y. V., & Walsh, N. A. (2006). Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food chemical toxicology, 44*(7), 1144-1150. doi:10.1016/j.fct.2006.02.002

Zulkifly, S., Hanshew, A., Young, E. B., Lee, P., Graham, M. E., Graham, M. E., . . . Graham, L. E. (2012). The epiphytic microbiota of the globally widespread macroalga

Cladophora glomerata (Chlorophyta, Cladophorales). *American Journal of Botany, 99*(9), 1541-1552. doi:10.3732/ajb.1200161